Metabolism
Clinical and Experimental

VOL 48, NO 9 SEPTEMBER 1999

Transforming Growth Factor Beta-1 and Beta-2 and Type II Receptor Functional Regulation of ALVA-101 Human Prostate Cancer Cells

A. Wayne Meikle, Richard E. Swope, Diana Y. Yin, Dan Fullmer, Steven M. Loop, and Darrell K. Murray

Transforming growth factor beta-1 (TGFβ-1) causes apoptosis of many epithelial cells, including the prostate, but other secondary effects of TGFβ-1 may be important in carcinogenesis. In a human prostate cancer cell line (ALVA-101), we determined the effects of TGFβ-1 and TGFβ type I and II receptor antibody on cell proliferation and TGFβ-1 receptor binding. TGFβ-1 and -2 and TGFβ type II receptor mRNA expression levels were determined by polymerase chain reaction (PCR) and Northern blot analysis. A dose-responsive suppression (0.03 to 10 ng/mL) was observed for cells treated with TGFβ-1 from 3 to 7 days ($P < .01$). Untreated cells had 1.1×10^3 (n = 3) TGFβ receptors per cell, with a K_d of 0.20 nmol/L (n = 3) as determined by Scatchard analysis; treatment for 3 days with TGFβ-1 (1 ng/mL) reduced the receptor number (0.9×10^3) and the K_d (0.12 nmol/L). Antibodies to TGFβ type I and II receptor stimulated proliferation with or without added TGFβ-1 (50% ± 5% above control, $P < .01$, n = 6). TGFβ-1 and -2 and TGFβ type II receptor mRNA expression was observed in untreated cells. In cells treated with TGFβ-1, TGFβ-1 mRNA was not affected by treatment, but expression levels of the TGFβ type II receptor and TGFβ-2 mRNA were moderately suppressed after 72 hours of treatment. Control cells actively produced TGFβ-1 as measured by radioimmunoassay. The active and inactive forms of TGFβ-1 were approximately equal, but TGFβ-2 was secreted in smaller quantities than TGFβ-1 and the inactive form of TGFβ-2 predominated, with very small amounts of the active form. Our results suggest that the human prostate cancer cell line ALVA-101 retains negative control of proliferation in response to TGFβ-1. Inhibition of endogenous TGFβ action by antibodies to its receptor enhances the growth of ALVA-101 human prostate cancer cells, suggesting that endogenous TGFβ exerts an inhibitory control on their growth and cellular function.

TRANSFORMING GROWTH FACTOR beta-1 (TGFβ-1) belongs to a family of growth factors that are biologically active as disulfide-linked dimers (25 kd).[1] It is produced in most cells and has widespread biological actions, including cell differentiation, growth promotion, and growth inhibition.[2-15] The expression of TGFβ-1 is higher in transformed versus nontransformed cells, and transformed cells produce more of the active form of TGFβ-1 than nontransformed cells.[16-18] As a stimulator of angiogenesis, TGFβ-1 may facilitate metastasis and tumor invasion.[6,13]

One of the multiple effects of TGFβs is their contribution to the programed cell death, or apoptosis, of epithelial cells, including the prostate.[19] In rat prostate and androgen-responsive human prostate cancer cells, androgen withdrawal results in an increase of TGFβ-1 production, which then inhibits cell growth and may function in apoptosis.[20] Re-treatment with androgen suppresses TGFβ-1 synthesis in prostate cells by decreasing mRNA, and cell growth increases.[21,22] TGFβ-1 not only suppresses the growth of androgen-responsive prostate cancer cell lines, but also some non–androgen-responsive cells. However, the escape of cancer cells from the growth-inhibitory effects of TGFβ-1 may be important in carcinogenesis.[21,22] This could occur because the cells lack TGFβ receptors or have paradoxical growth stimulation to TGFβ-1.

The growth influences of TGFβ-1 have been studied in several prostate cancer cell lines. PC-3[23] and DU145[24] are two non–androgen-responsive human prostate cancer cell lines that show growth inhibition by TGFβ-1,[25] as well as the Dunning R3327G rat adenocarcinoma cell line, which is androgen-responsive.[26] Colony formation of LNCaP, an androgen-responsive cell line,[27-29] is stimulated by TGFβ in vitro. Prostate cancer cell clones resistant to the growth-inhibitory effects of TGFβ-1 grow faster in vitro and in vivo and synthesize more of the active form of TGFβ-1 than clones inhibited by TGFβ-1.

From the Department of Medicine and Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Submitted June 3, 1998; accepted March 5, 1999

Supported in part by National Institutes of Health Grants No. R01 DK-45760 and DK-43344.

Address reprint requests to A. Wayne Meikle, MD, University of Utah School of Medicine, Division of Endocrinology, Salt Lake City, UT 84132.

0026-0495/99/4809-0001$10.00/0

Kim et al[30] have also shown that the LNCaP cell line is stimulated by androgen and androgen treatment of the cells enhances the growth suppression TGFβ-1 treatment. Therefore, androgen responsiveness does not predict growth responsiveness to TGFβ-1.

Most reports related to the influence of TGFβ-1 on prostate cancer have investigated the effects of TGFβ-1 on cell growth, but little is known about the functional regulatory actions of TGFβ-1 on prostate cancer cells. The human prostate cancer cell line ALVA-101 was selected for investigation because it is modestly androgen-sensitive,[31] and preliminary study suggested some resistance to the growth-inhibitory effects of TGFβ-1.

Our objectives were to determine the effects of TGFβ-1 on (1) the proliferation of ALVA-101 cells, (2) the binding of TGFβ-1 to its receptor, (3) the mRNA expression of TGFβ-1 and -2 and TGFβ type II receptor, and (4) the secretion of latent (inactive) and active forms of TGFβ-1 and -2.

