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Immunohistochemical Evidence of Autocrine Growth Factors in Adenocarcinoma of the Human Lung

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ABSTRACT

We immunohistochemically examined 131 primary human lung adenocarcinomas for the possible presence of autocrine factors. Transforming growth factor α (TGFα) and epidermal growth factor (EGF) were considered growth factors with epidermal growth factor receptor (EGFR) as the receptor. Of these tumors, 87 (66%) showed a high expression of TGFα, 66 (50%) showed a high expression of EGF, and 55 (42%) were positive for EGFR reactivity. In the EGFR-positive cases, the 5-year survival rates of patients with high TGFα and low TGFα were 36% and 85%, respectively ($P < 0.05$). The 5-year survival rates of patients with high EGF and low EGF were 25% and 77%, respectively ($P < 0.05$). In contrast, in the EGFR-negative cases, there was no statistical difference between the 5-year survival rates of patients with either high TGFα or EGF and low TGFα or EGF. Because autocrine growth mechanisms are present in adenocarcinoma of the human lung, these events may contribute to clarification of tumor development, and perhaps even to a better prognosis.

INTRODUCTION

There is a multiplicity of growth factors in various tissues from human embryonic and adult specimens as well as in transformed cells (1). EGF[2] is a single polypeptide chain of 53 amino acids first detected in the submaxillary glands of male mice. EGF promotes growth of cells of ectodermal and mesodermal origins (2, 3). The actions of EGF are mediated through binding to EGFR. Growth factors binding to EGFR include EGF and TGFα. The latter is composed of 50 amino acids with a 42% homology with EGF (4–6); the binding affinity to EGFR seems to be equal to that of EGF (7).

After binding to EGFR, TGFα or EGF activates the tyrosine kinase subunit and autophosphorylation of the receptor occurs (8, 9). These growth factors play an important role in cellular proliferation and differentiation (10, 11). With special reference to TGFα and EGFR, the hypothesis of an autocrine mechanism had gained wide acceptance (12).

We examined TGFα, EGF, and EGFR in lung adenocarcinoma to search for possible evidence of autocrine growth factors.

MATERIALS AND METHODS

The tissues examined were obtained at the time of surgery on 131 patients with primary adenocarcinoma of the lung. All the patients had been diagnosed and treated in The Department of Surgery II, Faculty of Medicine, Kyushu University, between 1974 and 1986. Patients who died within the first postoperative month or who underwent exploratory thoracotomy were excluded from the present analysis. The stage of the disease was classified according to the tumor, node, and metastasis classification of the International Union against Cancer (13), including a review of the surgical and pathological reports of the resected specimens. There were 66 patients with Stage I, 11 with Stage II, 32 with Stage IIIA, 11 with Stage IIIB, and 11 with Stage IV. Of these patients, 80 were men and 51 were women. The ages varied from 39 to 81 years (mean, 63 years). For all patients, the intraoperative decision was curative lobectomy with complete hilar and mediastinum lymph nodes dissection and no evidence of a residual tumor. The resected specimens were fixed in 10% formalin, and paraffin sections were prepared. These sections were stained with hematoxylin and eosin, and all tumors were reviewed by histological degree of differentiation of the WHO classification (14). Seventy tumors were well-differentiated, 42 moderately differentiated, 18 poorly differentiated, and 1 unclassified.

The primary anti-TGFα goat serum was obtained from BIOTOP (Washington, DC; Lot No. PA-125-G), the anti-EGF rabbit serum was from Wakunaga Pharmaceutical Co. Ltd. (Osaka, Japan; Lot No. 004B), and the anti-EGFR mouse serum was from Transformation Research Inc. (Framingham, MA; Lot No. 1096) (15). The staining was performed using the avidin-biotin-peroxidase complex method (16). The process of immunohistochemical staining was as follows: the deparaffinized sections were treated with 0.03% hydrogen peroxidase in methanol for 30 min at room temperature to inhibit endogenous peroxidase. After washing in phosphate-buffered saline and incubating with goat serum for TGFα, rabbit serum for EGF, mouse serum for EGFR (diluted 1:200, 30 min; Vector Laboratories, Burlingame, CA), each section was incubated at room temperature overnight with the primary antibody of TGFα at a dilution of 1:100, EGF at a dilution of 1:50, and EGFR at a dilution of 1:100. The sections were then exposed to a biotinylated secondary antibody and avidin with biotinylated horseradish peroxidase (Vector Laboratories) for 30 and 60 min. After these treatments, visualization of the peroxidase was achieved by the diaminobenzidine method. Each section was then stained with methyl green and examined under a transmission light microscope. Omission of the primary antibody resulted in negative staining.

