Transforming growth factor β_1 as a prognostic factor in pulmonary adenocarcinoma

I Takanami, T Imamura, T Hashizume, K Kikuchi, Y Yamamoto, S Kodaira

Abstract

Aims—To evaluate the efficacy of transforming growth factor β (TGF- β) for the prognosis of pulmonary adenocarcinoma.

Methods—TGF- β was detected immunohistochemically using the avidin-biotin-peroxidase complex technique in resected pulmonary adenocarcinomas from 88 patients.

Results—Of the 88 patients, 39 were TGF- β negative and 45 TGF- β positive. The five year survival rate was 56% for the TGF- β negative and 16% for the TGF- β positive group.

Conclusions—TGF- β can be used as a prognostic factor in pulmonary adenocarcinoma.

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Transforming growth factor β (TGF- β) affects cell growth and differentiation, and extracellular matrix and immunological function. This growth factor enhances or inhibits these processes depending on the cell type or condition, or the presence or absence of other factors.^{1,2} A recent experimental study using rat breast cancer cells showed that TGF- β enhances the production of extracellular matrix degradative enzymes.³ Another report found a correlation between TGF- β expression and cancer stage.⁴ Thus, TGF- β is thought to enhance cancer metastasis.

TGF- β has a dimeric structure with a molecular weight of 25 000. There are three types in humans (TGF- β_{1-3}) of which TGF- β_1 is the most prevalent.⁵ In our preliminary tests TGF- β_1 was expressed in all histological types of pulmonary carcinoma.

Adenocarcinoma is the most common histological type of pulmonary carcinoma, the incidence of which is increasing.⁶ Its biological behaviour is not completely understood, and the outcome of surgical and medical treatment remains unsatisfactory. Therefore, the present study was carried out to evaluate the efficacy of TGF- β_1 for the prognosis of resected pulmonary adenocarcinoma.

Methods

Of the patients with pulmonary adenocarcinoma who underwent resection at the First Department of Surgery, Teikyo University School of Medicine between 1980 and 1988, paraffin wax embedded resected specimens from 88 were available for examination.

Specimens from patients who died within one month of surgery and from those undergoing exploratory thoracotomy were not included in the study. Pulmonary adenocarcinomas were staged according to the tumour (T), lymph node (N), and metastasis (M) classification of the International Union against Cancer (1987). Of the patients, 36 had stage I, four stage II, 25 stage IIIa, two stage IIIb, and 21 stage IV cancer. The study population comprised 50 men and 38 women, aged from 28 to 81 years (mean 61 years). The degree of histological differentiation was determined according to the World Health Organisation classification (1982). The histological type was well differentiated in 44 patients, moderately differentiated in 29, and poorly differentiated in 15. The preoperative surgical indications included possible curative lobectomy or pneumonectomy accompanied by complete dissection of the hilar and mediastinal lymph nodes. All of the patients were followed for five to 13 years.

Resected specimens were fixed in formalin, embedded in paraffin wax and sectioned (3 μ m). These sections were stained with haematoxylin and eosin, and immunohistochemically for TGF- β_1 . Rabbit anti-human TGF- β_1 polyclonal antibody (King Brewing, Kakogawa, Hyogo, Japan) was used as the antibody against TGF- β_1 , the specificity of which had been confirmed using an enzyme linked immunosorbent assay and western blotting. Human placental tissue samples served as positive controls. TGF- β_1 was stained immunohistologically using the avidin-biotin-peroxidase complex (ABC) technique⁷ (Vectastain, Vector, Burlingame, California, USA). Briefly, deparaffinised sections were treated with 0.03% hydrogen peroxidase in methanol for 30 minutes at room temperature to inhibit endogenous peroxidase. After washing in phosphate buffered saline (PBS) and incubating with 10% normal swine serum (Vector), the sections were allowed to react at 4°C in the primary rabbit anti-human TGF- β_1 polyclonal antibody at a 1 in 50 dilution. The sections were then washed with PBS. Using biotinylated swine anti-rabbit serum (Vector) as the secondary antibody, the sections were allowed to react at room temperature for one hour, followed by colour development with diaminobenzine. Nuclei were stained with methyl green. Normal rabbit serum was used as a control. Assessment of staining was performed by at least two independent observers who had no knowledge of the tumour stage, grade, or

Department of
Pathology, Teikyo
University School of
Medicine
T Imamura

First Department of
Surgery
I Takanami
Y Yamamoto
S Kodaira

Department of
Surgery, National
Sanatorium,
Kanagawa Hospital
T Hashizume
K Kikuchi

Correspondence to:
Dr I Takanami, First
Department of Surgery,
Teikyo University of
Medicine, 11-1, Kaga 2-
Chome, I tabashi-Ku,
Tokyo 173, Japan

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Figure 1 TGF- β_1 staining in a tissue specimen from a patient with papillary adenocarcinoma ($\times 66$). Because of diffuse cytoplasmic staining of the cancer cells, this patient was assigned to the TGF- β_1 positive group.

patient history. The extent of the immunoreactivity was graded as absent (no detectable staining) (–), low (focal staining of less than 25% of the tumour cells) (+), moderate (staining of 25–74% of the tumour cells) (++) and strong (intense staining of more than 75% of the tumour cells) (+++).