MATERIALS AND METHODS

Cell Culture

The human prostate epithelial cancer cell line, ALVA-101 was isolated by Steve Loop (American Lake Veterans Administration Hospital, Tacoma, WA) and used for all experiments in this study. The cells were grown in RPMI 1640 medium supplemented with 5% fetal bovine calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin, and the medium was changed three times per week. The culture was maintained at 37°C with an atmosphere supplemented with 5% CO_2. The cells were split among 75-cm^2 flasks (25,000 cells/mL), multiwells (24 wells at 4×10^4 cells/well and 96 wells at 1,000 cells/well), and 100-mm plates. They were allowed to stabilize for 2 to 3 days after splitting. They were then treated in experiments as noted in the Results.

Cell growth was determined using three different methods in preliminary experiments. Cell proliferation was determined by direct cell counting (model ZM; Beckman Coulter, Fullerton, CA), ^{3}H-thymidine incorporation (5 μCi/mL for 2 hours), and Cell Titer 96 AQ assay (Promega, Madison, WI). All methods produced similar results (data not shown); for these studies, the results of the Cell Titer 96 AQ assay method are reported. This titer assay is a nonradioactive procedure for measuring metabolic function that directly correlates with cell number.[32] It measures dehydrogenase activity converting MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxymethoxphenyl)-2-(4-sulfophenyl)-2H-tetrolium, inner salt]) tetrazolium into formazan, and this product is determined by measuring absorbance at 490 nm compared with controls. Absorbance was measured using an enzyme-linked immunosorbent assay plate reader (Vmax Kinetic Microplate Reader; Molecular Devices, Menlo Park, CA). Cells were plated in 96-well plates at 1,000 cells/well in 200 μL medium, treated, and then assayed.

TGFβ-1 was purchased from Genzyme (Cambridge, MA) and fetal bovine calf serum from Hyclone (Logan, UT). Anti–TGFβ receptor type I (SC-399), a polyclonal antibody corresponding to amino acids 482 to 501 at the carboxy terminus of TGFβ (RI), and anti–TGFβ receptor type II (SC-400), a polyclonal antibody corresponding to amino acids 246 to 266 of TGFβ (RII), were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Native TGFβ production was determined in confluent ALVA-101 cells cultured in RPMI media without growth factors (serum-free) for 48 hours in 24-well plates. TGFβ present in the supernatants was assayed using the mink lung epithelial cell (MV 1Lu; American Type Culture Collection [ATCC], Rockville, MD) assay.[33] Following incubation, the conditioned RPMI media were removed from the ALVA-101 cells and then incubated with TGFβ-1–sensitive mink lung cells for 24 hours. ^{3}H-thymidine was then added and incubated for 6 hours. Cell growth of the mink lung cells was determined by thymidine uptake in treated (ALVA-101 cells) and untreated groups. Increased amounts of TGFβ-1 in the conditioned media reduced mink lung cell proliferation.

A second and more specific measurement of cellular production of TGFβ-1 and TGFβ-2 was performed using a commercial assay method (Quantikine; R&D Systems, Minneapolis, MN) on cells grown in 96-well plates. Active and latent forms of TGFβ were assayed. First, the active form was measured, and then the latent form was converted to the active form by treating culture supernatants with 10N HCl (final pH, 1.5 to 2.0) for 30 minutes at room temperature, followed by neutralization with equimolar NaOH.[34]

TGF binding was assayed using ALVA-101 cells cultured in 24-well culture dishes and grown until they were 70% to 80% confluent (about 4 days). The experimental groups were treated with TGFβ-1 (1 ng/mL) for 72 hours, and control groups were treated with phosphate-buffered saline (PBS). Before the assay, the cells were washed twice with 1 mL TGFβ-1 binding buffer (Dulbecco's modified Eagle's medium containing 0.1% wt/vol bovine serum albumin [BSA] and 25 mmol/L HEPES buffer, pH 7.4) to remove excess TGFβ-1.[35] For the TGFβ receptor assay, 200 μL assay medium containing ^{125}I-TGFβ-1 (0.02 to 0.2 μCi/well) and 100- to 500-fold unlabeled TGFβ-1 for determination of nonspecific binding were added to the appropriate wells after 4-hour incubations at room temperature. Unbound TGFβ-1 was removed by washing three times with ice-cold PBS containing 1% BSA. Bound TGFβ-1 was solubilized by incubation with 750 μL buffer containing 20 mmol/L HEPES, 1% Triton X-100, and 10% glycerol (pH 7.4) for 30 minutes at 37°C.[36] Aliquots (0.5 mL) were then counted on a Cobra Auto-Gamma counter (Packard Instrument, Meriden, CT). Nonspecific binding was calculated by determining cell-associated radioactivity in the presence of a 1,000-fold molar excess of unlabeled TGFβ-1. The nonspecific binding was much less than 5% of the total labeled ligand added and was subtracted from total counts to calculate specific binding. Binding data were analyzed according to the method of Scatchard.[37] ^{125}I-TGFβ-1 was obtained from Amersham (Arlington Heights, IL).

Reverse Transcription/Polymerase Chain Reaction Analysis

Reverse transcription/polymerase chain reaction (PCR) analysis was used to evaluate the presence of TGFβ-1 and -2 and TGFβ receptor type II expression in ALVA-101 prostate cancer cells from 10 minutes to 24 hours after TGFβ-1 (1 ng/mL) treatment. Total RNA was extracted using the Tri-Reagent RNA isolation protocol (Molecular Research Center, Cincinnati, OH) followed by removal of contamination DNA[38] using RNase-free DNase I (Boehringer Mannheim, Indianapolis, IN). The primers were synthesized by the DNA/Peptide Facility, Huntsman Cancer Institute (University of Utah, Salt Lake City, UT) and produced a 435–base pair (bp) fragment derived from nucleotides 1573 to 2006 of the HSTGFβ-1 mRNA[39] and a 455-bp fragment derived from nucleotides 609 to 1061 of the HUMTGFβIIR mRNA.[39] Reverse transcription and PCR were performed using the RNA PCR kit (808-0017) from Perkin Elmer (Branchberg, NJ).

