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Alterations of transforming growth factor β1 (TGF-β1) and TGFβ receptor expressions with progression in Dunning rat prostatic adenocarcinoma sublines

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Abstract Transforming growth factor-β1 (TGF-β1) inhibits epithelial cell proliferation in the normal prostate. Prostate tumours express high levels of TGF-β1, and seem to acquire resistance to its anti-proliferative effects with tumour progression. In this study, TGFβ variations with tumour progression were examined in the Dunning prostatic adenocarcinoma model. Expression of TGF-β1 and TGFβ receptor type I and type II (TGFβ-RI and TGFβ-RII) in rat dorsolateral prostate (DLP) and Dunning tumour sublines (PAP, AT-1, AT-2, AT-3 and MatLyLu) was examined in vitro and in vivo, using competitive reverse transcription-polymerase chain reaction (RT-PCR), Northern and Western blot, and immunohistochemistry. All tumours expressed elevated levels of TGF-β1 and TGFβ-RI mRNA, when compared with the DLP ($P \leq 0.05$). All tumours except MatLyLu also expressed elevated levels of TGFβ-RII mRNA ($P \leq 0.05$). Interestingly, TGFβ-RII protein levels were very low in the highly metastatic AT-3 and MatLyLu tumours in vivo, when compared with levels in the PAP, AT-1, and AT-2 tumours. This difference was not detected for the AT-1, AT-2, and AT-3 cells in vitro. Immunostaining of TGF-β1, TGFβ-RI, and TGFβ-RII was localised principally in normal and tumour epithelial cells, and occasionally in smooth muscle cells. In conclusion, high expression of TGF-β1 and TGFβ-RI and low expression of TGFβ-RII may contribute to tumour progression and metastasis in the Dunning prostatic adenocarcinoma model.

Key words Prostatic adenocarcinoma · TGF-β1 · TGFβ receptors · mRNA · Protein

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Introduction

Transforming growth factor-β1 (TGF-β1) is a multifunctional polypeptide, currently referred to as an inhibitor of epithelial cell growth [29]. In normal rat and human prostate, TGF-β1 inhibits epithelial cell proliferation and induces apoptosis [6, 23, 25, 37]. Paradoxically, prostate tumour cells express elevated levels of TGF-β1, when compared with their normal counterparts [35, 39], and tumour overproduction of TGF-β1 has recently been associated with poor clinical outcome in human prostate cancer [45]. Moreover, prostate tumour cells often show reduced sensitivity to the anti-proliferative effects of TGF-β1 both in vitro and in vivo [34, 46]. Steiner et al. [33] have shown that Dunning R3327 rat prostatic adenocarcinoma cells seem to acquire resistance to TGF-β1 growth inhibition with tumour progression. The slow-growing, androgen-sensitive, and moderately differentiated Dunning G subline is growth inhibited by TGF-β1 in vitro, while the fast-growing, androgen-insensitive, and anaplastic AT-2 and MatLyLu sublines are not. Taken together, these results indicate discrepancies between TGF-β1 effects in normal and cancerous prostatic cells.

The effects of TGF-β are mediated by membrane-bound serine-threonine kinase receptors of 53 and 70 kDa, TGF-β receptor I (TGFβ-RI) and TGFβ-RII [24]. Low expression or complete lack of these receptors has previously been described for several tumour systems [11, 14, 22, 30, 36]. The mechanism behind prostate tumour resistance to TGF-β1 growth inhibition is largely unknown, but may be due to alterations in the TGFβ receptor signalling pathway. Epithelial cells in human prostate tumours were recently shown to express low levels of TGFβ-RI and TGFβ-RII [15, 18, 47], and loss of epithelial immunoreactivity for these receptors has been associated with short cancer-specific survival in human prostate cancer [19, 45]. However, it is not known if lack of TGFβ receptor immunoreactivity indicates transcriptional or translational downregulation of the receptors or,

possibly, gene mutations that make detection of the proteins impossible with the antibodies used. Speculatively, low expression or functional defects of the epithelial TGFβ receptors may abolish direct TGF-β1 inhibition of tumour proliferation, without affecting possible tumour-promoting properties of TGF-β1 such as stimulation of angiogenesis [31, 49], cell motility and adhesion [24], and suppression of immune responses [40]. In fact, overexpression of TGF-β1 in the metastatic Dunning MatLyLu tumour has been demonstrated to result in increased tumour growth and metastatic capacity [34]. Moreover, TGF-β1 has been related to angiogenesis and metastasis in human prostate cancer [10, 45].

