Urol Res (2004) 32: 317–322
DOI 10.1007/s00240-003-0360-z

ORIGINAL PAPER

**Dionisios Mitropoulos · Aspasia Kiroudi
Evangelia Christelli · Efraim Serafetinidis
Anastasios Zervas · Ioannis Anastasiou
Constantinos Dimopoulos**

Expression of transforming growth factor β in renal cell carcinoma and matched non-involved renal tissue

Received: 18 April 2004 / Accepted: 30 July 2004 / Published online: 7 September 2004

Abstract TGFβ1 is one of several cytokines produced by proximal tubular and renal cancer cells. Previous studies have been mainly focused on determining plasma or serum TGFβ levels, its effect on RCC cultures, and the expression of TGFβ mRNA. Cancerous and autologous normal kidney samples were obtained from 24 patients treated by radical nephrectomy. TGFβ1 expression was determined using a semi quantitative Western blot analysis and immunohistochemistry. Blot densities and immunohistochemical expression intensities in normal and neoplastic tissue were compared, and subsequently correlated to tumor stage, histological type and nuclear grade. All tissue samples examined expressed TGFβ1; mean tumor to non-involved kidney spot density ratio correlated with advancing stage and higher nuclear grade. The overexpression of TGFβ1 in certain RCCs may partially explain their resistance to the growth suppression action of TGFβ. The correlation with tumor stage and grade indicates a possible role in the development of metastatic potential as well as in host's immune response modulation.

Keywords Renal cell carcinoma · Transforming growth factor beta · Immunohistochemistry · Western blot analysis

D. Mitropoulos (✉) · E. Serafetinidis · A. Zervas
I. Anastasiou · C. Dimopoulos
Department of Urology,
University of Athens Medical School, 75,
Mikras Asias St., 115-27 Athens, Greece,
E-mail: dmp@otenet.gr
Fax: +30-210-7701141

A. Kiroudi
Department of Histology and Embryology,
University of Athens Medical, School,
Athens, Greece

E. Christelli
Department of Experimental Surgery and Surgical Research,
University of Athens Medical School, Athens, Greece,

Introduction

Renal cell carcinoma (RCC) is the third most common genitourinary tumor, accounting for more than 2% of adult cancers today [19]. It is rather resistant to chemotherapy and radiotherapy and can often be cured if it is diagnosed and treated surgically when still localized to the kidney and to immediately surrounding tissue [34]. There are five distinct types of RCC currently accepted: conventional, papillary, chromophobe, collecting duct and unclassified [36]. As far as prognosis is concerned, stage and grade are considered the main prognostic factors along with tumor size, cell type, DNA content and nuclear morphometry, although they often fail to predict the clinical behavior of these tumors [38].

Transforming growth factor β1 (TGFβ1) is among several immunomodulators that have been reported to be produced by proximal tubular and renal cancer cells [16, 29]. TGFβ1 is the prototype of a large family of multifunctional peptides of which five isoforms termed TGFβ1, TGFβ2, TGFβ3, TGFβ1.β2 and TGFβ1.β3 have been identified in mammals [25]. It is usually secreted by normal cells in a biologically inactive or latent form [26] from which is produced the 25 kDa mature, active form of TGFβ1. TGFβ1 is multi-potent, with marked and sometimes opposing effects on cell proliferation, differentiation, adhesion and migration, extracellular matrix production, immunomodulation, and other activities, depending on the cell type and microenvironmental conditions [4, 20, 33]. Although TGFβ is one of the most potent inhibitors of normal cell growth, many malignancies of epithelial and hematopoetic origin are resistant to it, suggesting that developing resistance to growth inhibitory cytokines plays an important role in tumorigenesis [30].

