Pergamon

European Journal of Cancer, Vol. 34, No. 6, pp. 934–936, 1998
© 1998 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(97)10118-6

Short Communication

High Levels of Transforming Growth Factor-alpha (TGF-α) mRNA may Predict Local Relapses in Early Stage Urinary Bladder Cancer

P. Gazzaniga,[1] A. Gradilone,[1] I. Silvestri,[1] O. Gandini,[1] M. Napolitano,[1] R. Vercillo,[1] A. Vincenzoni,[2] M. Gallucci,[2] L. Frati[1] and A.M. Agliano'[1]

[1]Department of Experimental Medicine and Pathology, University of Rome 'La Sapienza' Viale Regina Elena 324, 00161 Rome; and [2]Division of Urology, Hospital 'Cristo Re', Rome, Italy

Elevated expression of transforming growth factor-alpha (TGF-α) gene has been previously reported in some types of human neoplasms, but its role in the pathogenesis of bladder cancer has still not been investigated. In the present study, we analysed 28 samples of early stage bladder tumours for the presence of TGF-α mRNA using reverse transcription–polymerase chain reaction (RT-PCR). We detected TGF-α mRNA in 71% (20/28) of these samples. When we related the expression levels of TGF-α with local relapses of patients during a follow-up of 2 years, we found that a high TGF-α expression level in bladder cancer was significantly associated with local relapses in patients with early stage tumours. The appearance of early relapses in tumours with high TGF-α expression levels may suggest the existence of an additional marker in the prediction of local relapses in patients with superficial disease. **© 1998 Elsevier Science Ltd. All rights reserved.**

Key words: TGF-α, bladder cancer, prognosis
Eur J Cancer, Vol. 34, No. 6, pp. 934–936, 1998

INTRODUCTION

TRANSFORMING GROWTH factor-alpha (TGF-α) is a 50 amino acid cleavage product which is structurally related to epidermal growth factor (EGF) and competes with it for binding to the same receptor [1, 2]. TGF-α is known to be involved in the generation and progression of some neoplasms in humans, such as head and neck tumours [3], duodenum adenocarcinomas [4], renal cancer [5], neuroendocrine tumours [6] and lung cancer [7]. The significance of its expression in the growth of transitional epithelium in bladder tumours is still unknown. In the present study, we related TGF-α mRNA levels to local relapses in early stage transitional cell carcinomas (TCC) of the bladder.

PATIENTS AND METHODS

Tissue samples of human bladder cancer were obtained from 28 patients by transurethral resection (TUR-B). Inclusion criteria in the study were: clinical staging up to T2b, no evidence of nodal (N0) or distant (M0) metastases, no radiotherapy or chemotherapy received before sampling, no previous local recurrences. After resection, patients were evaluated by cystoscopy every 3 months during the 2 years of follow-up. Random biopsies of urothelium, distant from the tumour, were taken from 10 patients as normal controls. Informed consent was obtained from all subjects.

Total RNA (1 μg) extracted from the frozen tissues was reverse transcribed with 50 units of MuLV reverse transcriptase. Aliquots of cDNA corresponding to 250 ng of RNA were separately amplified with beta-2 microglobulin and TGF-α primers [8]. Preliminary experiments were performed to determine the optimum thermal cycle number within the linear amplification range (33 cycles).

The polymerase chain reaction (PCR) products were size fractionated by agarose gel electrophoresis, stained with ethidium bromide and photographed. The negatives of the photographs were scanned with a scanning densitometer (UMAX). The values obtained for TGF-α were then related to the expression of beta-2 microglobulin in the corresponding sample.

Statistical analysis was performed using Student's *t*-test, using the Statview package, in order to evaluate the factors which may influence the relapse-free time.

Correspondence to A.M. Agliano'.
Received 22 Jul. 1997; revised 31 Oct. 1997; accepted 17 Nov. 1997.