The extent of the immunoreactivity of TGFα and EGF was separated into 2 groups as follows: (*a*) low, moderate to negative staining of less than 75% of the tumor cells; and (*b*) high, intense staining of more than 75% of the tumor cells. On the other hand, the extent of the immunoreactivity of EGFR was separated into two groups: (*a*) (+), immunoreactivity staining of more than 50% of the tumor cells; and (*b*) (−), that of less than 50% of the tumor cells. These assignments were made by persons with no knowledge of the clinical data.

The χ^2 test were used to analyze correlations among immunoreactivities of EGFR with TGFα or EGF and factors of sex, stage, curability of operation, and histological type of differentiation. The survival rate was calculated by the Kaplan-Meier method (17). Comparisons among survival rates were made by the generalized Wilcoxon test (18).

RESULTS

Immunoperoxidase reactivities for TGFα and/or EGF were evident in the cytoplasm of the malignant cells (Fig. 1, *A* and *B*). In the normal bronchial epithelium, both TGFα and EGF factors were weak along the brush borders of the epithelium. In the bronchial glands, both TGFα and EGF were present, in some cases.

The staining pattern for EGFR is shown in Fig. 1*C*. The cytoplasm and cell membrane of the tumor cells were densely stained. In the normal bronchial epithelium, there was a weak

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[2] The abbreviations used are: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; TGFα, transforming growth factor α.

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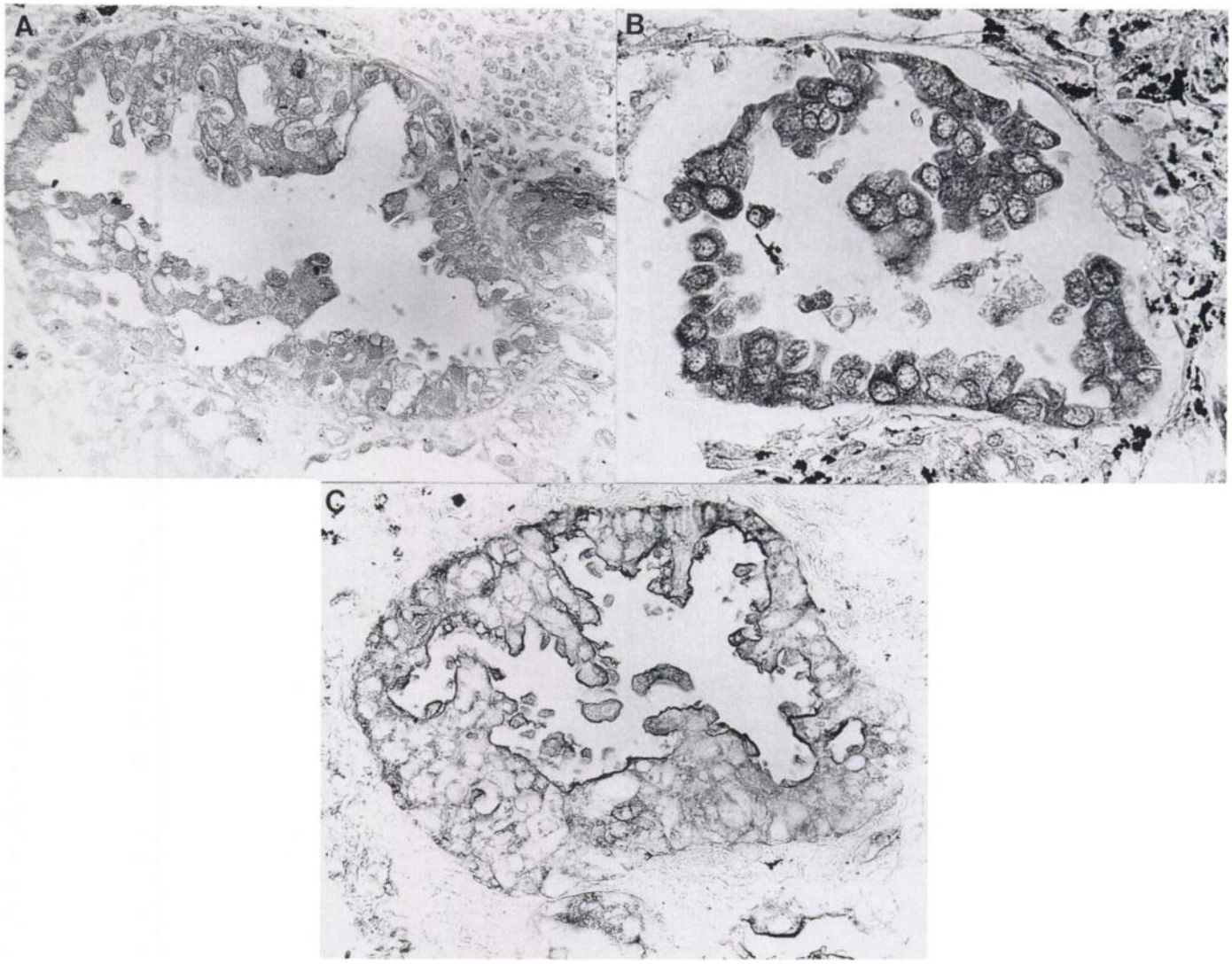

