Int. J. Cancer (Pred. Oncol.): **84,** 188–191 (1999)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION IN PRIMARY LARYNGEAL CANCER: AN INDEPENDENT PROGNOSTIC FACTOR OF NECK NODE RELAPSE

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Specimens of laryngeal squamous cell carcinoma (LSCC) were examined for epidermal growth factor receptor (EGFR) content using a radioreceptor method; 140 untreated consecutive patients with primary LSCC undergoing initial surgical resection were followed up for a median of 49 months (range 2–84 months) after surgery. Cox univariate regression analysis using EGFR as a continuous variable showed that EGFR levels were directly associated with the risk of lymph node metastasis. A significant relationship between EGFR status and cervical node metastasis was observed. The cutoff value of 20 fmol/mg protein was the best prognostic discriminator. The 5-year metastasis-free survival (MFS) was 66% for patients with EGFR$^-$ tumors compared with 15% for patients with EGFR$^+$ tumors. By multivariate analysis, the EGFR status appeared to be a significant independent prognostic factor for MFS. Our results suggest that the assessment of EGFR status at the time of diagnosis may identify a subset of LSCC patients highly susceptible to neck node metastases thus defining therapy accordingly. *Int. J. Cancer (Pred. Oncol.)* 84:188–191, 1999.

Despite advances in all areas of diagnosis and treatment, the prognosis of patients with laryngeal squamous cell carcinoma (LSCC) has remained unchanged during the last 2 decades. Furthermore, a slight increase in advanced stage patients indicates that efforts toward early detection have not been successful.

At present, prognosis is related to age, performance status, tumor size, tumor location, lymph node involvement, stage of disease, histological grading and treatment. Positive neck nodes have a decisive influence on prognosis in patients with head and neck cancer, especially laryngeal cancer, for which local recurrence is rarely observed. Indeed, regrouping stage III and IV patients as localized disease *vs*. regional metastasis appears to better predict survival (Shah *et al*., 1997).

The management of cervical lymph nodes represents a vitally important component of the overall treatment of patients with LSCC, particularly for supraglottic cancer. The treatment results of laryngeal cancer have improved greatly in recent years, for initial disease control as well as quality of life, due to the functional preservation of the larynx (Lefebvre and Bonneterre, 1996).

At present, great emphasis is also placed on the functional improvement and cosmetics in the management of neck cancer. The evolution of neck dissection from Crile's operation through modified radical, functional and comprehensive neck dissection to selective and limited neck dissection is representative of such a trend (Eicher and Weber, 1996).

While comprehensive neck dissection is widely reported as mandatory during initial treatment in cases with clinically positive neck nodes, no agreement exists as to whether to perform an elective selective neck dissection in LSCC patients with clinically negative neck nodes. Management of N0 neck nodes in LSCC includes elective jugular neck dissection, elective irradiation or observation, with no evidence of statistically different results in terms of survival (overall and disease-free) and salvageability of recurrent disease and quality of life (Collins and Muzaffer, 1998).

Elective ipsilateral or bilateral jugular neck dissection, which has been advocated as a therapeutic and staging modality, is currently the most common unsettled surgical option in LSCC (especially for supraglottic site) patients with clinically N0 neck nodes (Shah and Tollefens, 1974; Weber *et al*., 1994). However, the elective management of the neck in all cases of primary LSCC may lead to overtreatment with concomitant unnecessary increase in costs, duration of treatment and treatment-related morbidity (Eicher and Weber, 1996; Quraishi *et al*., 1997).

At present, evaluation of the development of regional metastases on the basis of clinicopathological parameters remains inadequate, since patients with identical clinicopathological features and treatment may differ widely in the development of regional disease and in response to therapy.

There has been continuous progress in understanding the molecular abnormalities underlying the pathogenesis of laryngeal cancer with the identification of altered dominant and recessive oncogenes, genetic instability and growth factor-linked signal transmission pathways. Much attention has been focused on the role of cyclin D1 gene (Bellacosa *et al*., 1996), telomerase activity (Hohaus *et al*., 1996), cathepsin D (Maurizi *et al*., 1996*a*), epidermal growth factor receptor (EGFR) expression and amplification (Scambia *et al*., 1991; Maurizi *et al*., 1992), and methyl-p-hydroxy-phenyllactate-esterase (MeHPLAase) activity (Maurizi *et al*., 1998) in the prognosis of laryngeal cancer.