Previous studies on the role of TGFβ in RCC tumorigenesis and biological behavior have been mainly focused on the serum [13, 40] or plasma [11, 14, 41], TGFβ concentration, TGFβ production by and effect on RCC cultures in vitro [1, 5, 8, 10, 31], and the presence

of TGFβ mRNA in tumor samples [6, 9, 28]. To further elucidate the role of TGFβ1 in renal neoplasms we studied its protein level expression in neoplastic and autologous normal tissue specimens in relation with other clinicopathological parameters.

Materials and methods

Patients and tissue samples

Primary RCC tumors and autologous non-involved kidney samples were obtained from 24 consecutive patients treated by radical nephrectomy. There were 16 males and eight females with a mean age of 64 ± 12 (mean $\pm$ SD) years (range, 40–77 years). Tumors were graded according to the World Health Organisation classification, and the stage was based on the TNM staging classification of the International Union Against Cancer, 1997 [36]. Of the 24 patients studied, RCC was diagnosed as stage $T_2N_0M_0$ in 11, $T_3N_0M_0$ in ten, and $T_3N_+M_0$ in three individuals. Three tumors were classified as nuclear grade I, 13 of them as grade II, and eight as grade III. Tissue samples were specifically encoded in order to disclose patients' identity.

Protein extraction

Tissue samples (minimum weight 5 g) from the neoplastic and adjacent non-neoplastic parts of the excised kidney were weighed and diced into very small (< 5 mm) pieces. These were homogenized in NP-40 lysis buffer (50 mM Hepes, 0.05 mM $ZnCl_2$, 2 mM EDTA, 1% NP40) in the presence of proteinase inhibitors (1 mM PMSF, 0.5 mg/ml leupeptin, 50 mg/ml pepstatin A, 15 mg/ml benzamidine, 2 mg/ml aprotinin) [12]. The temperature was maintained at 4°C through all procedures. Samples were sonicated (Soniprep 150, MSE) prior to centrifugation at 5,000 rpm for 30 min at 4°C. The protein concentration of supernatants was determined by the bicinchoninic acid method (Sigma) [35].

Immunoprecipitation and Western blot analysis

Total protein (1 mg) was immunoprecipitated with 5 µl antibody against TGFβ1 (mouse IgG1 monoclonal antibody, RD Systems). The mixture was incubated overnight at 4°C on a rotary mixer. Next morning, 30 µl of protein A–Sepharose CL-4B beads (Pharmacia) was added in each sample and the mixture was incubated for 3 h at 4°C on a rotary mixer. Immunoprecipitates were collected by centrifugation at 2,500 rpm for 5 min at 4°C. The pellet was washed three times with 0.5 ml washing buffer for beads (50 mM Hepes, 1% Triton X, 0.1% SDS, 150 mM NaCl, 100 mM NaF, 2 mM $NaVO_4$, pH 7.4) and resuspended in 40 µl Laemli buffer. Samples were boiled for 5 min and loaded on 10% polyacrylamide gel [21]. Electrophoresis was performed according to the method of Laemli [18], and the gels were equilibrated in Towbin's transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3) [37]. Proteins were transferred to a nitrocellulose membrane (0.45 µm) (Amersham) by electroblotting at 15 V for 50 min using a BioRad semi-dry system. The membrane was washed briefly twice with TBS (Tris buffered saline, pH 7.5) and the blocking of the unspecific binding of antibody was performed by incubating the membrane for 1.5 h at room temperature with blocking solution (50 mM Tris, 150 mM NaCl, 0.2% Tween 20 pH 7.4, 5% w/v skim milk powder). The blots were probed with 2 µg/ml of primary antibody solution (as determined by dot blot analysis) by incubating overnight at 4°C. The membrane was then washed four times with TBST (TBS-Tween 20). TGFβ1 protein levels were detected by the alkaline phosphatase-labeled antibody mixture. The optimum concentration was 650 mU/ml as determined by dot blot analysis [22]. The dot blot analysis used a reaction of immunoglobulins and incubation with ready to use substrate according to manufacturer's instructions (Boehringer Mannheim).

