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Epidermal growth factor receptor (EGFR) in laryngeal cancer

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Summary

Altered expression of growth factors and growth factor receptors is frequently described in human tumour cell lines.
The authors analysed the expression of epidermal growth factor receptor (EGFR) in 154 cases of laryngeal squamous cell carcinoma and its relationship to the clinical outcome of the patients. The difference in EGFR expression between control epithelium and cancer tissue was significant (p=0.0109).
There was no significant correlation between EGFR expression and sex and age of the patients, T stage, lymph node status, site and histopathological grading of the tumour. Univariate analysis revealed no correlation between EGFR expression and survival rates. In multivariate analysis only two variables showed significant correlation with prognosis: N status (p=0.0006) and, to a lesser degree, T stage (p=0.014) (Table II). We concluded that immunohistological examination of EGFR on paraffin section is not a valuable prognostic factor in laryngeal carcinoma. EGFR expression may be valuable tool in differentiating malignant from benign laryngeal epithelium.

Key words: epidermal growth factor receptor, laryngeal cancer

Introduction

One growth factor that may participate in the malignant transformation of upper respiratory tract squamous epithelial cells is epidermal growth factor (EGF). EGF stimulates proliferation of cells by interaction with its surface receptor (EGFR). The epidermal growth factor receptor gene, located on chromosome 7p12–13, codes for a 170 kDa transmembrane growth-regulating receptor glycoprotein, which has an intristic tyrosine-specific kinase activity. EGFR regulates cell growth in cell lines and in variety of cancers (7, 19, 22).
The overexpression of the EGFR gene has been reported to be a useful diagnostic and prognostic marker in head and neck squamous cell carcinomas (5).
EGFR has been described in many human neoplasms, and its expression might represent a
parameter of poor prognosis in breast (1), esophageal (13), ovarian (17) and oral (20) tumours. In a number of tumours increased of EGFR has been associated with tumour size and advanced stage (6, 12, 23).

There are some reports concerning overexpression of EGFR in premalignant lesions of the head and neck region, mainly in the larynx (3, 11, 19, 21). Gale et al. (3) revealed that expression of EGFR progressively increases with the grade of epithelial hyperplastic lesions of the larynx.

The purpose of our study was to investigate the expression of EGFR in laryngeal squamous cell carcinomas and its relationship to the clinical outcome of the patients.

Material and methods

One hundred fifty four patients who underwent biopsy for carcinoma of the larynx were selected for this study. All patients were diagnosed between 1991 and 1994. There were 26 females and 128 males. The mean age was 61,3±10,1 years. Tumour staging was performed according to TMN criteria (Table I).

All cases were squamous carcinomas. The histopathological grading of tumours (G1–G3) done on the hematoxylin-eosine sections was as follows: 39 cases – G1, 61 cases G – II and 54 cases – GIII.

After diagnosis the patients were treated by surgery and/or radiotherapy. Eighty nine patients underwent complete surgical excision of tumour and free margins. Thirty two patients were treated by radiotherapy alone and thirty three patients underwent surgery and subsequent radiotherapy.

A minimum follow-up of 3 years or to a patient’s death was available for all the cases.

The tissue specimens, in the form of paraffin blocks, were available in the Department of Pathology, School of Medicine in Wroclaw. Normal laryngeal epithelium obtained from

<table>
<thead>
<tr>
<th>Table I. Clinicopathological findings and EGFR expression in laryngeal cancer.</th>
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<tbody>
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<td>Features</td>
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<td>Sex</td>
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<td>Female</td>
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<tr>
<td>Male</td>
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<td>Age (yr)</td>
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<td>&lt;55</td>
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<tr>
<td>55-65</td>
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<tr>
<td>&gt;65</td>
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<tr>
<td>Site of tumour</td>
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<tr>
<td>Epiglottic</td>
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<td>Glottic</td>
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<tr>
<td>Subglottic</td>
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<td>Transglottic</td>
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<td>T stage</td>
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<td>T4</td>
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<td>Lymph node metastasis</td>
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<td>N₀</td>
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<td>N₁</td>
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<td>Histopathological grade</td>
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<td>G1</td>
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<td>GII</td>
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<td>GIII</td>
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<tr>
<td>Clinical follow-up</td>
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<tr>
<td>No recurrence</td>
</tr>
<tr>
<td>Recurrence</td>
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</tbody>
</table>
twenty five patients with benign lesions of the larynx was examined as the control group.

Immunohistochemical staining procedure

Formalin-fixed and paraffin-embedded tissue sections (4µm) were stained using the streptavidin-biotin-peroxidase method. The sections were deparaffinized in xylene and rehydrated in alcohol. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxidase in methanol for 15 minutes. Primary anti-EGFR antibody from DAKO (Epidermal Growth Factor Receptor mouse monoclonal antibody NCL-EGF) was applied in a dilution of 1:20 and incubated for 1 hour at 25°C followed by incubation with biotinylated antimouse immunoglobulin. The slides were incubated with streptavidin-peroxidase complex for 40 minutes at 25°C and then with chromogenic substrate solution for peroxidase (DAKO DAB) for 15 minutes. Specimens were counterstained with haematoxylin, and then observed using light microscope. Negative controls included omission of the antibody from the assay. Initial assessment was made at low microscopic power to examine the distribution of malignant cells and to ascertain whether there were any obvious variations in staining. The EGFR immunoreactivity was assessed on the cell membranes. The number of positive cells per high-field was assessed. The expression of EGFR was scored as follows: 0 = no staining, 1+ (moderate staining) = 0–50% positively stained cells, 2+ = (strong staining) 25–100% positively stained cells.

