ORIGINAL ARTICLE
Turkish Respiratory Journal 2006; 7(1): 15-18
Lung Pathology

Evaluation of Transforming Growth Factor-β_1, Fibronectin and Collagen Type IV in Non-Small Cell Lung Carcinoma

Gülay Özbilim[1], İrem Hicran Özbudak[1], İlknur Küçükosmanoğlu[1], Levent Dertsiz[2], Gökben Yıldırım Küpesiz[1], Mehtap Türkay[3]

[1]Akdeniz University School of Medicine, Pathology Department, Antalya, Turkey
[2]Akdeniz University School of Medicine, Chest Surgery Department, Antalya, Turkey
[3]Akdeniz University School of Medicine, Public Health Department, Antalya, Turkey

Abstract

Objective: Carcinoma of the lung is one of the most frequent and mortal cancers on earth. Transforming growth factor-β_1 (TGF-β_1) is a multifunctional polypeptide which regulates immunofunctions, angiogenesis, adhesion, extracellular matrix collection, cell development and differentiation. The purpose of this study was to determine the expression of TGF-β_1 and also of fibronectin and collagen type IV and the differences in expression between histopathologic subtypes. Design: Retrospective histopathologic study. **Material and Methods:** In this study we used resection materials of 54 non-small cell lung carcinomas (NSCLC) [40 squamous cell carcinoma (SCC), 14 adenocarcinoma (AC)]. Sections were taken from paraffin-embedded blocks and immunohistochemically stained with TGF-β_1, fibronectin and collagen type IV. Cytoplasmic staining density and percentage of stained areas were evaluated semiquantitatively. SPSS 10 program was used for statistical analysis. **Results:** In our study, 40 (74%) of 54 cases were SCC and 14 (26%) were AC. Statistically significant differences in TGF-β_1 and collagen type IV staining percentages between SCC and AC were found (p=0.032, p=0.002). Correlation between staining percentage area and density was found for each of the three markers separately (p=0.000). **Conclusion:** When these three markers were evaluated inside themselves, no correlation was found between staining percentage and density. A significant relation between histological grade and staining percentage was determined only in TGF-β_1 (p=0.001). TGF-β_1 staining percentage was 40% in Grade 1, 30% in Grade 2, and 7.4% in Grade 3.

Keywords: TGF-β_1, fibronectins, collagen type IV, lung neoplasms

Received: March 25, 2006 Accepted: March 28, 2006

INTRODUCTION

Lung cancer is one of the most common and mortal types of cancer worldwide. Non- small cell lung carcinoma (NSCLC), in which early metastatic spread is common, represents approximately 80% of all lung carcinomas. Metastasis is a complex process that occurs via the interaction between the tumor cells and the extracellular matrix (ECM) [1]. Transforming growth factor-β_1 (TGF-β_1) inhibits the proliferation of normal epithelial cells [2]. Reduced expression of TGF-β_1 receptors is associated with loss of TGF-β_1 sensitivity and increase in tumor progression in most tumors [1]. The ECM plays an important role as a barrier in the spreading of tumor cells. Collagen type IV is an important structural component of the basal membrane. Fibronectin, together with the basal lamina, is a non-collagenous glycoprotein [2-5].

The aim of this study was to assess the presence of fibronectin and collagen type IV, which are components of the ECM, and of TGF-β_1 in NSCLC, and to investigate whether there is a difference in their expression between different histopathologic subtypes of NSCLC.

MATERIAL AND METHODS

Fifty-four lung resection specimens diagnosed as NSCLC were used in this study. Five micrometer thick sections were taken from paraffin-embedded tumor tissue specimens, and immunohistochemical studies were performed with the labeled streptoavidin-biotin phosphatase method with TGF-β_1 antibody (1/500, Santa Cruz), fibronectin (1/800, DAKO) and collagen type IV (1/500, DAKO) staining.

The specimens were examined on light microscope. Immune staining was evaluated according to the brown-colored staining intensity as mild (+1), moderate (+2), or severe (+3). The percentage of the staining area was determined by the semiquantitative technique in the cytoplasm of tumor cells for TGF-β_1, in the tumor stroma for collagen type IV and in the cytoplasmic and stromal positivity for fibronectin.

The t-test was used for statistical evaluation of percentage of staining area. Chi-square test was applied to assess the lymphatic and/or vascular invasion. SPSS 10.0 software was used for statistical analyses.