Immunohistochemistry

Immunohistochemistry for TGFβ1 was performed on paraffin-embedded, formalin-fixed tissue sections. The sections were deparaffinized in xylene and rehydrated with a graded alcohol series. Endogenous peroxidase activity was inactivated by incubating in 3% H_2O_2 for 10 min. Antigen retrieval was performed by immersing the tissue sections in 0.1 M citrate buffer (pH 6.0) and microwaving at 800 W for 15 min. The tissue was then incubated with the primary antibody against TGFβ1 (the same used for immunoprecipitation and Western blot analysis) at 1:100 concentration in a humidified chamber at 4°C overnight. After applying the secondary antibody, reactivity was visualized with an avidin-biotin complex immunoperoxidase system (Dako).

Evaluation of blots and slides, and statistical analysis

TGFβ1 expression was measured in human RCC and non-involved renal tissue samples using a semi quantitative Western blot analysis. Tumor to non-involved kidney spot density ratios were calculated after measuring the corresponding spot densities with a computer program (Image-Pro Plus).

Immunohistochemistry specimens were classified based on the intensity of staining as follows: weak or absent staining ($< 10\%$ of cells), intermediate (10–25%), focally strong (25–50%), and strong ($> 50\%$ of cells).

Mean spot ratio variance between groups was estimated using the ANOVA test. Categorical data were analyzed using the χ^2-test.

Results

All tissue samples (neoplastic and non-neoplastic) examined expressed TGFβ1. Mean spot density ratio ranged from 1.0 to 3.5 (mean ± SE: 1.97 ± 0.16). Western blot analysis was successfully reproduced in eight randomly chosen tissue sample pairs. Repeated spot density measurements showed negligible variability. A representative immunoblot showing marked overexpression of TGFβ1 in tumor specimens is shown in Fig. 1. Mean spot density ratio increased significantly with advancing stage ($F = 3.6$, $= 0.045$) and nuclear grade ($F = 4.98$, $P = 0.017$) (Table 1). In clear cell carcinomas, the tumor to non-involved kidney TGFβ1 expression density ratio was 2.08 ± 0.17 (range 1.0–3.5). The corresponding values for the other subtypes were 1.0 for papillary and chromophobe, and 1.0 and 2.8 for the two cases of unclassified tumors, respectively.

A representative result of the immunohistochemistry for TGFβ1 in non-involved and cancerous tissues is shown in Fig. 2. In the normal kidney tissue, positive immunoreactivity was observed in the cells of proximal and distal convoluted tubules but not in glomeruli. In cancerous tissue TGFβ1 was found either in the cytoplasm or on the plasma membrane of the neoplastic cells. Staining intensity was intermediate in 11, focally strong in four, and strong in nine cases. Staining intensity in tumor specimens was not independent of tumor to non-involved kidney density ratio ($\chi^2 = 18.1$, $P = 0.001$). Moreover, mean spot density ratio increased significantly with staining intensity ($F = 18.96$, $P < 0.001$) (Table 1).

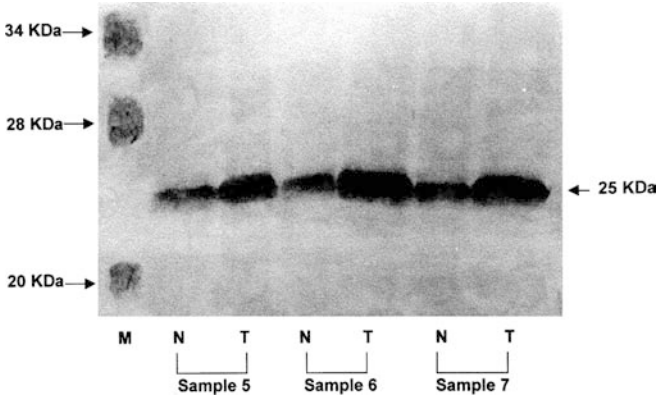

Fig. 1 Representative immunoblots for TGFβ1 in tumor specimens (*T*) and autologous clinically normal tissue (*N*) derived from three patients. TGFβ1 is overexpressed in tumor specimens. *M* Marker lane; the mature-active form of TGFβ1 has a molecular weight of 25 kDa