In order to study TGFβ variations with prostate tumour progression, we decided to quantify and localise the TGF-β1, TGFβ-RI, and TGFβ-RII expression in Dunning rat adenocarcinoma sublines. The tumour sublines were chosen to represent different prostate tumour grades, and included Dunning R3327 PAP (slow-growing, androgen-sensitive, well-differentiated, and non-metastatic [20]), AT-1 and AT-2 (fast growing, androgen-insensitive, and anaplastic with low and moderate metastatic capacity, respectively [16]), and AT-3 and MatLyLu (fast growing, androgen-insensitive, anaplastic, and highly metastatic to lymph nodes and lungs [16]). Since the tumours originate from a spontaneous tumour in the rat dorsolateral prostate (DLP) [9], the TGFβ expression in the tumour sublines was compared to the TGFβ expression in the DLP.

Materials and methods

Tissue preparation

Small pieces of the well-differentiated, androgen-sensitive Dunning R3327 PAP rat prostatic adenocarcinoma, and of the anaplastic, androgen-insensitive, and metastatic Dunning MatLyLu tumour were subcutaneously transplanted into male Copenhagen × Fisher and Copenhagen rats, respectively. The anaplastic, androgen-insensitive AT-1 and AT-2 cells, and the metastatic AT-3 cells were grown in culture, before subcutaneous inoculation into male Copenhagen rats. Cell culturing conditions as well as transplantation and inoculation procedures were the same as previously described [16, 20]. "The principles of laboratory animal care" of the NIH were followed, as well as specific national laws. The PAP tumour subline originated from Dr N. Altman (Papanicolaou Cancer Research Institute, Miami, Fla.), and the AT-1, AT-2, AT-3 cell-lines and the MatLyLu tumour subline from Dr J.T. Isaacs (Johns Hopkins Hospital, Baltimore, Md.). The rats were housed in a controlled environment (25°C, 40%–60% humidity) on a 12 h light/12 h dark schedule with free access to water and pelleted food. When the tumours had reached a size about 1–2 cm^3, the animals were killed by decapitation. The DLP and tumour tissues were quickly removed, frozen in liquid nitrogen, and stored at −70°C. Some frozen pieces were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight, before being embedded in paraffin and subjected to immunohistochemical analysis.

Total RNA preparation and competitive reverse transcription-polymerase chain reaction (RT-PCR)

The TRIzol extraction method (Life Technologies, Täby, Sweden) was used to isolate total RNA from frozen prostatic tissues and from AT-1, AT-2 and AT-3 cells, grown to about 75% confluence in vitro. Total RNA concentrations were determined by spectrophotometer counting. RNA concentration and integrity were ensured by ethidiumbromide staining after agarose gel electrophoresis (according to standard procedures).

The mRNA levels for TGF-β1, TGFβ-RI, TGFβ-RII, and for the housekeeping gene cyclophilin [7] was quantified, by using competitive RT-PCR and PCR primers as previously described [21, 44]. Briefly, 100 ng of total RNA was reverse transcribed together with appropriate amounts of internal RNA standards (IS) [21, 44] for TGF-β1, TGFβ-RI, TGFβ-RII and cyclophilin. Each RNA sample was titrated with three amounts (double samples) of IS, in the range of 8.5–170 and 17–340 amol for TGF-β1, 0.021–0.42 and 0.10–1.0 amol for TGFβ-RI, 0.46–4.6 amol for TGFβ-RII, and 70–1400 amol for cyclophilin (DLP and tumour samples, respectively). After RT completion, the samples were devided into four PCR tubes, in order to amplify cDNA for TGF-β1 (248 bp), TGFβ-RI (177 bp), TGFβ-RII (215 bp), and cyclophilin (362 bp) separately. During 30 PCR cycles (95°C, 30 s; 59°C, 30 s; 72°C, 45 s), the TGFβ templates were competitively amplified with cDNA for their corresponding IS (266, 163, 199 and 315 bp, respectively). Resulting PCR products were analysed in an automatic laser fluorescence system (ABI PRISM 377 DNA sequencer, Perkin Elmer). The data was processed by the ABI PRISM GeneScan software (Perkin Elmer), and RNA levels were calculated from template to IS cDNA ratios, as previously described [21].

Northern blot

Total RNA (20 μg/well) was size fractionated through 1% agarose gel electrophoresis and transferred onto nylon membranes (Hybond-N, Amersham Sweden AB, Solna, Sweden), according to standard procedures [3]. TGFβ-RII, cyclophilin, and actin probes were synthesised due to PCR amplification of the TGFβ-RII- and cyclophilin-IS cDNA fragments [31], as well as of an actin cDNA fragment of 162 bp [21], that had been inserted into the pCRII vector (Invitrogen, BV, Leek, The Netherlands). The PCR reactions were performed in the presence of 75 μCi [α-32P]dCTP (3000 Ci/mmol, Amersham Sweden), as previously described [26]. The radiolabelled probes were purified by using QIAquick PCR Purification Kit (Stratagene, La Jolla, Calif.), and counts were determined with a Beckman LS 1801 counter. Filters were prehybridised for 20 min in Quickhyb solution (Stratagene), hybridised for 3 h with 1.25×10^6 CPM/ml probe, and washed according to the manufacturer's instructions. Thereafter, filters were exposed to autoradiography films (Hyperfilm-MP, Amersham Sweden) for 5–7 days. The filter used for TGFβ-RII detection was boiled in 0.1 × standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), before rehybridisation with the cyclophilin and actin probes.