Fig. 1. *A*, immunostaining for TGFα in human lung adenocarcinoma, high TGFα pattern. × 280. *B*, immunostaining for EGF in human lung adenocarcinoma, high EGF pattern. × 440. *C*, immunostaining for EGFR in human lung adenocarcinoma, EGFR(+) pattern. × 300.

but positive staining for EGFR along the brush border of the epithelium. There was no staining in the bronchial glands.

Of the 131 specimens examined, there were 55 (42%) with immunoreactivity of EGFR(+) and 76 (58%) with that of EGFR(−). Data assessed included factors of sex, tumor status, node status, metastasis status, stage, pathological grade of differentiation, and curability of operation according to the immunoreactive intensities of TGFα and EGF, as shown in Tables 1 and 2. In cases of EGFR(+), the incidence of a high extent of TGFα or EGF was greater in patients with M_1 than in those with M_0 disease, the differences being statistically significant ($P < 0.05$).

The 5-year survival rates of patients with high TGFα and low TGFα were 38 and 65%, respectively, with a statistically significant difference ($P < 0.05$). The 5-year survival rates of patients with high EGF and low EGF were 35 and 58%, respectively, with a statistically significant difference ($P < 0.05$). In contrast, the 5-year survival rates of patients with EGFR(+) and EGFR(−) were 48 and 46%, respectively, but with no statistical difference.

In EGFR(+) cases, the 5-year survival rates of patients with high TGFα and low TGFα were 36 and 85%, respectively (Fig. 2*A*), with a statistically significant difference ($P < 0.05$). However, in EGFR(−) cases, the 5-year survival rates of patients with high TGFα and low TGFα were 39 and 56%, respectively (Fig. 2*B*). There was no statistical difference. In the EGFR(+) cases, the 5-year survival rates of patients with high EGF and low EGF were 25 and 77%, respectively (Fig. 3*A*), with a statistically significant difference ($P < 0.05$). In the EGFR(−) cases, the 5-year survival rates of patients with high EGF and low EGF were 44 and 48%, respectively (Fig. 3*B*), with no statistical difference. Therefore, in the presence of these growth factors, as expressed by receptors in the tumor, the survival rate was poor.