Discussion

Cytokines are multifunctional peptides with many biological activities, being synergistic or antagonistic with one or more cytokines. Several potent immunomodulatory cytokines, such as TGFβ1 or IL-6, are produced by proximal tubular cells or cell cultures derived from RCC of proximal tubuli origin (conventional RCC) [5, 16, 29]. These cytokines may exert tumor-promoting and immunosuppressive effects [2, 30]. TGFβ1 is one of the mammalian TGFβ isoforms which are homologous peptide growth factors that act by binding to a single common receptor complex. TGFβ family proteins are implicated in many biological processes including embryogenesis, tissue repair, and the regulation of hematopoesis and the immune response. An interesting feature is that TGFβ family proteins are potent growth suppressors of different normal cell types, while, on the other hand, many tumors are resistant to TGFβ [23]. In this regard, we investigated the expression of active TGFβ1 in RCC samples using a semi-quantitative Western blot analysis, immunohistochemistry, and possible correlation with pathological data.

While most studies have been focused on the presence of TGFβ1 mRNA in RCC samples [6, 9, 28], the detection of TGFβ1 as a protein product was reported only recently [3, 31]. We were able to confirm these studies by using both immunohistochemistry and Western blot analyses. Since TGFβ has been shown to play an important role in maintaining the renal histological structure as well as tubular function, the constant expression in normal renal samples was of no surprise. However, the fact that renal carcinoma cells also express TGFβ1 shows that it is potentially a regulator of both normal and neoplastic cellular activity. We and others

Table 1 Correlation of tumor to non-involved blot density ratio to tumor stage, nuclear grade and TGFβ1 immunohistochemical staining intensity

	No. of patients	Mean ± SE	Range	Statistics (ANOVA)
Stage				
$T_2N_0M_0$	11	1.61 ± 0.25	1.00–3.50	
$T_3N_0M_0$	10	2.11 ± 0.21	1.00–3.00	
$T_3N_+M_0$	3	2.83 ± 0.18	2.60–3.20	$F = 3.6$, $P = 0.045$
Nuclear grade				
I	3	1.26 ± 0.26	1.00–1.80	
II	13	1.77 ± 0.21	1.00–3.20	
III	8	2.56 ± 0.22	1.40–3.50	$F = 4.98$, $P = 0.017$
Staining intensity				
Intermediate	11	1.32 ± 0.10	1.00–2.00	
Focally strong	4	2.10 ± 0.41	1.00–2.80	
Strong	9	2.71 ± 0.17	1.90–3.50	$F = 18.96$, $P < 0.001$

