Transforming growth factor $\beta_1$ as a prognostic factor in pulmonary adenocarcinoma

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Abstract

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

Methods—TGF-$\beta$ 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-$\beta$ negative and 45 TGF-$\beta$ positive. The five year survival rate was 56% for the TGF-$\beta$ negative and 16% for the TGF-$\beta$ positive group.

Conclusions—TGF-$\beta$ can be used as a prognostic factor in pulmonary adenocarcinoma.

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Transforming growth factor $\beta$ (TGF-$\beta$) 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. A recent experimental study using rat breast cancer cells showed that TGF-$\beta$ enhances the production of extracellular matrix degradative enzymes. Another report found a correlation between TGF-$\beta$ expression and cancer stage. Thus, TGF-$\beta$ is thought to enhance cancer metastasis.

TGF-$\beta$ has a dimeric structure with a molecular weight of 25 000. There are three types in humans (TGF-$\beta_1, \ldots$) of which TGF-$\beta_1$ is the most prevalent. In our preliminary tests TGF-$\beta_1$ 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. 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-$\beta_1$ 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 μm). These sections were stained with haematoxylin and eosin, and immunohistochemically for TGF-$\beta_1$. Rabbit anti-human TGF-$\beta_1$ polyclonal antibody (King Brewing, Kakogawa, Hyogo, Japan) was used as the antibody against TGF-$\beta_1$, 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-$\beta_1$ was stained immunohistochemically using the avidin-biotin-peroxidase complex (ABC) technique (Vestinac, Vector, Burlington, California, USA). Briefly, deparaffinised sections were treated with 0.03% hydrogen peroxide 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-$\beta_1$, 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 diaminobenzene. 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...
Figure 1 TGF-β1 staining in a tissue specimen from a patient with papillary adenocarcinoma (×66). Because of diffuse cytoplasmic staining of the cancer cells, this patient was assigned to the TGF-β1 positive group.

Results
Following immunostaining for TGF-β1, 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 TGF-β1 negative, 15 (17%) had low, 15 (17%) moderate, and patients with low, moderate and strong staining were defined as TGF-β1 positive. Data assessed included the TNM stage, the pathological grade of differentiation and the extent of TGF-β1 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-β1 negative and 16% for the positive group (p < 0·01; fig 2). Multivariance analysis revealed that TGF-β1 and N stage had a significant affect on prognosis (table).

Discussion
Most of the studies on the role of TGF-β1 in cancer have been performed on cell lines. Although the influence of TGF-β1 differed with each cell line, its primary function in epithelium was thought to be inhibition of cell growth and differentiation. One study found that in cell lines TGF-β1 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. Another report indicated that the administration of a small dose of TGF-β1 to breast cancer cells resulted in the increased production of gelatinase, heparinase, and urokinase-type plasminogen activator.

Findings in cell lines, however, cannot be applied directly to a living organism. In the thyroid TGF-β1 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. The expression of TGF-β1 is also increased in breast cancers, suggesting that this factor is involved in the invasion and metastasis of breast cancer.

TGF-β1 messenger RNA (mRNA) has been detected in human cancer tissue, the expres-
sion of which is greater in malignant than in normal tissue. The degree of TGF-β1 mRNA expression is positively correlated with TGF-β1 protein levels. Activation of the TGF-β gene is thought to cause excessive expression of TGF-β1 mRNA, which, in turn, is translated into the protein.

On immunohistological staining, TGF-β1 was detected in 56% of the patients studied. There were clear prognostic differences between the TGF-β1 negative and positive groups. Expression of TGF-β1 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. 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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