Northern Blot Analysis

Northern blots were used to quantify the expression of TGFβ-1 and -2 and TGFβ receptor type II. The concentration of RNA was determined spectrophotometrically at 260 nm. Total RNA (25 to 30 μg per lane) was size-fractionated on a 1.0% agarose-formaldehyde gel. The RNA was transferred to nitrocellulose using the Turboblotter-Downward Transfer System (Schleicher and Schuell, Keene, NH), cross-linked (UV Stratalinker 1800; Stratagene, La Jolla, CA), and then incubated at 42°C for 4 hours in prehybridization mixture (50× Denhardt solution, 100 μg/mL denatured salmon testes DNA, 50% vol/vol formamide, 0.1% sodium dodecyl sulfate [SDS], and 20%

vol/vol diethylpyrocarbonate [DEPC] H_2O). The membrane was then hybridized overnight at 42°C with a ^{32}P-labeled *Eco*RI 2.14-kilobase (kb) fragment of TGFβ-1 cDNA probe (ATCC), a 2.35-kb fragment of TGFβ II cDNA (ATCC), or a 455-bp fragment of TGFβ receptor type II cDNA probe from the above-mentioned PCR product (specific activity, 1.5 to 1.8 × 10^9 dpm/μg). After hybridization, the membrane was washed at room temperature in 1× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate)/0.1% SDS and placed in a PhosphorImager cassette (Molecular Dynamics, Sunnyvale, CA) for 4 hours. To standardize the RNA load, the membrane was sequentially hybridized with a β-actin cDNA probe 1.1-kb insert from the ATCC. The amount of specific RNA was measured using the Molecular Dynamics PhosphorImager.

Statistical Analysis

Statistical analyses included ANOVA or Student's *t* test to determine statistically significant differences at a *P* level of .05 or less. Data are expressed as the mean ± SEM.

RESULTS

TGFβ-1 Effects on Cell Proliferation

The dose-response effects of TGFβ-1 (0.01 to 10 ng/mL) on ALVA-101 cell proliferation were determined from 1 to 7 days of treatment (Fig 1A). Doses of 0.3 ng/mL or greater significantly ($P < .05$, n = 9) slowed cell growth at intervals between 3 and 5 days. Since 1 ng/mL produced comparable effects versus the higher doses, other experiments were performed with this dose.

Figure 1B shows various incubation periods from 1 to 7 days using 1 ng/mL TGFβ-1, and indicates that significant inhibition ($P < .05$, n = 8) occurred by 3 days of treatment.

Effects of Antibodies to the TGFβ Type I and II receptor

Antibodies to the TGFβ type I and II receptor were used to investigate whether endogenously produced TGFβs might affect cell proliferation by blocking their growth-inhibitory influences. Figure 2 shows the effects of 3 days of treatment with antibody to TGFβ type I and II receptor. Both type I and II TGFβ antibody treatment ($P < .01$, n = 36) for 24 and 48 hours increased proliferation to approximately 50% above the control value without antibody, and TGFβ-1 treatment had no influence on the responses to the antibodies (data not shown). These results suggest that ALVA-101 cells can be stimulated to grow by blocking the binding of TGFβs to the TGFβ type I and II receptors. The lack of growth at 72 hours may be due to a reduction of the antibody concentration to the point that endogenous production of TGFβ is not sufficiently neutralized.

TGFβ-1 Receptor Binding

We determined if these cells have high- and low-affinity receptor binding of exogenous radiolabeled TGFβ-1. Scatchard analyses of TGFβ-1 binding to ALVA-101 cells are shown in Fig 3. TGFβ-1 treatment (n = 3) for 3 days modestly affected the binding constant or binding sites of the cells. The function of active binding sites for TGFβ-1 can be blocked by TGFβ type I and II receptor antibodies (Fig 2). These antibodies compete with endogenous TGFβ-1 for the available binding sites and thereby block the inhibitory effects of TGFβ-1 (data for endogenous TGFβ are reported in Fig 7).