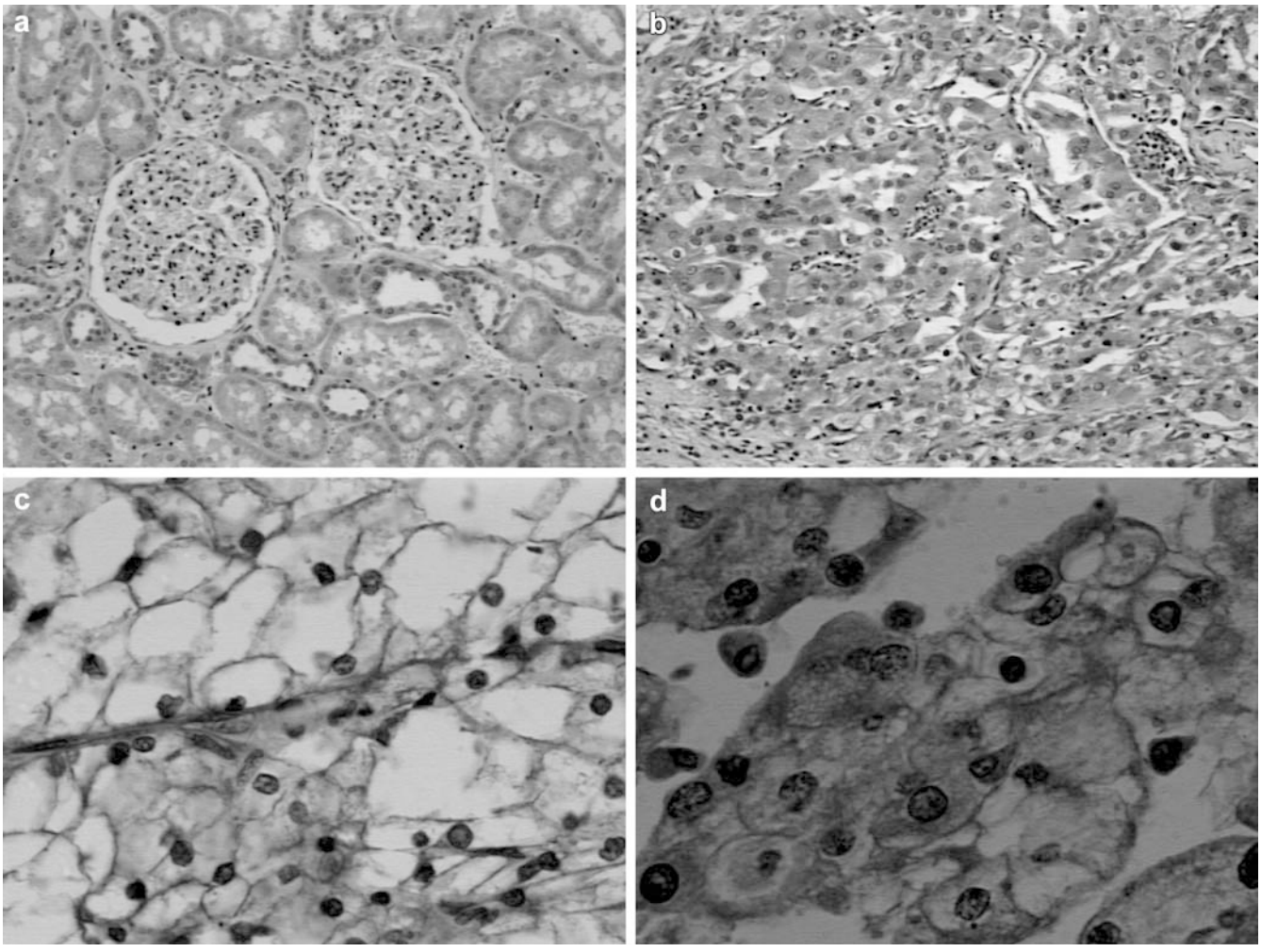

Fig. 2 Immunohistochemical expression of TGFβ1. **A** Normal kidney: positive immunoreactivity is observed in the cells of proximal and distal convoluted tubules but not in glomeruli. **B**–**D** Renal cell carcinoma: TGFβ1 is localized on the cytoplasm or on the plasma membrane of neoplastc cells. Original magnification: **A**, **B** ×10; **C**, **D** ×40

[3] showed that in certain RCC samples TGFβ1 expression was more intense in comparison with the corresponding autologous non-involved renal tissue. This may partially explain the resistance of these tumors to the growth suppression autocrine action of TGFβ. In vitro studies using renal cancer cell lines indicated that some of these are resistant to the growth inhibitory action of TGFβ. This was attributed to possible changes in the expression of TGFβ receptors and its signaling molecules [17, 31]). The fact that TGFβ1 expression varied significantly between normal and tumor samples indicates that TGFβ1 accumulation is related only to individual tumor characteristics and not to differences in the metabolic status between normal and cancerous tissues.