Protein extraction and Western Blot

Frozen prostatic tissue specimens were cut into small pieces and transferred to an extraction buffer containing 0.5% NP-40, 0.5% NaDOC, 0.1% SDS, 50 mM TRIS-HCl; pH = 7.5, 150 mM NaCl, 1 mM NaF, and Complete Protease Inhibitor Cocktail (Boehringer Mannheim, Bromma, Sweden). AT-1, AT-2 and AT-3 cells, grown to about 75% confluence in vitro, were resuspended in the same buffer after isolated by scraping and centrifugation (2 × 5 min, 1200 rpm) in PBS. Samples were incubated on ice, with constant shaking for 30 min, and then sonicated for 2 × 10 s. After another incubation on ice, samples were centrifuged for 15 min (13 000 *g*, 4°C), and resulting supernatants were recovered. Protein concentrations were determined with the D_c Protein Assay (Bio-Rad Laboratories, Sundbyberg, Sweden). Electrophoresis was carried out in 10% SDS-polyacrylamide gels, using 40 μg protein per lane. Fractionated proteins were electroblotted onto nitrocellulose membranes (Hybond ECL, Amersham, Sweden) in the Ancos semi-dry electroblotting system (Ancos Denmark, Höby, Denmark). Membranes were stained with 1% Ponceau-red, 0.5% acetic acid, in order to demonstrate equal loading of proteins,

before being blocked in 5% dry milk in PBS, 0.1% Tween 20 (PBST) for 3 h in room temperature. Incubations with primary antibodies were made for 1 h with dilutions of 1:1000 for the TGFβ-RI and TGFβ-RII antibodies (V-22 and L-21, respectively; Santa Cruz Biotechnology, Santa Cruz, Calif.), and 1:4000 for the actin antibody (Anti-actin, Boehringer Mannheim). Immunoreactions were detected with the ECL technique, according to the manufacturer's instructions (Amersham, Sweden). The specificity of the immunoreactions was examined by preincubation of TGFβ-RI and TGFβ-RII antibodies with 50-fold (w/w) excess of the corresponding control peptides (V-22P and L-21P, respectively, Santa Cruz Biotechnology). The filters used for TGFβ-RI and TGFβ-RII detection were washed in 0.0625 M TRIS; pH = 6.8, 2% SDS, 0.7% mercaptoethanol for 30 min in 52°C and in PBST for 4 × 5 min, before reincubation with the actin antibody. Fluorescence signals were quantified using densitometer scanning (personal Densitometer, Molecular Biosystems, Kebo Lab, Sweden).

Immunohistochemistry (IHC)

Paraffin sections (4 μm) were deparaffinated and rehydrated according to standard procedures, washed with PBS and heated in a microwave oven at 600 W for 2 × 7.5 min and 1 × 5 min in 0.01 M citrate buffer, pH = 6.0, as earlier described [38]. To quench endogenous peroxidase activity, slides were immersed in 1% H_2O_2 in methanol for 20 min. Unspecified binding was blocked in 5% normal goat serum (0.1% BSA in PBS) for 30 min prior to antibody incubations. Incubations with primary antibodies were made overnight, at 4°C, with dilutions of 1:200 for the TGF-β1 antibody (sc-146, Santa Cruz Biotechnology) and 1:1000 for the TGFβ-RI and TGFβ-RII antibodies (the same as in the Western blot experiments). Immunoreactions were detected with biotinylated secondary antibodies followed by the ABC technique (Vectastain ABC elite kit, Vector Laboratories, Burlingame, Calif.) with aminoethylcarbazole as chromogen, according to the manufacturer's instructions. Sections were counterstained with Mayer's hematoxylin solution. The specificity of the immunoreactions was examined as in the Western blot experiments. The sc-146P control peptide (Santa Cruz Biotechnology) was used to block the TGF-β1 antibody.

Statistical analysis

The statistical significance of differences between groups was analysed using the Mann-Whitney U-test. Data are presented as means with SEM. $P \leq 0.05$ was considered as statistically significant.