EGFR overexpression or amplification may be a helpful predictor of tumor aggressiveness or invasiveness and of metastatic potential for a cost-effective treatment of cervical lymph nodes to improve neck management. In previous studies, EGFR expression has been linked with a higher probability of relapse and poor prognosis and relative resistance to chemotherapy and radiotherapy (Maurizi *et al*., 1992, 1996*b;* Stanton *et al*., 1994; Dassonville *et al*., 1993). EGFR activation appears also to be implicated in critical processes involved in tumor invasion and metastasis (Eccles *et al*., 1994–95).

In this study, the clinical role of EGFR expression in the prediction of metastasis-free survival (MFS) in primary LSCC patients was investigated on a large patient population with a long follow-up period, and belonging to a single institution.

MATERIALS AND METHODS

Patients and tumor samples

One hundred forty untreated consecutive primary LSCC patients have been studied. The clinicopathological features of the patients are shown in Table I. Tumor site was classified as supraglottic, glottic or transglottic (when the extent of disease did not permit identification of the original site). Tumors were staged according to the TNM classification and graded as well (G1), moderately (G2) and poorly (G3) differentiated. At our institution, all primary laryngeal cancer patients receive standard therapeutic management

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Received 8 August 1998; Revised 30 October 1998

including therapeutic surgical treatment (curative surgery) of the primary tumor (T) related to the lesion extension and location, therapeutic neck dissection in case of lymph node involvement at clinical presentation (N+) and observation under strict follow-up conditions with surgical salvage for recurrent neck disease in clinically N0 neck tumors. Seventy-three patients underwent radical laryngectomy and 67 had conservative surgery (*i.e.*, cordectomy, hemilaryngectomy, horizontal supraglottic laryngectomy). Thirty-seven patients had a therapeutic comprehensive neck dissection at time of the initial treatment. None of the patients received preoperative chemotherapy or radiotherapy. The patients with locoregional relapse underwent salvage surgery or irradiation.

EGFR assay

Tissue specimens were frozen on dry ice shortly after surgical removal and stored at −80°C until processed. A representative section of specimens was retained for histopathologic examination, which revealed that most of the cells were cancer cells. The membrane fraction and cytosol were prepared as described elsewhere (Scambia *et al*., 1991).

The membrane pellet was resuspended in TENG plus 10 nM $MgCl_2$. Aliquots of the suspension (100 μl containing 300–500 μg protein) were incubated with ^{125}I-EGF (3.2 nM; NEN, Boston, MA) in the presence or absence of unlabeled EGF (1 μM) for 16 hr at room temperature in a final volume of 400 μl. Binding was stopped by the addition of 3 ml of TENG plus 0.1% BSA. Pellets were obtained by centrifugation at 2,000*g* for 20 min at 0°C and counted in a gamma-counter for 1 min. Protein concentration was measured by the Bradford (1976) method. Results are expressed as fmol/mg of membrane protein. Receptor characterization has been reported elsewhere (Scambia *et al*., 1991).

TABLE I – DISTRIBUTION OF EGFR LEVELS ACCORDING TO CLINICOPATHOLOGIC PARAMETERS IN 140 PRIMARY LARYNGEAL CANCER PATIENTS

	Number	% EGFR		
		>8 fmol/mg protein	>16 fmol/mg protein	>20 fmol/mg protein
Total	140	53	26	20
Age (years)				
<60	46	39	22	17
>60	94	59	29	21
Tumor site				
Glottic	13	46	15	15
Supraglottic	47	54	24	17
Transglottic	80	52	30	22
T classification				
T1	22	52	14	14
T2	57	46	23	16
T3	41	54	29	22
T4	20	70	45	35
Lymph node involvement				
N0	103	51	26	21
N+	37	68	31	12
Histological grading				
G1	28	43	21	18
G2	71	56	27	21
G3	41	52	30	20

Statistical analysis

The Wilcoxon rank sum non-parametric test was used to analyze the distribution of EGFR levels according to clinicopathological characteristics. The Cox-Mantel method was used to evaluate the prognostic role of logarithmically transformed EGFR values as a continuous variable (Cox, 1972).