TGFβ has been found to induce angiogenesis in vivo, possibly due to cellular migration and extracellular deposition [32, 39]. Considering that the growth of solid tumors such as RCC is angiogenesis-dependent, TGFβ1 overexpression in certain RCCs may suggest a promoting role for angiogenesis and therefore a negative impact on patient outcome. Other studies have been focused on the immunosuppressive role of TGFβ1 [24]. These observations have been further confirmed with other studies [28] in which tumors continue to progress in vivo despite the presence of tumor infiltrating lymphocytes, suggesting an ineffective, poor host immune response to the tumor. The suppression of tumor infiltrating lymphocytes' effector function could be due to the local over-production of the immunosuppressive TGFβ1.

Our results also showed that the overexpression of TGFβ1 was related to the tumor stage and grade, indicating that TGFβ1, which is produced by both normal and neoplastic cells, is implicated in malignant transformation and the development of aggressive biological behavior. Although this disagrees with a recently published report [3], our findings comply with previous observations on tumors acquiring resistance to TGFβ relatively late in carcinogenesis, being correlated with the development of invasiveness [7, 15, 27]. In our study, there was no correlation found between TGFβ1 expression and histological type, confirming that TGFβ1 is a ubiquitous substance.

Data on the specificity and sensitivity of TGFβ1 used as a tumor specific marker are rather controversial. Increased levels of latent TGFβ1 were measured in plasma samples from patients with RCC, but only metastatic disease resulted in significant elevation [11, 13, 41]. The issue of chronic kidney damage as the cause of elevated TGFβ1 levels was addressed by Junker et al [14], who studied the plasma levels of latent TGFβ1 in an extended set of patients with various renal diseases. The conclusion was that the elevated plasma TGFβ1 levels are common in at least two chronic renal diseases. The restoration of renal function normalizes the TGFβ1 mRNA levels leading to speculation that the chronic elevation of TGFβ1 may predispose to renal cancer. Although previous studies [11, 13, 14, 40, 41] have confirmed that increased serum levels of TGFβ1 are commonly found in patients with renal cell carcinoma, its source has yet to be determined. Various cells have been identified to be able to secrete the latent form of TGFβ1 that can be activated by acidification or alkalinization [40]. Thus, it is possible that tumor cells may not be the only source of TGFβ1, which may also be secreted by adjacent and circulating cells when stimulated by the tumor cells. Another question that has been raised is whether TGFβ1 is predominately secreted in the latent form or not. Previous studies showed that TGFβ1 was secreted mainly in the active form without excluding the possibility that it is inactive locally and the low pH encountered within tumors is in the range necessary for its activation [13].

In conclusion, our data comply with previous studies that showed an enhanced expression of TGFβ1 mRNA and protein in tumor samples and increased serum TGFβ1 levels in RCC patients. The correlation of TGFβ1 expression intensity with tumor stage and nuclear grade indicates its possible role in the development of metastatic potential as well as in modulating the host's immune response. More studies comparing TGFβ1 expression in tumor and normal tissue supported by adequate follow-up observation may define a more specific relation between the overexpression of TGFβ1 and the development of aggressive biological behavior.

Acknowledgements The authors acknowledge the skillful technical contribution of Penelope Chiara (medical arts), Despina Karandrea and Vasiliki Dotsika.