DISCUSSION

The TGFα or EGF bind EGFR and stimulate the autophosphorylation of EGFR (8, 9). Clustering of the EGF-EGFR complexes is thought to trigger DNA synthesis and to be associated with cell growth and proliferation (10). Growth factors were seen to have a close link to oncogenes that not only directly code for growth factors or their receptors but also

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Table 1 *Relationship among the immunoreactivities of TGFα, EGFR, and various clinicopathological factors in patients with lung adenocarcinoma*

	EGFR(+)		EGFR(−)	
	High TGFα	Low TGFα	High TGFα	Low TGFα
Sex				
Male	20 (25)[b]	10 (12)	31 (39)	19 (24)
Female	21 (41)	4 (8)	15 (29)	11 (21)
T[c]				
1	19 (37)	8 (16)	13 (25)	11 (22)
2	13 (24)	6 (11)	23 (43)	12 (22)
3	5 (36)	0	5 (36)	4 (28)
4	4 (33)	0	5 (42)	3 (25)
N				
0	22 (27)	12 (14)	29 (35)	20 (24)
1	3 (22)	0	9 (64)	2 (14)
2	16 (47)	2 (6)	8 (23)	8 (23)
M				
0	34 (28)[a]	12 (10)	44 (37)	30 (25)
1	7 (64)	2 (18)	2 (18)	0
Stage				
I	16 (24)	10 (15)	23 (35)	17 (26)
II	2 (18)	0	7 (64)	2 (18)
IIIA	13 (41)	2 (6)	9 (28)	8 (25)
IIIB	3 (27)	0	5 (46)	3 (27)
IV	7 (64)	2 (18)	2 (18)	0
Differentiation				
Well	21 (30)	9 (13)	25 (36)	15 (21)
Moderately	15 (36)	3 (7)	16 (38)	8 (19)
Poorly	5 (28)	2 (11)	5 (28)	6 (33)
Unknown				1
Curability				
Curative	25 (25)	11 (11)	40 (39)	25 (25)
Noncurative	16 (53)	3 (10)	6 (20)	5 (17)
Total	41 (31)	14 (11)	46 (35)	30 (23)
	55 (42)		76 (58)	

[a] Difference is statistically significant ($P < 0.05$).
[b] Numbers in parentheses, percentages.
[c] T, tumor status; N, node status; M, metastasis status.

Table 2 *Relationship among the immunoreactivities of EGF, EGFR, and various clinicopathological factors in patients with lung adenocarcinoma*

	EGFR(+)		EGFR(−)	
	High EGF	Low EGF	High EGF	Low EGF
Sex				
Male	13 (16)[b]	17 (21)	25 (31)	25 (31)
Female	19 (37)	6 (12)	9 (18)	17 (33)
T[c]				
1	14 (28)	13 (25)	11 (22)	13 (25)
2	11 (20)	8 (15)	13 (24)	22 (41)
3	4 (29)	1 (7)	5 (35)	4 (29)
4	3 (25)	1 (8)	5 (42)	3 (25)
N				
0	17 (20)	17 (20)	24 (29)	25 (31)
1	2 (14)	1 (7)	5 (36)	6 (43)
2	13 (38)	5 (15)	5 (15)	11 (32)
M				
0	25 (21)[a]	21 (18)	33 (27)	41 (34)
1	7 (64)	2 (18)	1 (9)	1 (9)
Stage				
I	12 (18)	14 (21)	17 (26)	23 (35)
II	1 (9)	1 (9)	4 (36)	5 (46)
IIIA	10 (31)	5 (16)	7 (22)	10 (31)
IIIB	2 (18)	1 (9)	5 (46)	3 (27)
IV	7 (64)	2 (18)	1 (9)	1 (9)
Differentiation				
Well	17 (24)	13 (19)	17 (24)	23 (33)
Moderately	12 (29)	6 (14)	13 (31)	11 (26)
Poorly	3 (17)	4 (22)	4 (22)	7 (39)
Unknown				1
Curability				
Curative	18 (18)	18 (18)	29 (29)	36 (35)
Noncurative	14 (46)	5 (17)	5 (17)	6 (20)
Total	32 (24)	23 (18)	34 (26)	42 (32)
	55 (42)		78 (58)	

[a] Difference is statistically significant ($P < 0.05$).
[b] Numbers in parentheses, percentages.
[c] T, tumor status; N, node status; M, metastasis status.

amplify the mitogenic signals generated by a growth factor, at its receptor (19).