Table 1. *Correlation between transforming growth factor-alpha (TGF-α) expression levels and relapse-free time in bladder cancer patients*

Patient no.	Gender	Age (years)	Grading	Staging	Relapse-free time (months)	TGF-α expression (by TGF-α/beta 2 microglobulin ratio)	
						Tumoral tissue	Normal tissue
1	F	74	G1	T2	8	High level	n.t.
2	M	66	G3	T2	2	High level	n.t.
11	M	72	G2	T2	6	High level	Neg.
12	M	64	G2	T2	6	High level	Neg.
16	M	66	G1	Tis	6	High level	n.t.
17	M	68	G3	T2	6	High level	n.t.
22	F	72	G2	T2	3	High level	n.t.
24	M	59	G2	T2	6	High level	Neg.
25	M	73	G3	T2	3	High level	n.t.
27	M	69	G2	T1	3	High level	n.t.
3	M	66	G1	Ta	d.f	Low level	Neg.
4	M	58	G1	T1	12	Low level	Neg.
7	M	69	G3	T2	d.f.	Low level	n.t.
10	M	77	G1	T1	d.f.	Low level	n.t.
13	M	49	G3	T2	6	Low level	Low level
15	F	76	G3	T2	d.f.	Low level	Neg.
18	M	58	G1	T1	d.f.	Low level	n.t.
19	M	78	G2	Ta	d.f.	Low level	n.t.
20	M	75	G2	Ta	d.f.	Low level	n.t.
21	M	27	G2	T2	d.f.	Low level	Neg.
5	M	72	G2	Tis	d.f.	Neg.	Neg.
6	M	66	G1	Ta	d.f.	Neg.	n.t.
8	M	73	G2	T2	d.f.	Neg.	n.t.
9	M	76	G3	T2	d.f.	Neg.	n.t.
14	M	65	G3	T2	d.f.	Neg.	Neg.
23	M	73	G2	Tis	d.f.	Neg.	n.t.
26	M	48	G3	T1	3	Neg.	n.t.
28	M	71	G2	Ta	d.f.	Neg.	n.t.

d.f., patients who are disease-free after 24 months of follow-up; high level, TGF-α > beta 2 microglobulin gene expression; low level, TGF-α < beta 2 microglobulin gene expression; neg., negative TGF-α expression; n.t., not tested.

RESULTS

Reverse transcription–PCR (RT-PCR) analysis showed the presence of TGF-α mRNA in 71% (20/28) of the tumoral samples with variable expression levels among patients, when compared with the expression of a gene constitutively expressed, such as beta-2 microglobulin. On the basis of the TGF-α/beta-2 microglobulin expression ratio, patients were divided into three subgroups: the first with a ratio > 1 (10/28, 36%), the second with a ratio < 1 (10/28, 36%) and the third with absence of TGF-α expression (8/28, 29%). No expression was detectable in any of the normal counterparts, except in one case.

We correlated the expression levels of TGF-α in neoplastic tissues with local recurrence incidence during a 2-year follow-up. As shown in Table 1, local relapses were observed in 100% (10/10) of patients from the first group, in only 20% (2/10) of patients from the second group and in 12% (1/8) of patients from the third group. Furthermore, among patients from the second and third groups, 83% (15/18) of patients are currently disease free.

Thus, among the 28 patients examined, 13 (46%) had local recurrences; among the patients with relapses, 77% (10/13) showed levels of TGF-α expression higher than those of beta-2 microglobulin. In the univariate analysis performed, TGF-α expression levels were found to be statistically significant for predicting local tumour recurrence ($P < 0.001$; Table 2). Furthermore, TGF-α expression levels were found

Table 2. *Statistical analysis of clinicopathological variable in bladder cancer patients*

Clinicopathological variable	Number of patients	Median relapse-free time	*P* value
TGF-α Level			
High level	10	4.9	< 0.001
Low or no level	18	21.3	
Age (years)			
< 70	15	12.9	NS
≥ 70	13	18.1	
Gender			
Male	25	15.8	NS
Female	3	11.6	
Grade			
1	7	17.4	NS
2	12	16	
3	9	12.8	
Stage			
TA	5	24	0.04
T2	15	12.6	

TGF-α, transforming growth factor-alpha; high level, TGF-α expression > beta 2 microglobulin expression; low level, TGF-α expression < beta 2 microglobulin expression; no level, negative TGF-α expression; NS, not significant.

to be significantly different among Ta and T2 stages ($P = 0.03$), since in all the Ta tumours, TGF-α, when expressed, was present at low levels. In addition, we found that relapse-free time was related to clinical staging, as expected, but not to histological grading. No correlation was observed between the age and gender of patients with tumour recurrence.

DISCUSSION

Molecular markers of disease progression in patients with early stage bladder cancer have been identified: mutated *p53* [9], bcl-2/bax expression ratio [10] and EGF-receptors (EGF-Rs) [11] have been correlated to propensity to relapse and unfavourable outcome. A high percentage of early stage TCC shows high expression of EGF-Rs in the superficial and deeper layers of urothelium [12]. Such density seems to favour the progression of disease. In such context, an overexpression of TGF-α may be involved in the progression of bladder TCC, probably through autostimulation of transitional cells mediated by an EGF-R related pathway.