References

1. Atlas I, Mendelsohn J, Baselga J, Fair WR, Masui H, Kumar R (1992) Growth regulation of human renal carcinoma cells: role of transforming growth factor alpha. Cancer Res 52:3335
2. Border W, Noble N (1995) Targeting TGFβ for treatment of diseases. Nat Med 1:1000
3. Cardillo MR, Lazzereschi D, Galdini O, Di Silverio F, Colletta G (2001) Transforming growth factor-beta pathway in human renal cell carcinoma and surrounding normal-appearing renal parenchyma. Anal Quant Cytol Histol 23:109
4. Cerwenka A, Bevec D, Majdic O, Knapp W, Holter W (1994) TGF-beta 1 is a potent inducer of human effector T cells. J Immunol 153:4367
5. Chang SG, Shin C, Rho SK, Kim DK, Kim JH (1998) Cytokine production in primary histoculture by human normal kidney, renal cell carcinoma and benign renal angiomyolipoma tissues. Anticancer Res 18:4195
6. Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Wiiliams RD, Bringman TS, Berger WH (1987) Synthesis of messenger RNAs for transforming growth factors α and β and the epidermal growth factor receptors by human tumors. Cancer Res 47:707
7. Filmus J, Kerbel RS (1993) Development of resistance mechanisms to the growth-inhibitory effects of transforming growth factor-beta during tumor progression. Curr Opin Oncol 5:123
8. Gomella LG, Sargent ER, Linehan WM, Kasid A (1989) Transforming growth factor-beta inhibits the growth of renal cell carcinoma in vitro. J Urol 141:1240
9. Gomella LG, Sargent ER, Wade TP, Anglard P, Linehan WM, Kasid A (1989) Expression of transforming growth factor α in normal human adult kidney and of transforming growth factors α and β1 in renal cell carcinoma. Cancer Res 49:6972
10. Hall S, Hsieh T-C, Ng C-Y, Mallouh C, Wu JM (1996) Control of renal carcinoma TC-1 growth, cyclin/kinase and nm23 expression by IFN-γ and TGF-β. Anticancer Res 16:1755
11. Hegele A, Vagra Z, Von Knobloch R, Heidenreich A, Kropf J, Hofmann R (2002) TGFβ1 in patients with renal cell carcinoma. Urol Res 30:126
12. Herman ME, Katzenellenbogen BS (1994) Alterations in transforming growth factor α and β production and cell responsiveness during the progression of MCF-7 human breast cancer cells to eostrogen–autonomous growth. Cancer Res 54:5876
13. Junker U, Knoefel B, Rebstock K, Steiner T, Wunderlich H, Junker K, Reinhold D (1996) Transforming growth factor beta 1 is significantly elevated in plasma of patients suffering from renal cell carcinoma. Cytokine 8:794
14. Junker U, Haufe CC, Nuske K, Rebstock K, Steiner T, Wunderlich H, Junker K, Reinhold D (2000) Elevated plasma TGFbeta1 in renal diseases: cause or consequence ? Cytokine 12:1084
15. Kerbel RS (1992) Expression of multi-cytokine resistance and multi-growth factor independence in advanced stage metastatic cancer. Malignant melanoma as a paradigm. Am J Pathol 141:519
16. Knoefel B, Nuske K, Steiner T, Junker K, Kosmehl H, Rebstock K, Reinhold D, Junker U (1997) Renal cell carcinomas produce IL-6, IL-10, IL-11 and TGF beta-1 in primary cultures and modulate T-lymphocyte blast formation. J Interferon Cytokine Res 17:95
17. Kundu SD, Kim IY, Zelner D, Janulis L, Goodwin S, Engel JD, Lee C (1998) Absence of expression of transforming growth factor-beta type II receptor is associated with an aggressive growth pattern in a murine renal cell carcinoma cell line, Renca. J Urol 160:1883
18. Laemli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680
19. Landis SH, Murray T, Bolden S, Wingo PA (1999) Cancer statistics 1999. CA Cancer J Clin 49:8
20. Lyons RM, Moses HL (1990) Transforming growth factors and the regulation of cell proliferation. Eur J Biochem 187:467
21. Maniatis T, Fritch EF and Sambrook KJ (1982) Detection and Analysis of proteins expressed from cloned genes. In: Maniatis T, Fritch EF, Sambrook KJ (eds) Molecular cloning: a laboratory manual. Cold Spring Harbor, New York, p 18.47
22. Maniatis T, Fritsch EF, Sambrook KJ (1982) Detection and analysis of proteins expressed from cloned genes. In: Maniatis T, Fritch EF, Sambrook KJ (eds) Molecular cloning: a laboratory manual. Cold Spring Harbor, New York, p 18.60
23. Markowitz SD, Roberts AB (1996) Tumor suppressor activity of the TGFβ pathway in human cancers. Cytokine Growth Factor Rev 7:93