Results

Expression of TGFβ1, TGFβ-RI, and TGFβ-RII mRNA in rat DLP and Dunning sublines in vivo

By using competitive RT-PCR, TGF-β1, TGFβ-RI, TGFβ-RII, and cyclophilin mRNA were detected in the rat DLP and Dunning tumours (Fig. 1). The specificity of these RT-PCR reactions has previously been determined [21, 44]. The mRNA for the housekeeping gene cyclophilin, which was expected to be equally expressed in the tissues [7], was quantified with the aim of correcting the TGF-β1, TGFβ-RI and TGFβ-RII mRNA levels for RNA degradation, etc., as recently described [21, 44]. Surprisingly, the cyclophilin mRNA content was elevated in the anaplastic AT-1, AT-2, AT-3, and MatLyLu tumours, when compared with the normal DLP,

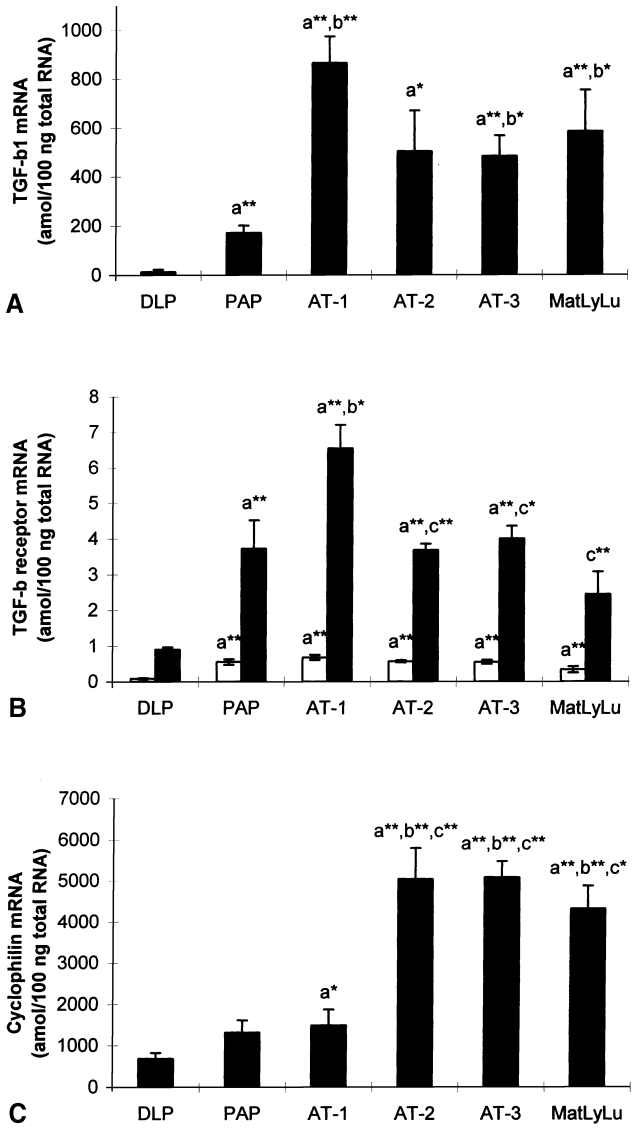


Fig. 1 Competitive reverse transcription-polymerase chain reaction (RT-PCR) results showing transforming growth factor β1 (TGF-β1) (**A**), TGFβ-RI (*open columns*) and TGFβ-RII (*solid columns*, **B**), and cyclophilin (**C**) mRNA levels in 100 ng total RNA from rat dorsolateral prostate (DLP) and Dunning PAP, AT-1, AT-2, AT-3, and MatLyLu prostatic adenocarcinoma sublines in vivo. Values are given as means ± SEM of 4–6 rats in each group, and comparations are made between the tumours and the DLP (a* $P \leq 0.05$, a** $P \leq 0.01$), the anaplastic AT-1, AT-2, AT-3 and MatLyLu tumours and the well-differentiated PAP tumour (b* $P \leq 0.05$, b** $P \leq 0.01$), and the moderately to highly metastatic AT-2, AT-3 and MatLyLu tumours and the AT-1 tumour with low metastatic capacity (c* $P \leq 0.05$, c** $P \leq 0.01$)

and in the AT-2, AT-3, and MatLyLu tumours, when compared with the PAP or the AT-1 tumours ($P \leq 0.05$, Fig. 1C). Therefore, no correction for cyclophilin was made in the analysis of TGF-β1 or its receptor mRNA in this study. Instead, RNA concentration and integrity were ensured by etidiumbromide staining after agarose electrophoresis (results not shown).

