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The Loss of Expression of Transforming Growth Factor-β Receptors Correlates with the Histopathologic Tumor Grade in Bladder Transitional Cell Carcinoma Patients

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Abstract

Transforming growth factor-β (TGF-β), a pleiotropic growth factor, is a potent inhibitor of cellular proliferation in cells of epithelial origin. Recently, it has been suggested that a loss of sensitivity to TGF-β through a loss of expression of TGF-β receptors TβR-I and TβR-II - is associated with tumor initiation and progression. Therefore, to investigate the relationship between TGF-β receptors expression and carcinogenesis of bladder TCC, this study examined the expression of TβR-I and TβR-II in 46 bladder TCC patients using immunohistochemistry. Since histopathological grade is a widely accepted marker of prognosis, the results were compared in relation to the three grades of bladder TCC. The results demonstrated that the loss of TGF-β receptors expression is associated with increasing histopathological grades of bladder TCC. Specifically, both TβR-I and TβR-II were readily detected in all 10 normal bladder mucosa specimens. Likewise, all 6 specimens of grade I TCC samples expressed high levels of both TGF-β receptors. However, among grade II TCC samples, TβR-I and TβR-II were detected in 78% and 89%, respectively: among grade III TCC samples, TβR-I and TβR-II were detected in 45% and 41%, respectively. These results suggested that loss of sensitivity to TGF-β may play a role in the progression of TCC from low to high grade disease.

Key Words: Bladder transitional cell carcinoma, TGF-β receptor, tumor grade

INTRODUCTION

Bladder transitional cell carcinoma (TCC) is the second most common urological malignancy, which affected 54,500 Americans in 1997.[1] Although the etiology of the disease has not been completely defined, it is thought to be multifactorial, including genetic, dietary, and environmental causes. At initial presentation, approximately 20% of bladder cancers are invasive, whereas 80% are superficial. Approximately 20% of patients who present with superficial disease will eventually progress to muscle invasive cancer.[2] To prevent tumor recurrence, intravesical instillation of BCG, IFN or chemotherapeutic agents is performed. Nevertheless, some bladder tumors recur at different sites of the bladder and several tumors invade bladder muscles. There is latent micrometastasis in 50% of muscle invasive bladder cancer at diagnosis. In case of micrometastasis, local treatment such as radical cystectomy or radiotherapy has limitations. Most micrometastases become clinically evident within a year and almost all bladder cancer patients with micrometastasis die within two years. About 5% of micrometastases remain as latent cancer over 5 years. Half of the patients with muscle invasive bladder cancer have no micrometastasis and can be cured with local treatment.[2] Due to the varying clinical course of bladder cancer, it is difficult to determine treatment modality for patients. Therefore, additional information that will permit a more accurate prediction of the clinical behavior of bladder TCC is necessary.

Transforming growth factor-β (TGF-β) is a 25-kDa multifunctional growth factor expressed by many cell lines and tissue types. Although this growth factor has been implicated in immunosuppression and angiogenesis, it usually acts as a growth inhibitor in most cells, especially those of the epi-

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thelial lineage.[3,4] Malignant epithelial cells, though, are frequently resistant to TGF-β and express elevated levels of TGF-β. The exact mechanism whereby TGF-β inhibition is lost during transformation remains poorly understood. There is, however, evidence that suggests that TGF-β receptors (TβRs) play an important role during cacinogenesis; especially in neoplasms of the breast,[5] colon,[6] esophagus,[7] and thyroid[8] in that the loss of expression of TβRs correlated with the progression of cancer.