The χ^2 test was used to analyse correlations between TGF- β_1 staining and sex, staging (T, N, and M), and the degree of histological differentiation. Survival rates were calculated using Kaplan-Meier's method. Survival rates were compared using the log rank test. Multivariate analysis was performed using Cox's proportional hazards regression model. Calculations were carried out using the SAS statistical package (SAS Institute, Cary, North Carolina, USA). Significance was set at $p < 0.05$.

Results

Following immunostaining for TGF- β_1 , diffuse cytoplasmic staining of cancer cells was observed (fig 1). Cancer stroma in a small number of patients were also stained. Normal alveoli, normal bronchial epithelium, and smooth muscle were slightly stained.

Of the 88 patients examined, 39 (44%) had no evidence of staining and were defined as

Multivariate analysis of the clinicopathological factors and TGF- β_1 in patients with pulmonary adenocarcinoma

Parameter	No of cases	Risk ratio	p value
T		1.490	0.1650
1	34		
2	46		
3	6		
4	2		
N		1.488	0.0225
0	46		
1	4		
2	36		
3	2		
M		1.242	0.7551
0	67		
1	21		
Stage		1.221	0.4307
I	36		
II	4		
IIIa	25		
IIIb	2		
IV	21		
Differentiation		1.366	0.0859
well	44		
moderately	29		
poorly	15		
Sex		0.640	0.1257
male	50		
female	38		
TGF- β_1		1.992	0.0316
negative	39		
positive	49		

TGF- β_1 negative, 15 (17%) had low, 15 (17%) moderate, and patients with low, moderate and strong staining were defined as TGF- β_1 positive. Data assessed included the TNM stage, the pathological grade of differentiation and the extent of TGF- β_1 staining. The five year survival rates of patients with no low, moderate and strong staining were 56, 20, 17, and 14%, respectively. The overall five year survival rate was 56% for the TGF- β_1 negative and 16% for the positive group ($p < 0.01$; fig 2). Multivariate analysis revealed that TGF- β_1 and N stage had a significant affect on prognosis (table).

Discussion

Most of the studies on the role of TGF- β in cancer have been performed on cell lines. Although the influence of TGF- β differed with each cell line, its primary function in epithelium was thought to be inhibition of cell growth and differentiation.⁸ One study found that in cell lines TGF- β caused a reduction in the synthesis of collagenase, an enzyme lysing the extracellular matrix, stromelysin, and cathepsin L; enhanced the synthesis of protease inhibitors; and inhibited lysis of the extracellular matrix.⁹ Another report indicated that the administration of a small dose of TGF- β to breast cancer cells resulted in the increased production of gelatinase, heparinase, and urokinase-type plasminogen activator.³ Findings in cell lines, however, cannot be applied directly to a living organism. In the thyroid TGF- β_1 was detected in malignant but not in benign or normal tissue suggesting that increased synthesis of TGF may be linked to an increase in cell number, and hence the size of the tumour.¹⁰ The expression of TGF- β_1 is also increased in breast cancers, suggesting that this factor is involved in the invasion and metastasis of breast cancer.⁴

TGF- β_1 messenger RNA (mRNA) has been detected in human cancer tissue, the expres-

Figure 2 Survival curves of patients with pulmonary adenocarcinoma classed according to TGF- β_1 expression.

sion of which is greater in malignant than in normal tissue.¹¹ The degree of TGF- β_1 mRNA expression is positively correlated with TGF- β protein levels. Activation of the TGF- β gene is thought to cause excessive expression of TGF- β_1 mRNA which, in turn, is translated into the protein.¹²

On immunohistological staining, TGF- β_1 was detected in 56% of the patients studied. There were clear prognostic differences between the TGF- β_1 negative and positive groups. Expression of TGF- β_1 in malignant tissue from patients with adenocarcinoma differed from that observed in cell lines. TGF- β activity is mediated by type I receptors and the TGF- β inhibitory element (TIE) binding protein.¹³ Expression of both type I receptor and TIE binding protein was lower than that in normal tissue, resulting in reduced inhibition of TGF- β .¹⁴

In conclusion, autocrine or paracrine stimulation, or both, of cell proliferation by TGF- β is important for tumour growth and progression. As the expression of TGF- β is increased, and that of type I receptor and TIE binding protein reduced, in malignant tissue this growth factor can be used as a prognostic marker in pulmonary adenocarcinoma.

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