The tumours expressed higher levels of TGF-β1 mRNA than the DLP in vivo (13, 66, 38, 37 and 45

times, for PAP, AT-1, AT-2, AT-3 and MatLyLu tumours, respectively; $P < 0.05$, Fig. 1A). Moreover, the anaplastic AT-1, AT-2, AT-3 and MatLyLu tumours contained more TGF-β1 mRNA than the highly differentiated PAP tumour (5.1, 2.9, 2.8 and 3.4 times, respectively), although the difference between PAP and AT-2 were non-significant ($P = 0.2$, Fig. 1A). In accordance with the TGF-β1 mRNA results, the amount of TGFβ-RI mRNA was higher in all tumours, when compared with the DLP (6.3, 7.7, 6.5, 6.2 and 3.8 times, respectively; $P \leq 0.01$, Fig. 1B). No significant differences were found between the TGFβ-RI mRNA levels in the tumour sublines. Furthermore, all tumours except the MatLyLu tumour demonstrated higher TGFβ-RII mRNA levels than the normal tissue (4.1, 7.2, 4.0 and 4.4 times, respectively; $P \leq 0.01$, Fig. 1B). Interestingly, the moderately metastatic AT-2 tumour and the highly metastatic AT-3 and MatLyLu tumours showed significantly lower levels of TGFβ-RII mRNA than the anaplastic AT-1 tumour with low metastatic capacity (0.56, 0.63 and 0.37 times, respectively; $P \leq 0.05$, Fig. 1B).

Northern blot experiments were made to strengthen the RT-PCR results regarding the TGFβ-RII and cyclophilin mRNA variations in the Dunning tumours in vivo, but also with the aim of analysing another potential housekeeping gene, actin, in these tumours. The TGFβ-RII, cyclophilin, and actin mRNAs were detected as single bands with the approximate lengths of 5.4, 0.9 and 2.2 kb, respectively (Fig. 2). Confirming the RT-PCR results, Northern blot analysis showed a weaker TGFβ-RII band in the metastatic MatLyLu tumour than in the AT-1 tumour, and more intense cyclophilin bands in the tumours than in the DLP. In accordance with the cyclophilin mRNA levels, the actin mRNA levels were higher in the tumours than in the DLP.

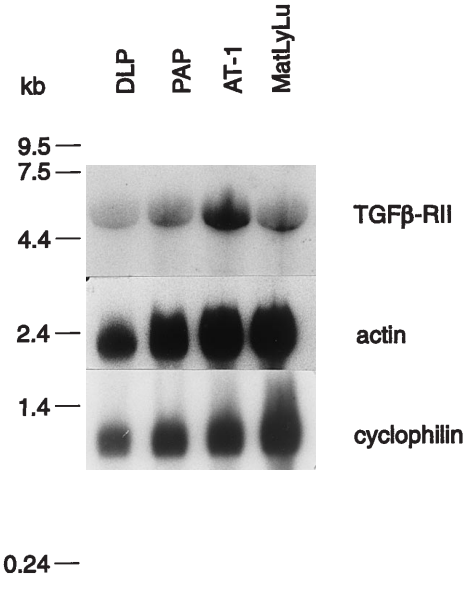


Fig. 2 Northern blot results showing TGFβ-RII, cyclophilin, and actin mRNA levels in rat DLP and Dunning PAP, AT-1 and MatLyLu prostatic adenocarcinoma sublines in vivo. Twenty μg of total RNA, originating from four rats in each group, was loaded per well. Single bands with the approximate lengths of 5.4, 0.9, and 2.2 kb were detected with the TGFβ-RII, cyclophilin and actin probes, respectively

Protein expression of TGF-β1, TGFβ-RI, and TGFβRII in rat DLP and Dunning sublines in vivo

By using Western blotting, protein expression of TGFβ-RI, TGFβ-RII, and actin was detected in the rat DLP and Dunning tumours in vivo (Fig. 3). The TGFβ-RI antibody recognised a protein with the approximate weight of 53 kDa in all tissues (Fig. 3A). In addition, a protein with slightly higher protein weight was detected in some of the tumours. Signals from these proteins were undetectable in the control experiment, where preblocked antibodies were used (Fig. 3A). These results suggest specific detection of the expected 53 kDa TGFβ-RI, as well as of a protein that could be an alternative form of TGFβ-RI. In accordance with the mRNA results for TGFβ-RI, the TGFβ-RI protein levels were higher in the Dunning tumours than in the normal DLP (Fig. 3A, C). The TGFβ-RII antibody detected one strong and two weaker protein bands of approximately 70 kDa, which were specifically blocked out by the control peptide (Fig. 3B). One protein band about 90 kDa was also detected by the TGFβ-RII antibody. The intensity of this band was not changed in the blocking experiments, and therefore the ~90 kDa band was considered as non-specific. Interestingly, the ~70 kDa protein bands were hardly detectable in the highly metastatic AT-3 and MatLyLu tumour tissues (Fig. 3B), suggesting very low expression of the TGFβ-RII protein in these tumours. The relative expression of the TGFβ-RII protein was lower in the DLP tissue (0.032 times) and in all tumours with anaplastic morphology (0.44, 0.37, 0.025 and 0.014 times for AT-1, AT-2, AT-3 and MatLyLu, respectively), than in the highly differentiated PAP tumour (Fig. 3C). In line with the mRNA result for actin, protein levels of actin were higher in the tumours than in the normal tissue (Fig. 3A, B). Ponceau-red staining of membranes ensured equal loading of proteins on the polyacrylamide gels (results not shown).