Statistical methods

Differences between grouped data were analysed by the Mann-Whitney test or chi-squared test as appropriate.

Prognostic factors analysed for their influence on survival were age and sex of the patients, site and size of the tumour, appearance of lymph node metastasis, histopathological grading of tumour, and EGFR expression. Any deaths resulting from causes other than the primary cancer were excluded from the statistical analysis.

Univariate survival analyses were based on the Kaplan-Meier product-limit estimates of survival distribution.

Differences between survival curves were tested statistically using generalised Wilcoxon test. The relative importance of multiple prognostic factors on survival was estimated using the Cox proportional hazards regression model.

All data were processed with S-PLUS statistical software (MathSoft, Seattle WA).

A p value less than 0.05 was considered significant.

Results

Of 154 laryngeal carcinomas 96 (62.4%) showed evidence of positive immunostaining for EGFR. In positively stained carcinomas, typical staining pattern was predominantly of membrane staining although some cytoplasmatic was noted.

Of 25 control laryngeal epithelium 5 (20%) showed evidence of positive immunostaining for EGFR. In positive cases the membrane staining were limited to the lower layer of epithelium (Figure 1).

The difference in EGFR expression between control epithelium and cancer tissue was significant (p=0.0109).

The EGFR expression correlated with the patients clinical course. The EGFR expression was higher in cases with recurrence than in cases free of disease after treatment but the difference was again on statistical borderline (p=0.0521).

There was no significant correlation between EGFR expression and sex and age of the patients, T stage, lymph node status, site and histopathological grading of the tumour (Table I).

Univariate analysis revealed no correlation between EGFR expression and survival rates (p=0.849).
To determine which parameter is an independent prognostic variable in laryngeal carcinoma, a multivariate analysis was performed. By testing the association of response with covariates in the Cox model, only two variables showed significant correlation with prognosis: N status (p=0.0006) and, to a lesser degree, T stage (p=0.014) (Table II).

Table II. Multivariate analysis of Cox’s proportional hazards model in patient’s with laryngeal cancer.

<table>
<thead>
<tr>
<th>Variables</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>NS*</td>
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<tr>
<td>Sex</td>
<td>NS</td>
</tr>
<tr>
<td>Site of tumour</td>
<td>NS</td>
</tr>
<tr>
<td>T status</td>
<td>p=0.014</td>
</tr>
<tr>
<td>N status</td>
<td>p=0.0006</td>
</tr>
<tr>
<td>Grading</td>
<td>NS</td>
</tr>
<tr>
<td>EGFR</td>
<td>NS</td>
</tr>
</tbody>
</table>

Discussion

The study of growth factors and their receptors is an important field of research in the biology of cancer. Several transformed cells abnormally synthesise and respond to their own growth factors.

EGFR expression in head and neck carcinomas has been previously reported (8, 16, 20).

Di Marco et al. (2) assumed that the altered expression of EGFR found in laryngeal carcinomas is by itself one of the multistep processes occurring in tumour development and that it might have a role as a prognostic factor in this group of carcinomas.

Correlation of expression with poor survival was shown in oral carcinoma (19), maxillary sinus carcinoma (12), laryngeal carcinoma (10).

Uhlman et al. analysed dysregulation of EGFR in premalignant lesions in the larynx. The results indicate that EGFR could be a useful biomarker for multistep carcinogenesis and may serve as prognostic indicator in dysplastic lesions.

The aim of our work was to analyse the correlation between EGFR expression and clinicopathological features of the tumour. We also
estimated whether EGFR expression could be a prognostic marker of laryngeal carcinoma.

Our results confirm observations of Scambia et al. (17) that higher EGFR expression are present in laryngeal cancer than in normal epithelium. This suggests that uncontrolled cell growth may be mediated by abnormal EGFR expression.

We failed to find any significant difference in the EGFR expression in relation to histopathologic grading.

The EGFR expression was significantly less for benign lesions than for malignant epithelium, in accord with the findings of other authors (4, 10). This suggests that uncontrolled cell growth may be mediated by abnormal EGFR expression.

In normal laryngeal epithelium EGFR staining was confined to cells adjacent to the basal laminae. In invasive, poorly differentiated carcinoma positive EGFR stained cells were present in all tumour infiltration. When the tumour cells matured into squamous pearls, the EGFR staining disappeared. This observation is in accord with results of other authors (Störkel et al., 1993).

We have not observed positive association between EGFR expression and T stage, in accord with the findings of Maurizi et al. (10) but in contrast with Kusukawa et al. (9).

It is difficult to assess the correlation between lymph node status and expression of markers of cell proliferation. Some authors revealed such relation (9) but other authors including this study did not confirm this correlation (10, 15).

Our results revealed that EGFR expression didn’t correlate significantly with patient’s clinical outcome and survival. These findings are in line with the results obtained by Resnick et al. (15) and Kearsley et al. (8).

In multivariate analysis the only significant prognostic factor was appearance of nodal lymph metastases and T stage.

It appears that the immunohistological examination of EGFR on paraffin section is not a valuable prognostic factor in laryngeal carcinoma.

EGFR expression may be valuable tool in differentiating malignant from benign laryngeal epithelium.

References


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