Different cutoff values for EGFR were tested in the survival analysis and arbitrary values of 8, 16 and 20 fmol/mg protein were chosen. All medians and life tables were computed using the product-limit estimate according to Kaplan and Meier (1958), and the curve was examined by means of the log-rank test (Mantel, 1996). Multivariate analysis was performed using the Cox (1972) proportional hazards model. MFS was calculated from the date of first surgery to the date of neck node relapse (median follow-up 49 months; range 2–84 months).

RESULTS

The distribution of EGFR levels in the 140 primary larygeal cancer patients is shown in Table I. EGFR levels ranged from 0 to 169.9 fmol/mg protein with a median value of 8.4 fmol/mg protein. Using arbitrary cutoff values of 8, 16 and 20 fmol/mg protein, 53%, 26% and 20% of tumors, respectively, were considered as being $EGFR^+$. No difference in EGFR distribution in relation to sex, age, tumor site, T classification, lymph node involvement or histopathologic grading was observed (Table I). During the follow-up period, cervical lymph node metastatic recurrence involvement was observed in 54 cases. At the end of the study, 37 patients had died of cancer.

Cox univariate regression analysis using EGFR as a continuous variable showed that EGFR levels are directly associated with the risk of regional metastatic recurrence (χ^2 9.19, $p = 0.002$).

Figure 1 shows the MFS curve according to EGFR status. A significant relationship was found between EGFR positivity and a short MFS at the cutoff values chosen. The cutoff value of 20 fmol/mg protein was the best prognostic discriminator: the 5-year MFS was 66% [95% confidence interval (CI) 57–75] for patients with $EGFR^-$ tumors compared with 15% (95% CI 3–33) for patients with $EGFR^+$ tumors ($p = 0.0005$). T classification was also significantly correlated with neck node MFS in univariate analysis (Table II).

Table III shows the multivariate analysis of prognostic variables for MFS in laryngeal cancer patients. Tumor site, T classification and EGFR status remained the most important independent prognostic factors for MFS.

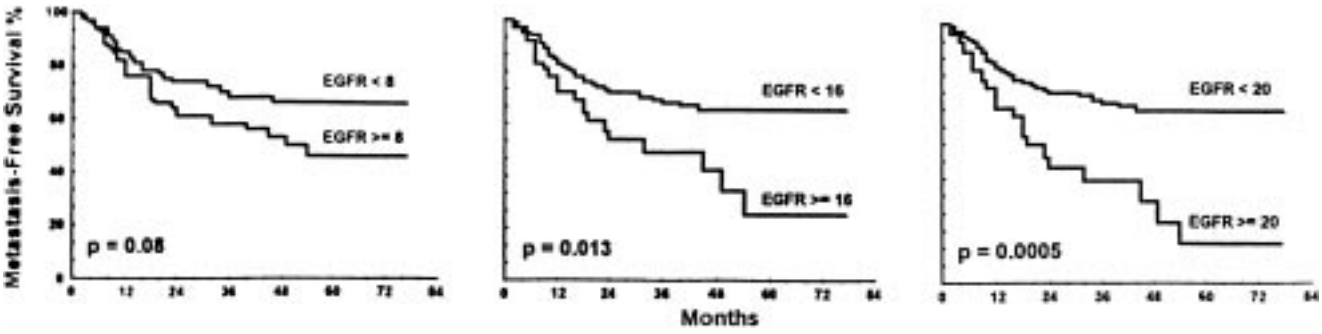

FIGURE 1 – MFS rate according to EGFR status in 140 primary laryngeal cancer patients (54 patients had lymph node involvement).

TABLE II – UNIVARIATE ANALYSIS OF PROGNOSTIC VARIABLES FOR MFS IN 140 PRIMARY LARYNGEAL CANCER PATIENTS

Prognostic variable	Number	MFS	
		% 5-year survival	*p*
Age (years)			
<60	46	60	
>60	94	55	N.S.
Tumor site			
Glottic	13	85	
Supraglottic	47	47	
Transglottic	80	56	—[1]
T classification			
T1–T2	78	68	
T3–T4	62	40	0.0011
Histopathological grading			
G1–G2	99	57	
G3	41	54	N.S.
Lymph node involvement			
N0	103	56	
N+	37	59	N.S.
EGFR status (fmol/mg protein)			
<16	103	65	
>16	37	25	<0.013
<20	112	66	
>20	28	15	<0.0005

[1]Glottic *vs*. supraglottic: $p = 0.12$; glottic *vs*. transglottic: $p = 0.07$; supraglottic *vs*. transglottic: p = N.S. (non-significant).