IHC experiments were performed to localise the expression of TGF-β1, TGFβ-RI and TGFβ-RII in the rat DLP and Dunning tumour sublines. TGF-β1 as well as the TGFβ receptors were localised principally in the epithelial cells in the DLP and Dunning tumour tissues, although smooth muscle cells (SMC) were occasionally stained (results shown for TGFβ-RII in Fig. 4). The tumours were homogeneously stained, while the DLP showed heterogeneous staining of both epithelial and SMC, ranging from negative to intense. Semi-quantitatively, the IHC results confirmed the Western blot results. The epithelial TGFβ-RII staining was more intense in the Dunning PAP, AT-1 and AT-2 tumours than in the rat DLP or in the metastatic Dunning AT-3 and MatLyLu tumours (results shown for DLP, and the

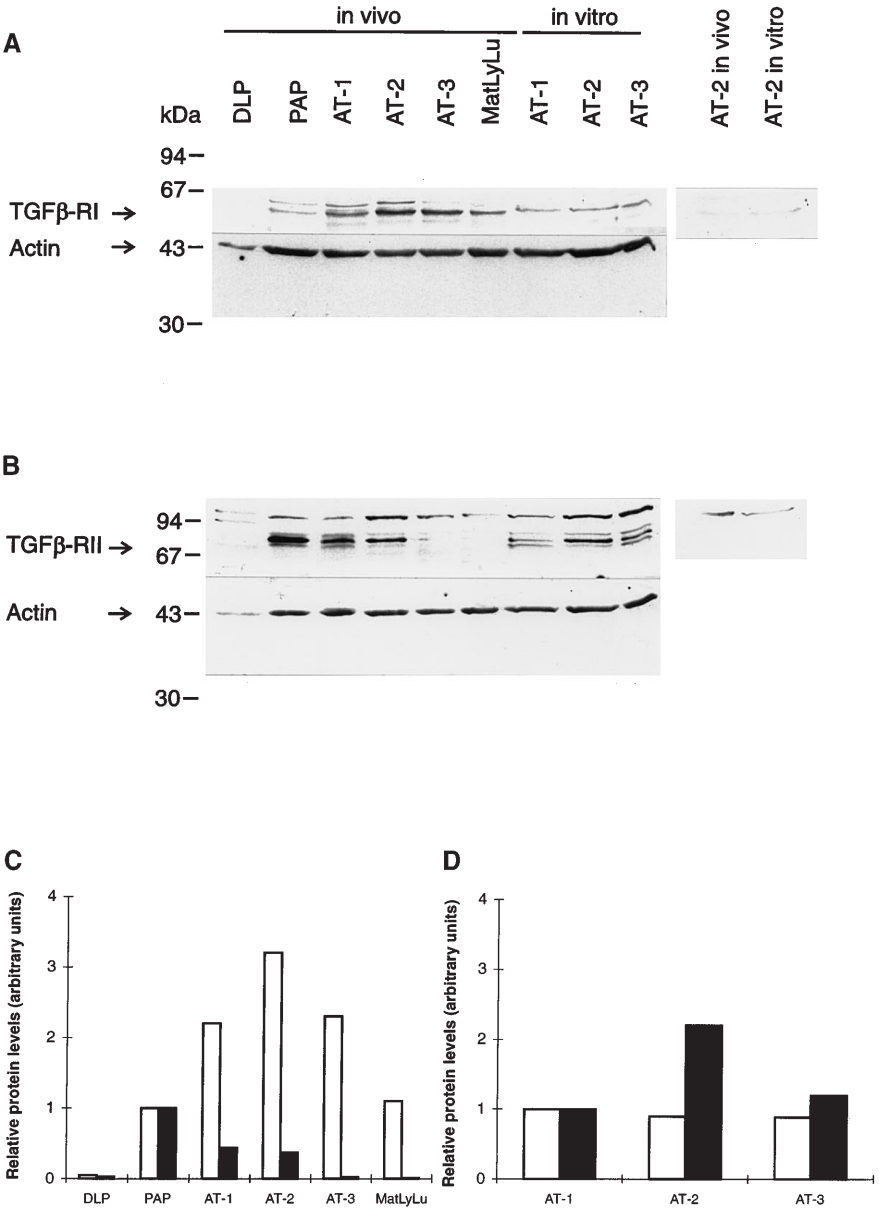


Fig. 3 Western blot results showing TGFβ-RI (**A**), TGFβ-RII (**B**) and actin protein levels in rat DLP and Dunning PAP, AT-1, AT-2, AT-3 and MatLuLy prostatic adenocarcinoma sublines in vivo and in AT-1, AT-2, and AT-3 cells in vitro. Forty