The presence of both growth factor and its receptor in the same tumor is regarded as autocrine secretion. Sporn and Roberts (19) proposed the term "autocrine secretion," which is self-stimulation whereby a cell secretes a hormone-like substance for which the cell itself has functional external receptors.

Derynck *et al.* (20) reported that human tumors or tumor cell lines carried TGFα messenger RNA with a relatively high level of EGFR messenger RNA. Sugiyama *et al.* (15) described the relationship between EGF and EGFR in cases of gastric cancer. Both the EGF- and EGFR-stained tissues had a higher rate of the infiltrative type, poorly differentiated type, scirrhous type, and deep invading type.

We examined TGFα, EGF, and EGFR using immunohistochemical approaches, and we compared the prognosis from the point of view of the relationship between the growth factor and the receptor. Cases that demonstrated high expression of growth factors with co-expression of receptor were observed in the more advanced stage tumors, for example M_1. Among the receptor-positive cases, a high expression of growth factors was associated with a significantly poorer prognosis, for both TGFα and EGF. However, in the receptor-negative cases, the amount of growth factor could not serve as a prognostic indicator. In cases of both receptor-positive and a low growth factor, there was a trend toward a better prognosis. However, a statistically significant difference was apparent only when comparing receptor-positive with the high growth factor group. These data suggest that the autocrine mechanism plays an important role in the advancement of a lung adenocarcinoma, and that when such a mechanism becomes operative, the prognosis will be poor.

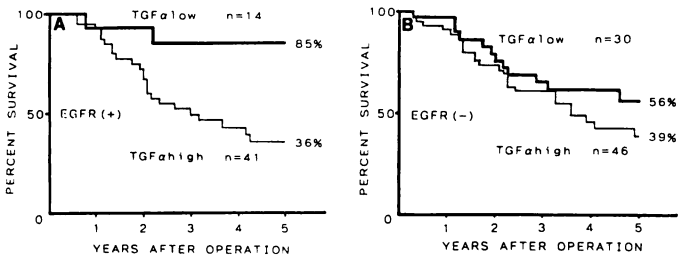

Fig. 2. *A*, survival curves of patients with EGFR(+) in lung adenocarcinoma according to the extent of TGFα. The difference is statistically significant between the 2 groups ($P < 0.05$). *B*, survival curves of patients with EGFR(−) in lung adenocarcinoma according to the extent of TGFα.

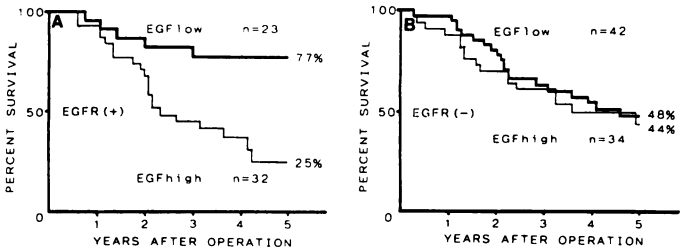

Fig. 3. *A*, survival curves of patients with EGFR(+) in lung adenocarcinoma according to the extent of EGF. The difference is statistically significant between the 2 groups ($P < 0.05$). *B*, survival curves of patients with EGFR(−) in lung adenocarcinoma according to the extent of EGF.

A most important prognostic factor in lung cancer is the pathological stage of the disease (13). Survival time was stratified according the stage and curability of the surgery. However,

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recurrences, regional and distant, are frequent in patients who undergo resection (21). Moreover, adjuvant chemotherapy is of little effect in the treatment of patients with non-small cell lung cancer (22). Taken together with the findings presented here, defined growth factors and their receptor have to be isolated and characterized. A malignant transformation may possibly be controlled to some extent if specific inhibitors of the action of growth factor or receptor are available.

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