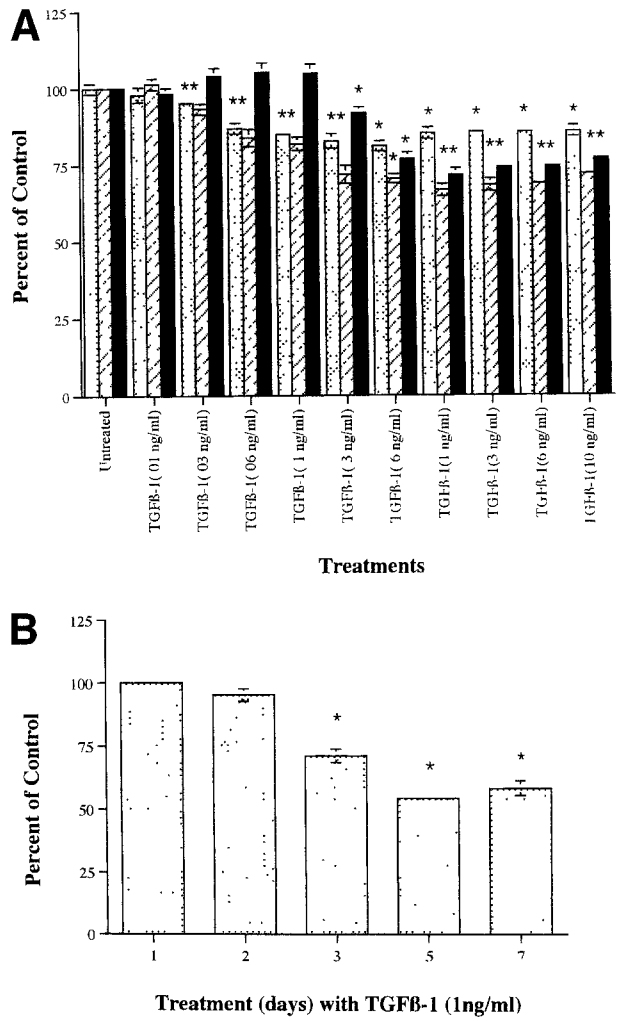

Fig 1. ALVA-101 human prostate epithelial cancer cells monitored for cell growth using a metabolic assay to determine cell numbers. (A) Cells treated at various time intervals (3 ▨, 5 ▨, and 7 ■ days) with different TGFβ-1 concentrations (0.01-10 μg/mL). Data are compared against control values set at a 100% growth rate. TGFβ-1 at 1 ng/mL significantly reduced cell growth for all 3 incubation times (*$P < .05$, mean ± SE, n = 8). (B) Cells treated with 1 ng/mL TGFβ-1 for 1 to 7 days. The growth rate was set at 100% under normal growing conditions, and this rate was compared following treated conditions. Cell growth was significantly reduced by TGFβ-1 treatment at days 3, 5, and 7 (*$P < .05$ or less, n = 8). All columns have standard errors applied; however, some have such low values that the error bar is not shown.

Effects of TGFβ-1 on mRNA of TGFβ-1 and -2 and Its Type II Receptor

Since TGFβ-1 inhibited cell growth and antibodies to the TGFβ receptor stimulated growth, the results suggested that TGFβ-1 had functional control in regulating either TGFβ-1 and -2 formation or TGFβ type I or II receptor. We then evaluated the effects of TGFβ-1 treatment (1 ng/mL) on TGFβ-1 mRNA (Fig 4). It was expressed in untreated ALVA-101 cells, and its expression remained constant for 72 hours of observation following treatment with TGFβ-1. This suggests that TGFβ-1 does not regulate its own message. The message for TGFβ-2 and TGFβ type II receptor was also expressed in untreated

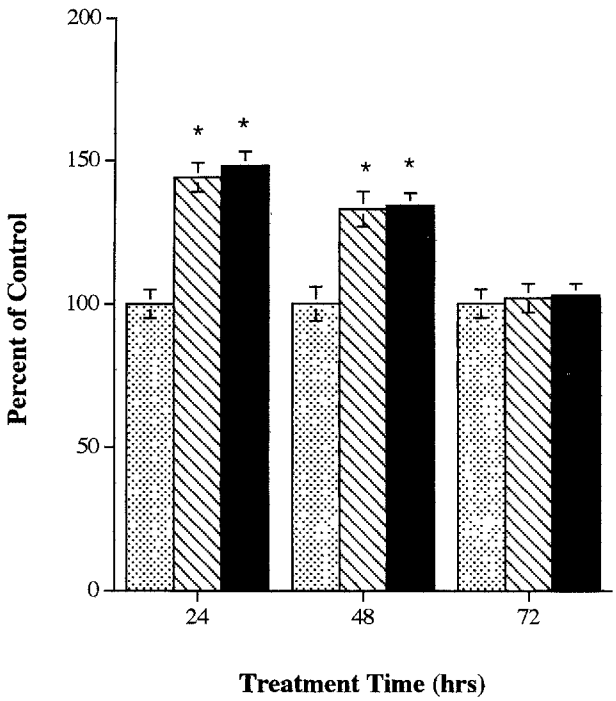

Fig 2. ALVA-101 prostate epithelial cancer cell growth determined as outlined in Fig 1. The growth rate was set at 100% under normal growing conditions, and this rate was compared following treated conditions. Cells were treated with and without antibody against TGFβ receptor I (anti TGFβ-I, ▨) and antibody against TGFβ receptor II (anti TGFβ II, ■) for 24, 48, and 72 hours at a concentration of 1 μg/mL. (▩) Control. Cell growth was significantly increased by these treatments at 24 and 48 hours (*$P < .05$ or less, mean ± SE, n = 36), but not at 72 hours. TGFβ-1 treatment had no influence on the response to the antibodies (data not shown). All columns have standard errors applied; however, some have such low values that the error bar is not shown.

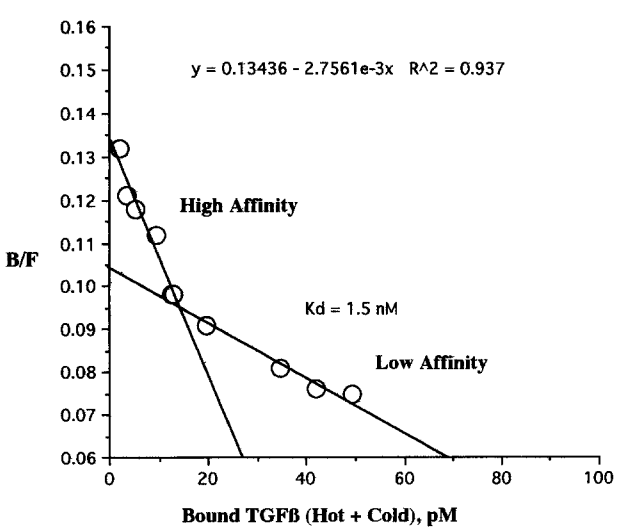

Fig 3. ALVA-101 prostate epithelial cancer cells evaluated for TGFβ-1 binding by Scatchard analysis. The horizontal axis represents bound TGFβ (pmol/L), and the vertical axis represents the bound/free (B/F) ratio. Both high and low binding affinities (K_d) are noted.