TABLE III – MULTIVARIATE ANALYSIS OF PROGNOSTIC VARIABLES FOR MFS IN 140 PRIMARY LARYNGEAL CANCER PATIENTS

Prognostic variable	MFS	
	RR[1]	*p*
Age (years)		
<60		
>60	1.43	0.25
Tumor site		
Supraglottic		
Transglottic	0.34	0.0015
T classification		
T1–T2		
T3–T4	3.20	0.0012
Histopathological grading		
G1–G2		
G3	1.74	0.10
Lymph node involvement		
N0		
N+	1.05	0.86
EGFR status (fmol/mg protein)		
<20		
>20	2.70	0.0013

[1]RR: relative risk taking into account all factors in the table.

DISCUSSION

In our previous studies, a higher EGFR expression in primary laryngeal tumors than in normal mucosa, and in poorly differentiated (G3) than in well/moderately (G2/G3) differentiated LSCC (Scambia *et al*., 1991) was reported. We also demonstrated that EGFR levels were significantly associated with a short relapse-free survival and overall survival and poor prognosis (Maurizi *et al*., 1992; Stanton *et al*., 1994).

To our knowledge, this is the largest study performed to date analyzing the metastatic potential of LSCC in a large single institution population of patients with a long follow-up. In our series, a close correlation was observed between the presence of high EGFR levels and a short MFS. Moreover, analysis of logarithmically transformed EGFR values showed that the risk of neck node relapse increased significantly with increasing EGFR values. More importantly, EGFR expression was revealed to be an independent predictor of short MFS, upon multivariate analysis, suggesting that EGFR assessment, together with that of clinicopathological parameters such as stage of disease, may improve the prognostic characterization of laryngeal cancer patients.

Although the role of the EGF/EGFR system in the development of malignant laryngeal SCC phenotype has already been described (Stanton *et al*., 1994), its actual role in tumor progression, tumor invasion and metastasis is not fully established. In mouse mammary carcinoma cell lines, EGF-dependent proteinase secretion has been shown to correlate with metastatic capacity. In addition, EGFR activation appears to be implicated in critical processes involved in metastases, *i.e*., adhesion to matrix proteins, migration and up-regulation of matrix metalloproteinases, key enzymes in tumor cell invasion and penetration of capillary sprouts during angiogenesis (Eccles *et al*., 1994–95). Interestingly enough, in a previous report, we have described a significant correlation between EGFR expression and cathepsin D, which was also significantly associated with a short MFS. The correlation between EGFR and the proteolytic enzyme cathepsin D suggests that the EGF/EGFR system may be involved in the regulation of cathepsin D synthesis and/or secretion (Maurizi *et al*., 1996*a*).

Although the application of experimental results to clinical practice is generally slow, we suggest that the evaluation of EGFR expression at the time of presentation may allow the identification of a subset of LSCC patients who are more susceptible to metastatic spread and permit therapy to be adapted accordingly. An aggressive neck management (*i.e*., bilateral elective jugular neck dissection or irradiation in N0 LSCC tumors; comprehensive or more radical ipsilateral neck dissection together with contralateral elective jugular neck dissection in N1 tumors and postoperative neck irradiation even if without extranodal spread) in $EGFR^+$ tumors may be proposed to avoid undertreatment.

On the other hand, in $EGFR^-$ tumors, a much less aggressive treatment (*i.e*., observation in N0 LSCC tumors; selective rather than comprehensive or more radical neck dissection in N1 tumors) may be proposed to avoid overtreatment. Although further studies are needed before specific conclusions can be drawn, our results suggest the possibility to limit elective neck dissection to selected LSCC patients who are likely to have neck node recurrence on the basis of tumor biological parameters. More particularly, in $EGFR^-$ LSCC patients with clinically negative neck nodes, observation may be considered an effective option. Multi-institutional and multi-disciplinary studies should allow us to meet this important challenge.

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