Corresponding Author: Gülay Özbilim
Akdeniz University School of Medicine, Pathology Department, Antalya, Turkey
Phone: +90242 2274343, E-Mail: gulayozbilim@hotmail.com

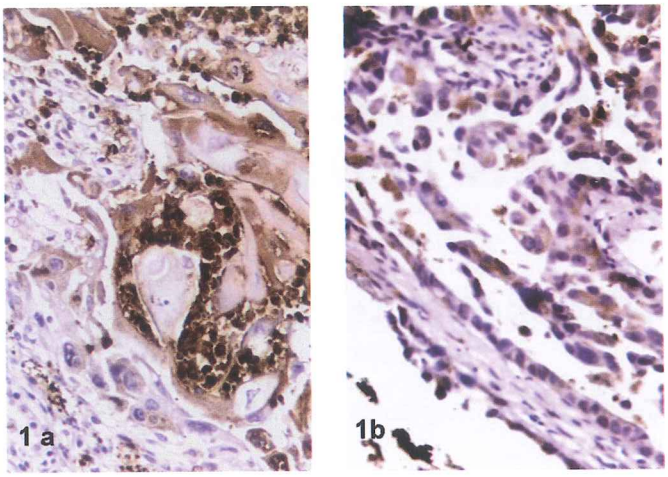

Figure 1. a,b. Immunohistochemical positivity of TGF-β_1 in SCC (a) and AC (b) of lung (TGF-β1 X 400).

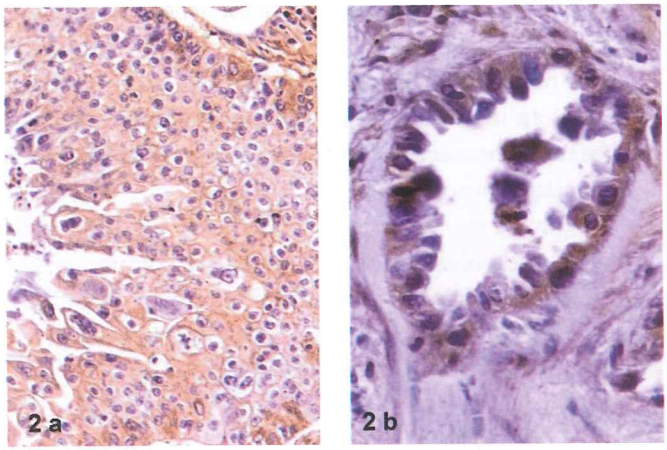

Figure 2. a, b. Immunostaining for fibronectin in SCC (a) and AC (b) of lung (fibronectin X 400).

RESULTS

The results are presented in Table 1. There were 40 cases (74%) with squamous cell carcinoma (SCC) and 14 cases (26%) with adenocarcinoma (AC). Of all cases, 2 were Grade 1, 30 were Grade 2 and 22 were Grade 3. Lymphatic and/or vascular invasion was detected in 8 cases (15%) on histological examination. Statistical findings for percentage of staining area are presented in Table 2.

There were significant differences between SCC and AC in the percentage of staining area of TGF-β_1 (p=0.002) and collagen type IV (p=0.032) (Tables 3, 4) (Figures 1, 2).

Chi-square test was applied to assess the lymphatic and/or vascular invasion. There was no significant difference between histologic groups in the eight cases which had invasion (p=0.325), and no significant difference was found between these groups when staining intensity of TGF-β_1, fibronectin and collagen type IV was evaluated (TGF-β_1 p=0.237; fibronectin p=0.834; collagen type IV p=0.208) (Figure 3).