μg of protein, originating from three rats in each group or from two in vitro experiments, was loaded per well. Proteins with the weights of about 53, 70 and 43 kDa were detected with the TGFβ-RI, TGFβ-RII and actin antibodies, respectively. In the last two lanes, where immunodetections were made with preblocked antibodies, neither TGFβ-RI nor TGFβ-RII was detected. Relative protein levels of TGFβ-RI (*open columns*) and TGFβ-RII (*solid columns*) are shown for the in vivo (**C**) and in vitro (**D**) results, when compared with the levels in the PAP tumour and the AT-1 cells, respectively

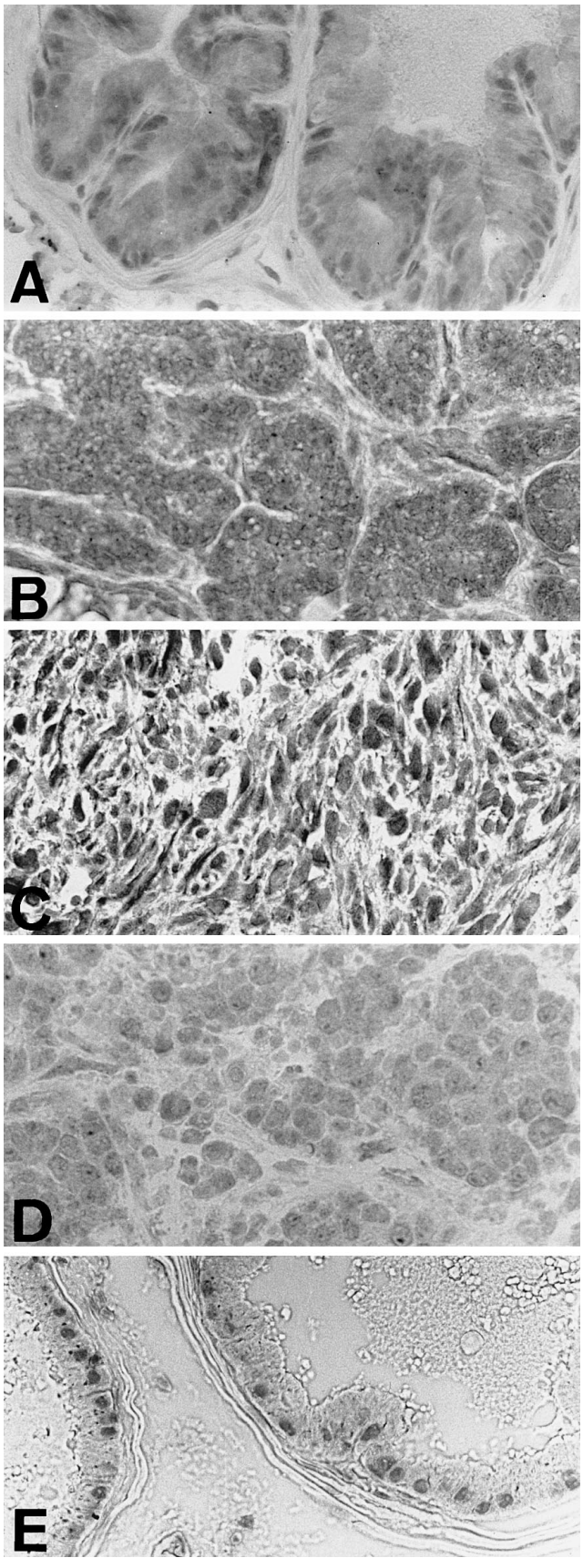
well-differentiated PAP, the anaplastic AT-1, and the anaplastic, highly metastatic MatLyLu tumours in Fig. 4). The TGF-β1 and TGFβ-RI protein staining was more intense in all tumours than in the DLP (results not shown). The specificity of the immunoreactions has previously been determined [8, 31], and control slides incubated with preblocked antibodies showed no staining in this study (results shown for TGFβ-RII in Fig. 4E).