24. Massague J (1990) The transforming growth factor β family. Annu Rev Cell Biol 6:597
25. Michel K, Tso CL, Mulders P, Kernion JD, Beldegrun A (1997) Expression of TGFβ1 in renal cell carcinoma (RCC); putative role of TGFβ as tumor infiltrating lymphocyte (TIL) suppressor. J Urol 157 [Suppl]:376
26. Miyazono K, Ichijo H and Heldin CH (1993) Transforming growth factor β: Latent forms, binding proteins and receptors. Growth Factors 8:11
27. Mulder KM, Ramey MR, Hoosen NM, Levine AE, Hinshaw XH, Brattain DE, Brattain MG (1988) Characterization of transforming growth factor-beta-resistant subclones isolated from a transforming growth factor-beta-sensitive human colon carcinoma cell line. Cancer Res 48:7120
28. Olive C, Cheung C, Nicol D, Falk MC (1998) Expression of cytokine mRNA transcripts in renal cell carcinoma. Immunol Cell Biol 76:357
29. Phillips AO, Steadman R, Topley N, Williams JD (1995) Elevated D-glucose concentrations modulate TGF-beta1 synthesis by human cultured renal proximal tubular cells .The permissive role of platelet-derived growth factor. Am J Pathol 147:362
30. Polyak K (1996) Negative regulation of cell growth by TGF beta. Biochim Biophys Acta 1242:185
31. Ramp U, Jaquet K, Reinecke P, Nitsch T, Gabbert HE, Gerharz CD (1997) Acquisition of TGF-beta 1 resistance: an important progression factor in human renal cell carcinoma. Lab Invest 76:739
32. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche WS, Wakefield LA, Heine UI, Liotta LA, Falanga V, Kehl JH, Fauci AS (1986) Transforming growth factor β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci U S A 83:4167
33. Sankar S, Mahooti–Brooks N, Bensen L, McCarthy TL, Centrella M, Madri JA (1996) Modulation of transforming growth factor beta receptor levels on microvascular endothelial cells during in vitro angiogenesis. J Clin Invest 97:1436
34. Sene AP, Hunt L, McMahon RF, Carroll RN (1992) Renal carcinoma in patients undergoing nephrectomy: analysis of survival and prognostic factors. Br J Urol 70:125
35. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Gocke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76
36. Storkel S, Eble J, Adlakha K, Amin M, Blute ML, Bostwick DG, Darson M, Delahunt B, Iczkowski K (1997) Classification of renal cell carcinoma: Workgroup No1, Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Cancer 80:987
37. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 76:4350
38. Van Brussel JP, Mickisch GH (1999) Prognostic factors in renal cell and bladder cancer. BJU Int 83:902
39. Wechsel HW, Bichler KH, Feil G, Loeser W, Lahme S, Petri E (1999) Renal cell carcinoma: relevance of angiogenetic factors. Anticancer Res 19:1537
40. Wunderlich H, Steiner T, Junker U, Knoefel B, Schlichter A, Schubert J (1996) Serum transforming growth factor β1 in patients with renal cell carcinoma. J Urol 157:1602
41. Wunderlich H, Steiner T, Kosmehl H, Junker U, Reinhold D, Reichelt O, Zermann DH, Schubert J (1998) Increased transforming growth factor beta 1 plasma level in patients with renal cell carcinoma: a tumor-specific marker? Urol Int 60:205