Table 1. Findings of the cases

Case	Diagnosis	Grade	Invasion	Fibronectin %-intensity		Collagen type IV %-intensity		TGF-β_1 %-intensity	
1	SCC	1	-	60	+2	-	-	40	+2
2	SCC	2	+	15	+1	-	-	-	-
3	SCC	2	-	-	-	-	-	15	+1
4	SCC	2	-	80	+2	-	-	40	+2
5	AC	2	-	50	+2	-	-	-	-
6	AC	1	-	90	+2	30	-	40	+2
7	SCC	2	-	90	+2	15	+1	30	+2
8	SCC	2	-	90	+2	15	+1	15	+2
9	SCC	2	+	90	+2	30	-	10	+2
10	SCC	2	+	90	+2	40	+2	80	+2
11	SCC	2	-	90	+2	-	-	30	+2
12	SCC	2	+	60	+2	15	+1	80	+2
13	SCC	2	-	60	+2	15	+2	30	+2
14	SCC	3	-	80	+2	15	-	20	+1
15	SCC	3	-	90	+2	40	-	15	+1
16	SCC	2	-	90	+2	40	-	40	+2
17	SCC	2	-	70	+2	60	-	20	+1
18	SCC	3	-	70	+2	15	-	-	-
19	SCC	2	+	70	+2	15	-	80	+2
20	SCC	2	-	90	+2	40	-	80	+2
21	SCC	3	-	15	+1	30	-	15	+2
22	AC	2	-	90	+2	80	+2	10	+2
23	SCC	2	-	80	+2	-	-	40	+2
24	AC	2	-	80	+2	-	-	-	-
25	AC	2	-	90	+2	80	+2	-	-
26	AC	2	+	80	+2	80	+2	5	+1
27	SCC	3	-	80	+2	-	-	15	+2
28	SCC	3	-	80	+2	-	-	-	-
29	SCC	2	-	80	+2	40	+2	40	+2
30	AC	3	-	80	+2	80	+2	10	+1
31	AC	2	-	90	+2	60	-	15	+2
32	SCC	3	-	80	+2	80	+2	5	+1
33	AC	2	-	80	+2	80	+2	15	+2
34	SCC	3	-	80	+2	20	+1	10	+2
35	SCC	3	-	90	+2	20	+1	10	+2
36	AC	2	-	15	+2	90	+2	-	-
37	SCC	3	-	80	+2	-	-	-	-
38	SCC	3	-	80	+2	-	-	15	+1
39	SCC	3	-	80	+2	-	-	5	+1
40	SCC	3	+	60	+2	20	+2	-	-
41	SCC	3	-	80	+2	-	-	-	-
42	SCC	3	-	80	+3	80	+2	-	-
43	SCC	3	-	90	+2	20	+2	15	+1
44	SCC	3	+	30	+1	-	-	-	-
45	AC	3	-	20	+2	5	+1	15	+1
46	SCC	2	-	60	+2	40	-	40	+2
47	SCC	3	-	20	+2	40	-	5	+1
48	AC	2	-	20	+2	20	+1	-	-
49	SCC	2	-	80	+2	80	+1	40	+1
50	AC		-	80	+2	-	-	10	+1
51	SCC	2	-	80	+2	20	-	60	+2
52	SCC	3	-	60	+1	60	+2	-	-
53	AC		-	90	+1	80	+1	-	-
54	SCC	2	-	90	+2	60	+2	60	+2

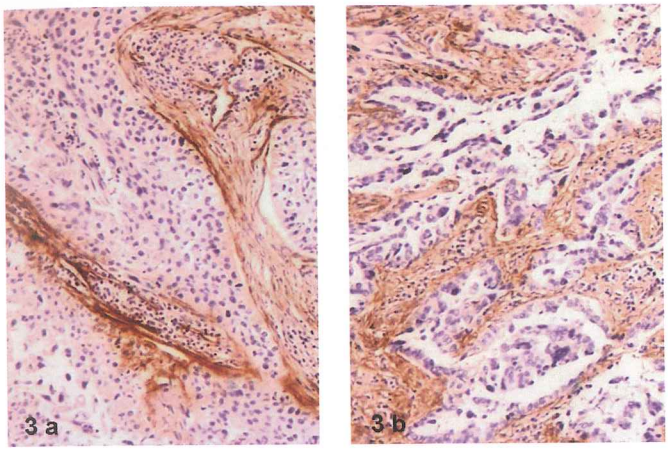

Figure 3. a,b. Stromal positivity of collagen type IV in SCC (a) and AC (b) of lung (collagen type IV X 400).

There was significant correlation between staining intensity and percentage of staining area for each marker separately (p=0.000 for TGF-β_1, fibronectin and collagen type IV). No significant correlation for percentage of staining area was found in dual comparisons between TGF-β_1-fibronectin, TGF-β_1-collagen type IV and fibronectin-collagen type IV.

There was a significant relation between histopathologic grades and the percentage of staining area of TGF-β_1. The mean percentages of TGF-β_1 staining area were 40% in Grade 1, 30% in Grade 2 and 7.4% in Grade 3 tumors. There was a significantly lower percentage of staining area in Grade 3 tumors compared to that in the lower grade tumors (p=0.001).