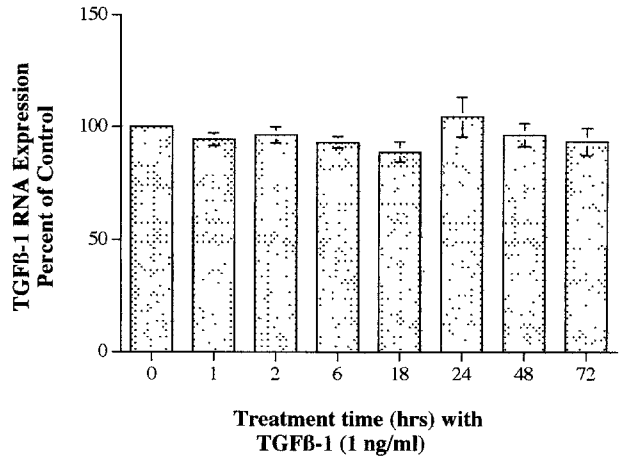

Fig 4. ALVA-101 prostate epithelial cancer cells evaluated for TGFβ-1 mRNA expression following treatment with TGFβ-1 (1 ng/mL). Expression was determined using Northern blot analysis, and no significant changes were noted from 0 to 72 hours of treatment (n = 12, mean ± SEM).

ALVA-101 cells (Figs 5 and 6). However, both TGFβ-2 and TGFβ receptor II mRNA decreased after treatment with TGFβ-1, indicating that TGFβ-1 treatment downregulates the mRNA of its receptor and TGFβ-2, and might explain the growth responses observed in treatment with TGFβ-1 and antibodies to the receptors.

Endogenous Formation of TGFβ-1 and -2

Native production of active and latent TGFβs was measured with the mink lung assay and by immunoassay to demonstrate that ALVA-101 cells produce and release TGFβs. After 3 days of incubation of ALVA-101 cells, the medium was removed and incubated with mink lung cells that are growth-sensitive to TGFβs. Compared with control medium without cell TGFβ-1,

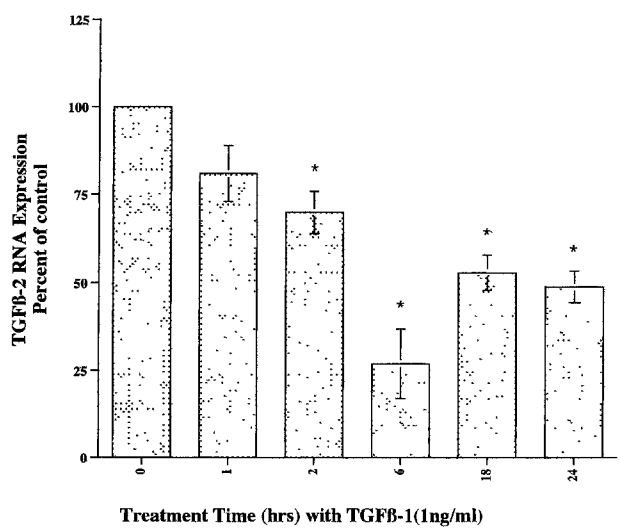

Fig 5. ALVA-101 prostate epithelial cancer cells evaluated for TGFβ-2 mRNA expression following treatment with TGFβ-1 (1 ng/mL). Expression was determined using Northern blot analysis, and a significant reduction was noted (n = 3, mean ± SEM, *$P > .05$ by ANOVA).

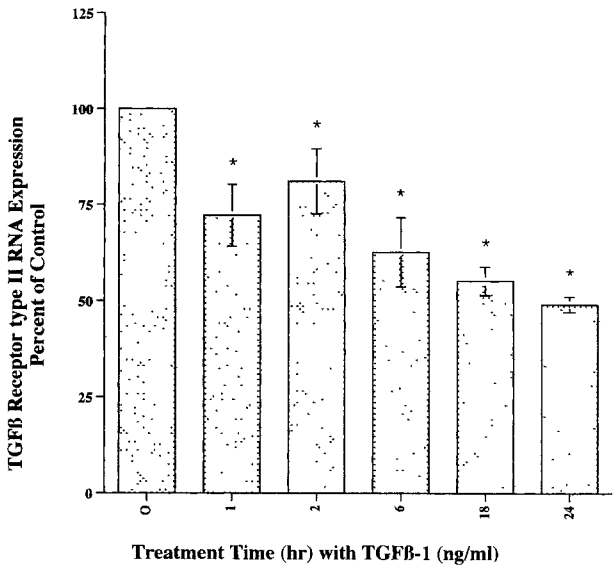

Fig 6. ALVA-101 prostate epithelial cancer cells evaluated for TGFβ receptor type II mRNA expression following treatment with TGFβ-1 (1 ng/mL). Expression was determined using Northern blot analysis, and a significant reduction was noted over the time course of treatment (n = 6, mean ± SEM, *$P > .05$). Experiments continued up to 72 hours, but no further decrease of RNA expression was noted.

mink lung cells demonstrated concentration-dependent growth suppression in response to the medium obtained from ALVA-101 cell incubations (data not shown), indicating that ALVA-101 cells produce biologically active TGFβs.

As determined by immunoassay, Fig 7A and B shows the total and active TGFβ-1 and -2 secreted into the media following treatment with TGFβ-1. Secretion of active TGFβ-1 was about 50% of the total quantity of TGFβ-1. In contrast, TGFβ-2 was predominately secreted in the inactive form and in lower quantities than TGFβ-1 (n = 4). Treatment with various concentrations of TGFβ-1 had minimal influence on the secretion of TGFβ-1 or -2 (data not shown).