There are three ubiquitously expressed TGF receptors: type I, type II, and type III (TβR-I, TβR-II, and TβR-III, respectively). TβR-III is a membrane proteoglycan that has a very short cytoplasmic tail and lacks any consensus-signaling motif,[9] whereas TβR-I and TβR-II are serine/ threonine kinases.[10,11] The current understanding of the mechanism of action of TβRs is that both TβR-I and TβR-II are required for TGF- signal transduction.[12] Since a heteromeric complex composed of TβR-I and TβR-II is required for TGF-β signal transduction, cancer cells may reduce the expression of either TβR-I or TβR-II to escape the growth inhibitory effect of TGF-β.[13]

Herein we investigated the expression of TβR-I and TβR-II in archival tissues of human bladder TCC. We report that the expression of TβR-I and TβR-II is frequently lost in high-grade bladder cancer cells.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed, paraffin-embedded tissue specimens of bladder cancer were obtained from 46 patients, who were randomly chosen from patients with superficial and invasive bladder cancer by transurethral resection or radical cystectomy at Baylor College of Medicine from 1985 to 1993. Normal samples of bladder mucosa were obtained from 10 patients with benign prostatic hyperplasia by punch biopsy at Soonchunhyang Medical School. The average age of patients with bladder cancer was 66.0 (range: 41 ~ 80), and 20 of 46 patients were superficial bladder cancer patients; the remainder had invasive bladder cancer.

Immunohistochemistry

Specific rabbit antisera against TβR-I and TβR-II were made against synthetic peptides corresponding to the intracellular juxtamembrane parts of the receptors by Dr. Kohei Miyazono (The Cancer Institute, Tokyo, Japan). The use of the antibodies in immunohistochemistry has been reported previously.[14-17]

Archival bladder cancer specimens were fixed in neutral buffered formalin and were sectioned at a thickness of 4 μm, deparaffinized in Xylenes (Fisher Scientific Co., Pittsburgh, PA, USA), and rehydrated in PBS. Endogenous peroxidase activity was inactivated by incubation in 0.3% H_2O_2 for 10 min. Following a preincubation with 2% normal goat serum to block nonspecific sites, the sections were incubated with primary antibodies in a humidified chamber for 18h at 4°C. Anti-TβR-I and anti-TβR-II antibodies were used at a concentration of 2 μg/ml. Antigenic binding sites were visualized with a serial incubation using biotinylated secondary antibody, followed by the avidin-biotin-horseradish peroxidase complex, and diaminobenzidine tetrahydrochloride before counterstaining with hematoxylin (ABC kit; Vector Laboratories, Burlingame, CA, USA). Negative control sections were processed in an identical manner by substitution of primary antibody with a normal rabbit IgG fraction. All negative control sections showed no color reactions.

Because immunohistochemistry is not quantitative, all cases were classified into either positive or negative staining for TβRs. Specimens were classified as negative if the staining level was comparable to that of the negative control slide. All negative cases were confirmed with at least two independent staining experiments. In addition, all stainings were reviewed by at least two investigators who were blinded as to grade of the sample.

Statistics

The Cochran-Armitage trend test was used to evaluate the association of TβR expression with the histologic grades. A p-value less than 0.05 was regarded as statistically significant. Statistical analyses were performed using the SAS program.

RESULTS

Expression of TβRs in normal bladder tissues

The expression of TβRs was initially investigated in normal bladder tissues. The result is shown in Fig.