DISCUSSION

TGF-β_1 is composed of two monomers that bind to each other by cysteine-cysteine disulfate bands [6]. It is secreted in biologically inactive form and is activated by low pH, urea, and heat and by proteases such as plasmin and cathepsin D. It has a strong growth inhibiting effect, particularly for epithelial tumors [7,8]. TGF-β_1 also directly stimulates the expression of the structural elements of the ECM and protease inhibitors, and reduces the levels of proteases, which disrupt the ECM [7].

Most prior studies have found that in embryonic human lung fibroblast cell cultures, expression of fibronectin and fibronectin receptors is increased by the existence of TGF-β_1 [9-11], and that TGF-β_1 is the most important regulating mediator of both collagen type I and type IV production in tissue fibroblasts [12]. It is also a main factor stimulating lung fibrosis and plays a role in the accumulation of the ECM [13]. TGF-β_1 has an immunosuppressive effect on the immune system [14-16].

Table 2. Statistical findings of the cases

Groups		N	Mean	P Value
Fibronectin	SCC	40	71	0.721
	AC	14	68.21	
Collagen Type IV	SCC	40	24.12	0.032
	AC	14	48.92	
TGF-β_1	SCC	40	25	0.002
	AC	14	8.57	

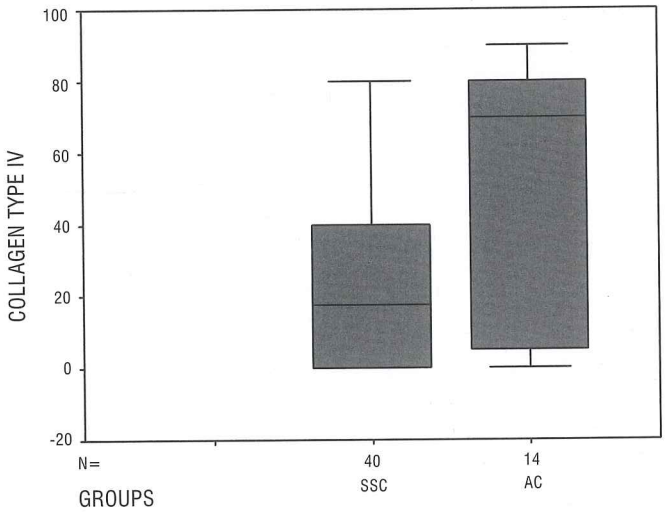

Table 3. Percentage of the staining area in collagen type IV

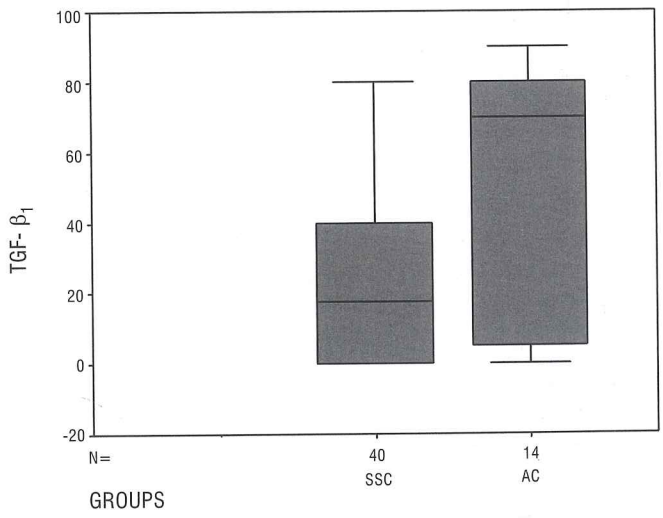

Table 4. Percentage of the staining area in TGF-β_1

In the present study, TGF-β1 was found negative in 28% of all cases. Nine TGF-β_1-negative cases constituted 22.5% of the SCC group. Eight cases were in Grade 3 and the other was in Grade 2. Six TGF-β_1-negative cases represented 48% of all AC patients. Five cases were in Grade 2

and 1 was in Grade 3. In this study, TGF-β_1 was found to be significantly low in patients with Grade 3 tumors.

We could not find any relation between the expression of TGF-β_1 and fibronectin as mentioned in a few reports recently [9-11]. Collagen type IV expression was not detected in 16 of our 54 cases (30%). There was no association between collagen type IV expression and the other markers. The conspicuous and statistically significant result was that collagen type IV staining was higher in AC than SCC when taking into account the percentage of staining area. In SCC, there was a lower staining for collagen type IV, which could explain the more frequent local lymph node metastases in SCC compared to AC. In our series, hilar and/or mediastinal lymph node metastases were detected in 14 cases (26%) (13 SCC, 1 AC). Interestingly, the percentage of TGF-β_1 staining area was also low in these cases.