DISCUSSION

TGFβ-1 is associated with apoptosis of the rat prostate.[21] The human prostate cancer cell line ALVA-101 is derived from an epithelial cell malignancy and is modestly androgen-responsive and therefore a good model to study the effects of TGFβs on the regulation of cell proliferation of a prostatic tumor. Our studies demonstrate for the first time that this cell line has high-affinity receptor binding for TGFβ-1 and mRNA expression of the growth factor and its receptor. Treatment with TGFβ-1 inhibits the growth of these cells, which have high-affinity binding receptors for TGFβ-1 as determined by Scatchard analysis. The cells produce both active and inactive forms of TGFβ-1 and -2 as measured by immunoassay and bioassay with mink lung cells. Since TGFβ-2 is produced in small quantities and TGFβ-1 does not downregulate its own message, the observation that TGFβ-1 downregulates mRNA for the TGFβ type II receptor may attenuate the growth-inhibitory effects of TGFβ-1.

Treatment of ALVA-101 cells with an antibody to the TGFβ type I and II receptor enhanced their growth. These findings may suggest that endogenous production of TGFβ growth factor(s) that bind to this receptor may partially restrain the growth of the cells. This observation is consistent with the growth suppression induced by exogenous treatment with TGFβ-1 and with the results showing that TGFβ-1 treatment reduces type II receptor mRNA.

TGFβ-1 had insignificant effects on its mRNA expression, but moderately reduced the message of TGFβ-2 and the type II receptor. When the receptor number or affinity were measured, TGFβ-1 treatment for only 3 days modestly reduced the receptor number or affinity. Longer treatment intervals may be needed to show a consistent relationship between the effects of TGFβ-1 treatment on surface receptor characteristics and numbers and mRNA expression of the type II receptor for

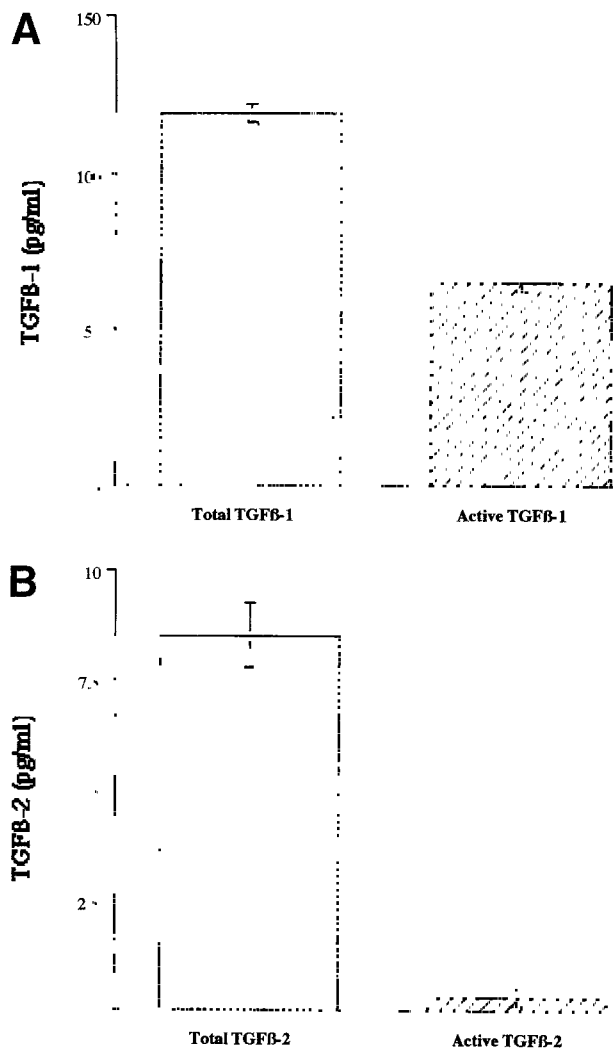

Fig 7. Native TGFβ-1 produced by untreated ALVA-101 prostate cancer cells and released into tissue culture media. TGFβ-1 was determined with an immunoassay that measures total and active forms of the growth factor. Substantial amounts of latent and active forms of TGFβ-1 are noted (mean ± SE, n = 4). (B) Native TGFβ-2 produced by untreated ALVA-101 prostate cancer cells and released into tissue culture media. TGFβ-2 was determined with an immunoassay that measures total and active forms of the growth factor. There were measurable amounts of the latent form of TGFβ-2, with little of the active form (mean ± SE, n = 4). Comparatively little active TGFβ-2 is produced in these cells compared with active TGFβ-1.

TGFβ. The cell-surface receptor expression may be slightly affected by treatment of the cells with TGFβ-1.

Our studies of ALVA-101 cells clearly demonstrate that they produce both TGFβ-1 and -2 and the TGFβ type II receptor. TGFβ-1 was produced in greater quantity than TGFβ-2 and substantial amounts of active TGFβ-1, but not TGFβ-2, were observed in the media, suggesting that any growth regulation of the cells is influenced by TGFβ-1 rather than TGFβ-2. However, despite reducing mRNA expression for TGFβ-2, TGFβ-2 production determined in the media of ALVA-101 cells was unaffected by treatment with TGFβ-1. The fact that antibodies to TGFβ receptors enhanced the growth of the cells suggests that TGFβ-1 production restrains but does not markedly suppress the growth of ALVA-101 prostate cancer cells.

Our results are consistent with the findings reported by Watt et al,[40] who observed that ALVA-101 cells produce an active form of TGFβ-1 as assessed with the mink lung assay. We also observed that ALVA-101 cells secrete biologically active TGFβs as assayed by the mink lung bioassay. In this system, antibody to TGFβ-1 inhibited the effects of TGFβ produced by ALVA-101 cells. Kim et al[30] showed that androgen treatment enhanced the growth-inhibitory response of TGFβ in another human prostate cancer cell line, LNCaP. Whether androgens have similar influences in ALVA-101 cells is unknown. However, androgens are known to suppress endogenous production of TGFβ and could therefore make the cells more sensitive to exogenous TGFβ.