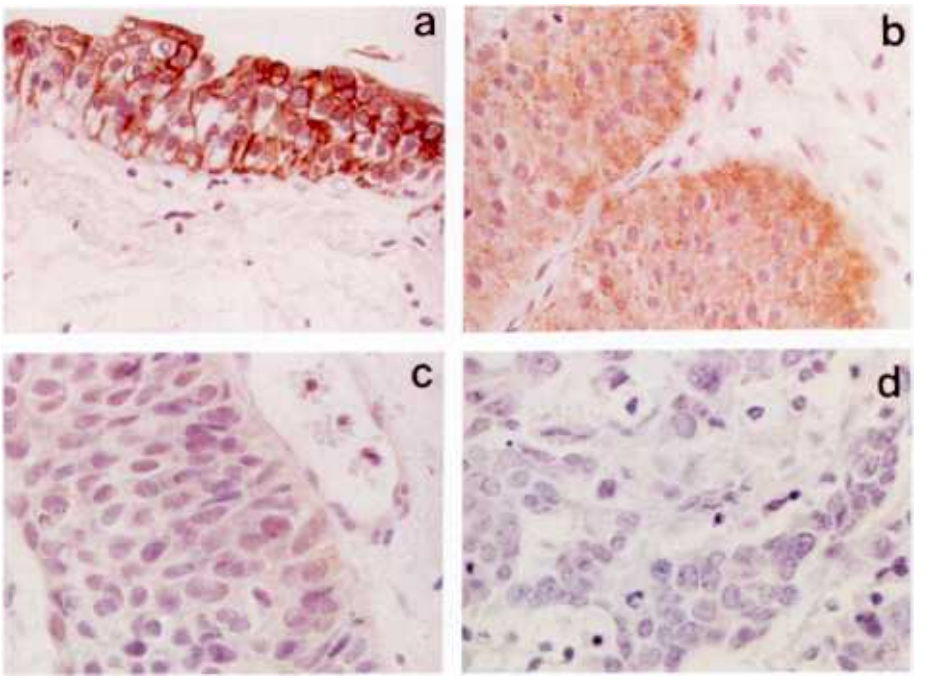

Fig. 1. Immunohistochemistry for TGF-β type I receptors in normal bladder mucosa and bladder cancer tissues. (a) Normal bladder mucosa, (b) grade I bladder cancer, (c) grade II bladder cancer, (d) grade III bladder cancer. Note the positive brown-staining cells. As in benign tissues, epithelial cells in bladder cancer tissue predominantly expressed TGF-β type I receptors. In addition, there was a significant decrease in the level of expression of TβR-I with increasing grades of bladder cancer.

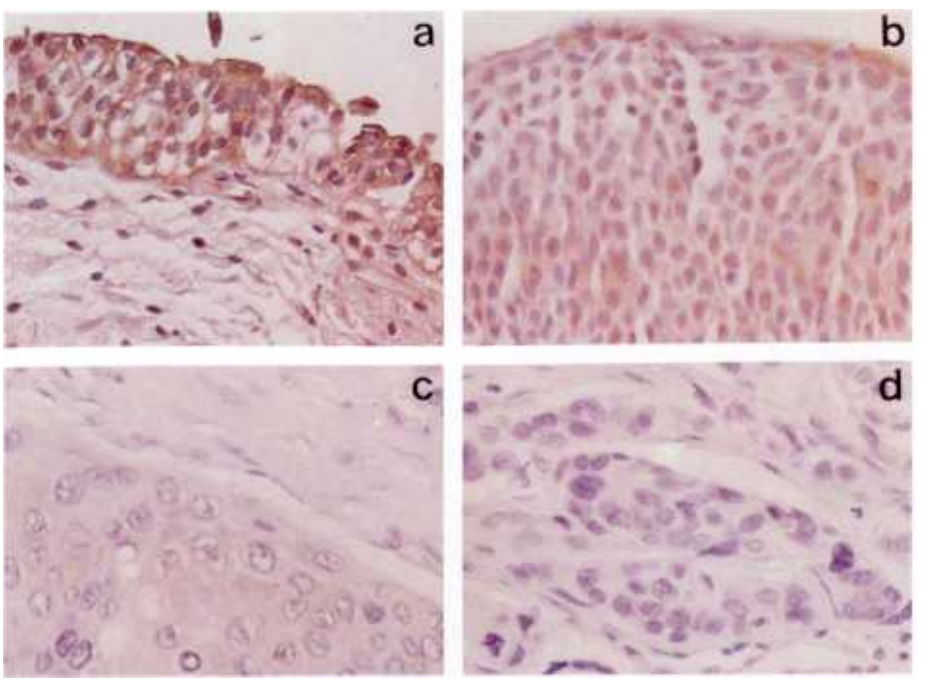

Fig. 2. Immunohistochemistry for TGF-β type II receptors in normal bladder mucosa and bladder cancer tissues. (a) Normal bladder mucosas, (b) grade I bladder cancer, (c) grade II bladder cancer, (d) grade III bladder cancer. TGF-β type II receptors were predominantly expressed by epithelial cells in malignant bladder tissues as in normal bladder mucosa. The level of expression of TβR-II significantly decreased with the increasing grade of bladder cancer.