Recently, some reports have indicated that TGF-β_1-positive NSCLC patients have a significantly longer survival [2,17]. In this study, the correlation between low percentage of TGF-β_1 staining and the presence of lymph node metastasis supports the view that low TGF-β_1 expression is prognostically worse.

In conclusion, TGF-β_1 and collagen type IV are important markers in NSCLC; their decreased expression is associated with lymph node metastasis and poorer prognosis.

REFERENCES

1. Han JY, Kim HS, Lee SH, et al. Immunohistochemical expression of integrins and extracellular matrix proteins in non-small cell lung cancer: correlation with lymph node metastasis. Lung Cancer 2003;41(1):65-70.
2. Colasante A, Aiello FB, Brunetti M, et al. Gene expression of transforming growth factor beta receptors I and II in non-small-cell lung tumors. Cytokine 2003;24(5):182-9.
3. Yoshida S, Shimizu E, Ogura T, et al. Stimulatory effect of reconstituted basement membrane components (matrigel) on the colony formation of a panel of human lung cancer cell lines in soft agar. J Cancer Res Clin Oncol 1997;123(6):301-9.
4. Bartolazzi A, Cerboni C, Flamini G, et al. Expression of alpha 3 beta 1 integrin receptor and its ligands in human lung tumors. Int J Cancer 1995;64(4):248-52.
5. Soderdahl G, Betsholtz C, Johansson A, et al. Differential expression of platelet-derived growth factor and transforming growth factor genes in small- and non-small-cell human lung carcinoma lines. Int J Cancer 1988;41(4):636-41.
6. Gupta MK, Qin RY. Mechanism and its regulation of tumor-induced angiogenesis. World J Gastroenterol 2003;9(6):1144-55.
7. Pelton RW, Moses HL. The beta-type transforming growth factor. Mediators of cell regulation in the lung. Am Rev Respir Dis 1990;142(6 Pt 2):S31-5.
8. Tucker RF, Shipley GD, Moses HL. Growth inhibitor from BSC-1 cells closely related to platelet type beta transforming growth factor. Science 1984;226(4675):705-7.
9. Eickelberg O, Kohler E, Reichenberger F. Extracellular matrix deposition by primary human lung fibroblasts in response to TGF-beta1 and TGF-beta3. Am J Physiol 1999;276(5 Pt 1):L814-24.
10. Raghu G, Masta S, Meyers D. Collagen synthesis by normal and fibrotic human lung fibroblasts and the effect of transforming growth factor-beta. Am Rev Respir Dis 1989;140(1):95-100.
11. Roberts CJ, Birkenmeier TM, McQuillan JJ. Transforming growth factor beta stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblasts. J Biol Chem 1988;263(10):4586-92.
12. Grande JP, Melder DC, Zinsmeister AR. Modulation of collagen gene expression by cytokines: stimulatory effect of transforming growth factor-beta1, with divergent effects of epidermal growth factor and tumor necrosis factor-alpha on collagen type I and collagen type IV. J Lab Clin Med 1997;130(5):476-86.
13. Zhang K, Flanders KC, Phan SH. Cellular localization of transforming growth factor-beta expression in bleomycin-induced pulmonary fibrosis. Am J Pathol 1995;147(2):352-61.
14. Roberts AB, Sporn MB, Assoian RK. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci U S A 1986;83(12):4167-71.
15. Clark RA, McCoy GA, Folkvord JM. TGF-beta 1 stimulates cultured human fibroblasts to proliferate and produce tissue-like fibroplasia: a fibronectin matrix-dependent event. J Cell Physiol 1997;170(1):69-80.
16. Kekow J, Wachsman W, McCutchan JA. Transforming growth factor beta and noncytopathic mechanisms of immunodeficiency in human immunodeficiency virus infection. Proc Natl Acad Sci U S A 1990;87(21):8321-5.
17. Boldrini L, Calcinai A, Samaritani E, et al. Tumour necrosis factor-alpha and transforming growth factor-beta are significantly associated with better prognosis in non-small cell lung carcinoma: putative relation with BCL-2-mediated neovascularization. Br J Cancer 2000;83(4):480-6.