TGFβ-1 also has been associated with the enhancement of prostate cancer growth in experimental animals. Studies by Barrack et al[10,41] showed that when a prostate cancer was transfected with TGFβ-1, growth of the cells increased in vitro and also in vivo following transplantation in nude mice. Their findings and the current results suggest that a partial avoidance of the growth-inhibitory effect of TGFβ-1 may give cells a growth advantage.

Few previous studies in human prostate cancer cells have included measurement of TGFβ-1 and -2 and type II TGFβ receptor mRNA, assayed secretion of these cytokines, or assessed the effects of TGFβ-1 on cell function as determined by molecular techniques.[16,20,25,28,42-46] Instead, the effects of these growth factors on the growth of the cells have been studied.

As demonstrated in our studies, quantitation of cell growth responses to these growth factors may require prolonged treatment periods to observe a growth influence. Further, the growth response to exogenous TGFβs does not predicate their potential functional effects on the TGFβ system or determine if endogenous TGFβ has growth-regulatory influences.

TGFβ has been demonstrated to be a potent growth inhibitor in human breast cancer cells in vitro.[47] In this cell system, TGFβ stimulates insulin-like growth factor (IGF) binding protein-3 (IGFBP-3) gene expression prior to inhibiting cell growth. IGFBP-3 was also shown to be inhibitory to human breast cancer cell growth. Because of the sequence of effects, it has been suggested that IGFBP-3 mediates the TGFβ inhibitory action. IGF-II and IGF-II analogs diminished TGFβ effects by blocking TGFβ-induced binding of IGFBP-3 to cell-surface receptors. IGFBP-3 was not evaluated in our cell system (ALVA-101) directly; however, we did incubate ALVA-101 cells with TGFβ and anti–epidermal growth factor receptor. These experiments did not alter TGFβ effects. These receptor interactions and mechanisms of action need to be investigated in detail in the ALVA-101 cell line in future studies.

In summary, ALVA-101 human prostate cancer cells predominately secrete TGFβ-1 compared with TGFβ-2. TGFβ-1 is secreted in approximately equal amounts as the active and inactive form, and mRNAs for TGFβ-1 and -2 and the type II receptor of TGFβ are expressed in ALVA-101 cells. Antibodies to the type I and II receptor stimulate cell growth, and TGFβ-1 treatment partially inhibits cell growth. It remains to be determined if these cells are less sensitive to the growth-inhibitory effects of the TGFβ system than normal cells. If they are less sensitive, this could give them a growth advantage over normal prostate cells and provide endogenous production of a growth factor endowing them with metastatic potential.

REFERENCES

1. Centrella M, Horowitz MC, Wozney JM, et al: Transforming growth factor-beta gene family members and bone. Endocr Rev 15:27-39, 1994

2. Iseki S, Osumi-Yamashita N, Miyazono K, et al: Localization of transforming growth factor-beta type I and type II receptors in mouse development. Exp Cell Res 219:339-347, 1995

3. Thesleff I, Vaahtokari A, Partanen AM: Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and other organs. Int J Dev Biol 39:35-50, 1995

4. Wall NA, Hogan BL: TGF-beta related genes in development. Curr Opin Genet Dev 4:517-522, 1994

5. Harris SE, Harris MA, Mahy P, et al: Expression of bone morphogenetic protein messenger RNAs by normal rat and human prostate and prostate cancer cells. Prostate 24:204-211, 1994

6. Battegay EJ: Angiogenesis: Mechanistic insights, neovascular diseases, and therapeutic prospects. J Mol Med 73:333-346, 1995

7. Yoshikawa H, Takaoka K: Bone remodeling and its regulators. Nippon Rinsho 52:2262-2267, 1994

8. Adzick NS, Lorenz HP: Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Ann Surg 220:10-18, 1994

9. Gressner AM: Activation of proteoglycan synthesis in injured liver—A brief review of molecular and cellular aspects. Eur J Clin Chem Clin Biochem 32:225-237, 1994

10. Morton DM, Barrack ER: Modulation of transforming growth factor beta 1 effects on prostate cancer cell proliferation by growth factors and extracellular matrix. Cancer Res 55:2596-2602, 1995

11. Timme TL, Truong LD, Slawin KM, et al: Mesenchymal-epithelial interactions and transforming growth factor-beta 1 expression during normal and abnormal prostatic growth. Microsc Res Tech 30:333-341, 1995

12. Cox DA: Transforming growth factor-beta 3. Cell Biol Int 19:357-371, 1995

13. Sunderkotter C, Steinbrink K, Goebeler M, et al: Macrophages and angiogenesis. J Leukoc Biol 55:410-422, 1994

14. Floege J, Eng E, Young BA, et al: Factors involved in the regulation of mesangial cell proliferation in vitro and in vivo. Kidney Int Suppl 39:47-54, 1993

15. Sunderkotter C, Goebeler M, Schulze Osthoff K, et al: Macrophage-derived angiogenesis factors. Pharmacol Ther 51:195-216, 1991

16. Merz VW, Arnold AM, Studer UE: Differential expression of transforming growth factor-beta 1 and beta 3 as well as c-*fos* mRNA in normal human prostate, benign prostatic hyperplasia and prostatic cancer. World J Urol 12:96-98, 1994

17. Eastham JA, Truong LD, Rogers E, et al: Transforming growth factor-beta 1: Comparative immunohistochemical localization in human primary and metastatic prostate cancer. Lab Invest 73:628-635, 1995

18. Glynne Jones E, Harper ME, Goddard L, et al: Transforming growth factor beta 1 expression in benign and malignant prostatic tumors. Prostate 25:210-218, 1994

19. Kyprianou N, Bains AK, Jacobs SC: Induction of apoptosis in androgen-independent human prostate cancer cells undergoing thymineless death. Prostate 25:66-75, 1994