1. In benign tissues, the expression of TβR-I and TβR-II was predominantly localized in epithelial cells. Specificity of the antibodies in bladder tissues was confirmed because the color reactions were successfully neutralized when the antibodies were preincubated with 100-fold molar excess of the corresponding peptide antigen (data not shown).

Expression of TβRs in malignant bladder tissues

To measure the levels of expression of TβRs in human bladder cancer cases, 46 archival samples were screened for TβR expression using immunohistochemistry. For comparison, 10 normal bladder mucosa were used. Figs. 1 and 2 show representative

Table 1. Expression of TβR-I and TβR-II in Normal Bladder Mucosa and Human Bladder Cancer Tissues

No.	Grade	TβR-I	TβR-II	No.	Grade	TβR-I	TβR-II
1	Normal	+	+	29	2	+	+
2	Normal	+	+	30	2	+	+
3	Normal	+	+	31	2	+	+
4	Normal	+	+	32	2	−	+
5	Normal	+	+	33	2	−	−
6	Normal	+	+	34	2	+	+
7	Normal	+	+	35	3	−	−
8	Normal	+	+	36	3	+	+
9	Normal	+	+	37	3	+	−
10	Normal	+	+	38	3	+	+
11	1	+	+	39	3	−	−
12	1	+	+	40	3	+	+
13	1	+	+	41	3	−	−
14	1	+	+	42	3	−	−
15	1	+	+	43	3	+	−
16	1	+	+	44	3	−	−
17	2	+	+	45	3	−	−
18	2	+	+	46	3	−	−
19	2	+	+	47	3	−	−
20	2	+	+	48	3	−	−
21	2	+	+	49	3	+	+
22	2	+	+	50	3	+	+
23	2	+	+	51	3	+	−
24	2	+	+	52	3	+	+
25	2	−	+	53	3	−	−
26	2	−	+	54	3	+	+
27	2	+	−	55	3	−	−
28	2	+	+	56	3	+	+

Table 2. Relationship of TβR Expression and Tumor Grade

Tissue	TβR-I					TβR-II				
	Positive		Negative		Significance	Positive		Negative		Significance
	No.	%	No.	%		No.	%	No.	%	
Normal	10	100	0	0	p=0.001	10	100	0	0	p=0.001
Grade I	6	100	0	0		6	100	0	0	
Grade II	14	78	4	22		16	89	2	11	
Grade III	10	45	12	55		9	41	13	59	

immunohistochemical staining for TβR-I and TβR-II, respectively. They show a wide variation in staining intensity for TβR expression among the human bladder cancer specimens. Since immunohistochemistry is not quantitative, the tissue samples were classified into either positive or negative staining for TβR expression. The negative cases were confirmed with at least two independent staining experiments. All stainings were reviewed by at least two investigators.

Of 46 bladder cancer patients, 6 were grade I, 18 were grade II, and 22 were grade III. Both TβR-I and TβR-II were readily detected in all 10 normal bladder mucosa specimens. Likewise, all 6 specimens of grade I bladder TCC samples expressed high levels of both TGF-β receptors. However, among grade II bladder TCC tissues, TβR-I and TβR-II were detected in 78% and 89%, respectively: among grade III bladder TCC tissues, TβR-I and TβR-II were detected in 45% and 41%, respectively (Table 1 and 2).

The results demonstrated that the loss of expression of both TβR-I and TβR-II was associated with increasing tumor grade in bladder cancer (TβR-I, $p=0.001$; TβR-II, $p=0.001$) (Table 2).