Our results indicate that a high expression level of TGF-α in superficial bladder cancer is correlated with poor prognosis in terms of short local relapse-free time of patients ($P < 0.001$). In addition, high TGF-α expression levels seem to be related to a more unfavourable prognosis compared with a higher T staging of the tumours. In fact, among subjects with T2 tumours, which represents the highest stage analysed in our study, local relapses were observed in 100% (8/8) of patients showing high TGF-α expression levels, in 25% (1/4) of those with low TGF-α expression levels and in none of those with negative TGF-α expression. Thus, a higher tumour category seems to be clearly associated with TGF-α expression.

Other studies confirmed that EGF-R expression is higher in malignant urothelium than in benign urothelium [13] and in Ta and T1 tumours EGF-R positivity was found to be associated with multiplicity, recurrence rate and tumour progression [14]. In such a context, a high expression of TGF-α might be responsible for activation of EGF-R.

No significant correlation was found among local recurrence and prognostic factors, such as age, gender and histological grade. Unfortunately, no information was available on other known prognostic factors, such as number and site of tumours.

We believe that the identification of a molecular profile in early stage bladder cancer would be of help in diagnosis and follow-up of those patients whose tumours have, independently of clinical stage, a higher propensity to unfavourable prognosis. Our findings may suggest the existence of an additional marker in the identification of high-risk patients in the post-TUR-B follow-up, although further studies with a larger number of patients should be performed.

1. Massague J. Transforming growth factor alpha. *J Biol Chem* 1990, **265**(35), 21393–21396.
2. Anzano MA, Roberts AB, Smith JM, *et al.* Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type α and type β transforming growth factors. *Proc Natl Acad Sci USA* 1983, **80**, 6264–6268.
3. Issing WJ, Liebich C, Wustrow TP, Ullrich A. Coexpression of epidermal growth factor receptor and TGF-α and survival in upper aerodigestive tract cancer. *Anticancer Res* 1996, **16**, 283–288.
4. Zhu L, Kim K, Domenico DR, *et al.* Adenocarcinoma of duodenum and ampulla of vater: clinicopathology study and expression of p53, c-neu, TGF-α, CEA and EMA. *J Surg Oncol* 1996, **61**, 100–105.
5. Hise MK, Jacobs SC, Papadimitriou JC, *et al.* Transforming growth factor alpha expression in human renal cell carcinoma. *Urology* 1996, **47**, 29–33.
6. Nilsson O, Wangberg B, Kolby L, *et al.* Expression of transforming growth factor alpha and its receptor in human neuroendocrine tumors. *Int J Cancer* 1995, **60**, 645–651.
7. Pecur L, Kapitanovic S, Sonicki Z. *et al.* Prognostic significance of transforming growth factor alpha in human lung carcinoma: an immunohistochemical study. *Anticancer Res* 1994, **14**, 2839–2843.
8. Albino AP, Davis BM, Nanus DM. Induction of growth factor RNA expression in human malignant melanoma: markers of transformation. *Cancer Res* 1991, **51**, 4815–4820.
9. Sidransky D, Von Eschenbach A, Tsai Y, *et al.* Identification of p53 gene mutations in bladder cancer and urine samples. *Science* 1991, **252**, 706–709.
10. Gazzaniga P, Gradilone A, Vercillo R, *et al.* bcl-2/bax mRNA expression ratio as prognostic factor in low grade urinary bladder cancer. *Int J Cancer* 1996, **69**, 100–104.
11. Messing E. Growth factors in human bladder tumors. *J Urol* 1987, **131**, 111A.
12. Neal DE, Marsh C, Bennet MK, *et al.* Epidermal growth factor receptors in human bladder cancer: comparison of invasive and superficial tumors. *Lancet* 1985, **1**, 366–368.
13. Wright C, Mellon K, Johnston P, *et al.* Expression of p53, c-erb B2 and the epidermal growth factor in transitional cell carcinoma of the human urinary bladder. *Br J Cancer* 1991, **41**, 517–521.
14. Messing MM. Clinical implications of the expression of epidermal growth factor receptors in human transitional cell carcinoma. *Cancer Res* 1990, **50**, 2530–2537.

Acknowledgement—This work was supported by a grant from MURST 60% and 40%.