20. Kyprianou N, Tu H, Jacobs SC: Apoptotic versus proliferative activities in human benign prostatic hyperplasia. Hum Pathol 27:668-675, 1996

21. Furuya Y, Isaacs JT: Differential gene regulation during programmed death (apoptosis) versus proliferation of prostatic glandular cells induced by androgen manipulation. Endocrinology 133:2660-2666, 1993

22. Furuya Y, Lin XS, Walsh JC, et al: Androgen ablation–induced programmed death of prostatic glandular cells does not involve recruitment into a defective cell cycle or p53 induction. Endocrinology 136:1898-1906, 1995

23. Franzen P, Ichijo H, Miyazono K: Different signals mediate transforming growth factor-beta 1–induced growth inhibition and extracellular matrix production in prostatic carcinoma cells. Exp Cell Res 207:1-7, 1993

24. Lokeshwar BL, Block NL: Isolation of a prostate carcinoma cell proliferation–inhibiting factor from human seminal plasma and its similarity to transforming growth factor beta. Cancer Res 52:5821-5825, 1992

25. Kim IY, Ahn HJ, Zelner DJ, et al: Genetic change in transforming growth factor beta (TGF-beta) receptor type I gene correlates with insensitivity to TGF-beta 1 in human prostate cancer cells. Cancer Res 56:44-48, 1996

26. Santen RJ, Mowszowicz I, Portois MC, et al: Androgen dependence of the Dunning R3327G cell line in monolayer culture. Prostate 11:377-387, 1987

27. Henttu P, Vihko P: Growth factor regulation of gene expression in the human prostatic carcinoma cell line LNCaP. Cancer Res 53:1051-1058, 1993

28. Hara Y: Analysis of anchorage independent growth of human prostate cancer cell line LNCaP. Nippon Hinyokika Gakkai Zasshi 83:1429-1435, 1992

29. Goldstein D, O'Leary M, Mitchen J, et al Effects of interferon beta ser and transforming growth factor beta on prostatic cell lines. J Urol 146:1173-1177, 1991

30. Kim IY, Zelner DJ, Sensibar JA, et al: Modulation of sensitivity to transforming growth factor-beta 1 (TGF-beta 1) and the level of type II TGF-beta receptor in LNCaP cells by dihydrotestosterone. Exp Cell Res 222:103-110, 1996

31. Liu X, Wiley HS, Meikle AW. Androgens regulate proliferation of human prostate cancer cells in culture by increasing transforming growth factor-alpha (TGF-alpha) and epidermal growth factor (EGF)/TGF-alpha receptor. J Clin Endocrinol Metab 77:1472-1478, 1993

32. Mosmann T: Rapid colorimetric assay for cellular growth and survival. Application to proliferation and cytotoxicity assays. J Immunol Methods 65:55-63, 1983

33. Kooistra A, van den Eijnden-van Raaij AJ, Klaij IA, et al: Stromal inhibition of prostatic epithelial cell proliferation not mediated by transforming growth factor beta. Br J Cancer 72:427-434, 1995

34. Lyons RM, Keski-Oja J, Moses HL. Proteolytic activation of latent transforming growth factor-beta from fibroblast-conditioned medium. J Cell Biol 106:1659-1665, 1988

35. Frolik CA, Wakefield LM, Smith DM, et al. Characterization of a membrane receptor for transforming growth factor-beta in normal rat kidney fibroblasts. J Biol Chem 259:10995-11000, 1984

36. Wade TP, Kasid A, Stein CA, et al: Suramin interference with transforming growth factor-beta inhibition of human renal cell carcinoma in culture. J Surg Res 53:195-198, 1992

37. Scatchard G: The attraction of protein for small molecules and ions. Ann NY Acad Sci 51:660-672, 1949

38. Gilman M: Preparation and analysis of RNA, in Ausubel FM, Brent R, Kingston RE, et al (eds): Current Protocols in Molecular Biology. New York, NY, Wiley, p 4.1.4

39. Lin HY, Wang XF, Ng-Eaton E, et al: Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase [published erratum appears in Cell 70:1068, 1992. Cell 68.775-785, 1992

40. Watt R, Ware JL, Levy J, et al: Transforming growth factor beta 1 regulation of human prostate cancer. Endocr Soc 74:1046A, 1992 (abstr)

41. Steiner MS, Barrack ER. Transforming growth factor-B1 overproduction in prostate cancer: Effects on growth in vivo and in vitro. Mol Endocrinol 6:15-25, 1992

42. Story MT, Baeten LA, Molter MA, et al: Influence of androgen and transforming growth factor beta on basic fibroblast growth factor levels in human prostate–derived fibroblast cell cultures. J Urol 143:241A, 1990 (abstr)

43. Wilding G, Zugneier G, Knabbe C, et al: Differential effects of transforming growth factor beta on human prostate cancer cells in vitro. Mol Cell Endocrinol 62:79-87, 1989

44. Muir GH, Butta A, Shearer RJ, et al: Induction of transforming growth factor beta in hormonally treated human prostate cancer. Br J Cancer 69:130-134, 1994

45. Story M, Hopp K, Meier D, et al: Influence of transforming growth factor beta 1 and other growth factors on basic fibroblast growth factor level and proliferation of cultured human prostate–derived fibroblasts. Prostate 22:183-197, 1993

46. Sokoloff MH, Tso CL, Kaboo R, et al: In vitro modulation of tumor progression–associated properties of hormone refractory prostate carcinoma cell lines by cytokines. Cancer 77:1862-1872, 1996

47. Oh Y, Muller HL, Ng L, et al: Transforming growth factor-beta–induced cell growth inhibition in human breast cancer cells is mediated through insulin-like growth factor–binding protein-3 action. J Biol Chem 270:13589-13592, 1995