DISCUSSION

Results of this study demonstrated that TβRs are preferentially expressed by the epithelial cells in human bladder mucosa, and that human bladder cancer cells frequently have reduced levels of expression of TβRs. The results also show an inverse correlation between the expression of TβRs and tumor grade in human bladder malignancy. Taken together, these observations provide valuable insight regarding the potential role of TGF-β in the human bladder and the mechanism responsible for the acquisition of TGF-β resistance in human bladder cancer cells.

TGF-β is a potent growth inhibitory factor in normal epithelial cells. In contrast, certain cancer cells have resistance to the growth inhibitory effects of TGF-β. The acquisition of resistance to TGF-β in bladder cancer is strongly related to the development and progression of cancer.[18-20] Patients with invasive bladder cancer have high serum levels of TGF-β1.[21] Although TGF-β suppresses growth of normal bladder mucosa and most bladder cancer cell lines in mice, LMC-19, a rat bladder cancer cell line, is resistant to the growth inhibitory effect of TGF-β and has a loss of expression of TβR-I.[22,23] Furthermore LMC19's malignant phenotype was reduced by the overexpression of TβR-I.[24] These results suggest that the loss of expression of TGF-β receptors may be an important event during bladder carcinogenesis.

Such a hypothesis is consistent with the results of the present study in which there was a significant association between the loss of expression of TβRs and increasing grades of tumor. Specifically, 11 of 13 bladder cancer cases that exhibited loss of expression of TβRs were grade III cases. Results of this study also indicated that the loss of expression of TβRs might be used as a prognostic marker in bladder cancer patients. Since the tumor grade is an excellent predictor of outcome among bladder cancer patients, the close association between the loss of expression of TβRs and a high grade suggests that the status of TβR expression can also be used as a prognostic marker in bladder cancer patients. Furthermore, because there were bladder cancer cases with grade III that still retained the expression of TβRs, the possibility exists that the loss of expression of TβRs may be a prognostic factor. Additional studies are under way to verify this concept.

Recently, it has been demonstrated that TβR-II is a cancer suppressor gene in a subset of colon carcinoma.[25] The potential role of TβR-I during carcinogenesis is less certain though. Intracellular signal transduction of TGF-β requires that TβR-I as well as TβR-II are needed.

In conclusion, the loss of TβRs is frequent and causes important changes in the development and progression of cancer. It is suggested that the loss of sensitivity to TGF-β plays an important role during bladder TCC carcinogenesis and that the loss of expression of TGF-β receptors may be a potential prognostic marker in bladder TCC.

REFERENCES

1. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, CA. 1997;47:5.
2. Catalona WJ. Urothelial tumors of the urinary tract. In: Walsh PC, Retic AB, Stamey TA, Vaughan, Jr., editors. Campbell's Urology, 6th ed. Philadelphia: W.B. Saunders; 1992. p.1094-158.
3. Moses HL, Tucher RF, Leof EB, Coffey RJ, Halper J, Shipley GD. Type beta transforming growth factor is a growth stimulator and a growth inhibitor. In: Feramisco J, Ozanne B, Stiles C, editors. Growth factors and transformation. New York: Cold Spring Habor; 1985. p.65-71.
4. Yang EY, Moses HL. Transforming growth factor beta-1

induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. J Cell Biol 1990;111:731-6.
5. Koli KM, Arteaga CL. Predominant cytosolic localization of type II transforming growth factor-beta receptors in human breast carcinoma cells. Cancer Res 1997; 57:970-7.
6. Jiang W, Tillekeratne MP, Brattain MG, Banerji SS. Decreased stability of transforming growth factor beta type II receptor mRNA in RER+ human colon carcinoma cells. Biochem J 1997;36:14786-903.
7. Garrigue-Antar L, Souza RF, Vellucci VF, Meltzer SJ, Reiss M. Loss of transforming growth factor-beta type II receptor gene expression in primary human esophageal cancer. Lab Invest 1996;75:263-72.
8. Lazzereschi K, Ranieri A, Mincione G, Taccogna S, Nardi F, Colletta G. Human malignant thyroid tumors displayed reduced levels of transforming growth factor beta receptor type II messenger RNA and protein. Cancer Res 1997; 57:2070-6.
9. Lopez CF, Cheifetz S, Doody J, Andres JL, Lane WS, Massague J. Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-β receptor. Cell 1991;67:785-95.
10. Lin HY, Wang X, Ng-Eaton E, Weinberg RA, Lodish HF. Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. Cell 1992;68:775-85.
11. Franzen P, Kijke P, Ichijo H, Yamashita H, Schulz P, Heldin C, et al. Cloning of a TGF-β type I receptor that forms a heteromeric complex with the TGF-β type II receptor. Cell 1994;75:681-92.
12. Chen F, Weinberg RA. Biochemical evidence for the autophosphorylation and transphosphorylation of transforming growth factor b receptor kinases. Proc Natl Acad Sci USA 1995;92:1565-9.
13. Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF-β receptor. Nature 1994;370:341-7.
14. Gullick WJ, Downward J, Waterfield MD. Antibodies to the autophosphorylation sites of the epidermal growth factor receptor protein - tyrosine kinase as probes of structure and function. EMBO J 1985;4:2869-77.
15. Waltenberger J, Wanders A, Fellstrom B, Miyazono K, Heldin C-H, Funa K. Induction of transforming growth factor- b during cardiac allograft rejection. J Immunol 1993;151:1147-57.
16. Iseki S, Osumi-Yamashita N, Miyazono K, Franzen P, Ichijo H, Ohtani H, et al. Localization of transforming growth factor- type I and type II receptors in mouse development. Exp Cell Res 1995;219:339-47.
17. Yamada N, Kato M, Yamashita H, Nister M, Miyazono K, Heldin C-H, et al. Enhanced expression of transforming growth factor-β and its type I and type II receptors in human glioblastoma tissues. Int J Cancer 1995;62:386-92.
18. Arteaga C, Bandon A, Von Hoff D, Osborne C. Transforming growth factor β: potential autocrine growth inhibitor of estrogen receptor-negative human breast cancer cells. Cancer Res 1988;48:3898-904.
19. Kimchi A, Wang XF, Weinberg RA, Cheifetz S, Massague J. Absence of TGF-β receptors and growth inhibitory response in retinoblastoma cells. Science 1988;240:196-240.
20. Wakefield LM, Sporn MB. Suppression of carcinogenesis: a role for TGF-β and related molecules in prevention of cancer. Immunol Ser 1990;51:217-43.
21. Eder IE, Stenzl A, Hobsch A, Cronauer MV, Bartsch G, Klocker H: Transforming growth factor-β1 and β2 in serum and urine from patients with bladder carcinoma. J Urol 1996;156:953-7.
22. Kawamata H, Azuma M, Kameyama S, Nan L, Oyasu R. Effect of epidermal growth factor/transforming growth factor-a and transforming growth factor-β1 on growth in vitro of rat urinary bladder carcinoma cells. Cell Growth Differ 1992;3:819-25.
23. Kawamata H, Kameyama S, Nan L, Kawai K, Oyasu R. Effect of epidermal growth factor and transforming growth factor β1 on growth and invasive potentials of newly established rat bladder carcinoma cell lines. Int J Cancer 1993;55:968-73.
24. Okamoto M, Oyasu R. Overexpression of transforming growth factor β type I receptor abolishes malignant phenotype of a rat bladder carcinoma cell line. Cell Growth Differ 1997;8:921-6.
25. Markowitz S, Wang J, Meyeroff L, Parsons R, Sun L, Lutterbaugh J, et al. Inactivation of the type II TGF-β receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cell. Science 1995; 268:1336-8.