Expression of TGFβ-RI and TGFβ-RII in AT-1, AT-2 and AT-3 cells in vitro

In contrast to the results found in vivo, showing extremely low levels of TGFβ-RII protein in the AT-3 tumour when compared with the AT-1 and AT-2 tumours (0.057 and 0.067 times, respectively, Fig. 3C), the TGFβ-RII protein was more equally expressed in these cell lines in vitro (Fig. 3D). Furthermore, when grown in culture, the AT-1, AT-2 and AT-3 cells contained comparable levels of TGFβ-RII mRNA (results not shown), as well of TGFβ-RI protein (Fig. 3D) and mRNA (results not shown).

Discussion

Prostate tumour cells of murine as well as of human origin express high levels of TGF-β1 [35, 39], and often show reduced sensitivity to TGF-β1 growth inhibition



[33, 34, 46]. The mechanism behind tumour cell resistance to TGF-β1 growth inhibition may be alterations in the TGF-β1 signalling pathways. In this study, the expression of TGF-β1, TGFβ-RI and TGFβ-RII in Dunning rat prostatic adenocarcinoma sublines with different tumour characteristics were investigated, in order to detect alterations that could possibly contribute to TGF-β1 resistance and tumour progression. The expression of the "housekeeping genes" cyclophilin and actin was also quantified, with the aim of correcting TGF-β1, TGFβ-RI and TGFβ-RII levels for RNA and protein degradation, etc. Surprisingly, both cyclophilin and actin levels were found to be elevated in the tumours, when compared with levels in the normal DLP. Furthermore, the cyclophilin expression seemed to increase with tumour progression. Our results suggest that cyclophilin and actin are unequally expressed in cells with different growing rates, and not adequate to use as housekeeping genes when comparing normal and tumour tissues, or tumours with different growth characteristics. Therefore, we did not correct for cyclophilin or actin expression in the analysis of TGF-β1 and its receptors in this study.

TGF-β1 expression was found to be elevated in all Dunning tumour sublines investigated, when compared with normal rat prostate. In accordance with previous studies [35, 39], these results show that TGF-β1 overproduction may be an early event in prostate cancer. TGF-β1 mRNA levels were, however, higher in the anaplastic AT-1, AT-2, AT-3 and MatLyLu tumours than in the well-differentiated Dunning PAP tumour, which may indicate increased expression of TGF-β1 with prostate cancer progression. Indeed, we recently demonstrated that overexpression of TGF-β1 is more common in high than low grade human prostate cancer [45].

Tumour overproduction of TGF-β1 may selectively suppress the growth of TGF-β1 sensitive tumour cells, while more malignant cells could be even growth stimulated by other TGF-β1 effects. TGF-β1 has been shown to promote tumour growth by stimulating angiogenesis [41], cell migration, invasion and metastasis [12, 27, 43], and by suppressing immune responses [1, 12] in several tumour systems. In line with this, we recently showed that overproduction of TGF-β1 is associated with angiogenesis, metastasis, and poor clinical outcome in human prostate cancer [45]. In the same study, loss of epithelial TGFβ-RII expression was associated with short prostate cancer-specific survival. Moreover, overproduction of TGF-β1 in the rat Dunning MatLyLu tumour has been demonstrated to result in increased tumour growth and metastatic capacity. These TGF-β1 tumour-promoting effects were not achieved by direct

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Fig. 4 Immunohistochemical results for TGFβ-RII in rat DLP (**A**) and Dunning PAP (**B**), AT-1 (**C**), and MatLyLu (**D**) prostatic adenocarcinoma sublines, showing TGFβ-RII staining in epithelial and stromal cells in the DLP and tumour tissues in vivo. Control slide incubated with preblocked antibody showed no staining (**E**)

stimulation of tumour cell proliferation [5], but instead at least partly obtained by stimulation of tumour cell motility [28] and suppression of host immune responses [5]. Tumour cell motility and invasion may be stimulated by TGF-β1 enhancement of proteolytic activity, as has been demonstrated for human prostate tumour cells with reduced sensitivity to TGF-β1 growth inhibition [8, 32]. Taken together, these results indicated to us that there may be alterations on the TGFβ receptor level that possibly make prostate cancer cells insensitive to TGF-β1 growth inhibition, without abolishing other TGF-β1 effects.

In the present study, Western blotting and IHC experiments demonstrated extremely low levels of the TGFβ-RII protein in the highly metastatic AT-3 and MatLyLu tumours in vivo. Furthermore, the normal DLP and the tumours with low and moderate metastatic capacity (AT-1 and AT-2) showed lower levels of TGFβ-RII protein than the non-metastatic PAP tumour. A similar trend in decreased TGFβ-RII expression with prostate cancer progression could be seen on the mRNA level, although the results were not as clear-cut as on the protein level. TGFβ-RII was found to be expressed predominantly in the epithelial cells, and the low levels of TGFβ-RII mRNA and protein in the DLP could therefore at least partly be explained by the relatively low number of epithelial cells in this tissue. The low levels of TGFβ-RII in the anaplastic tumours, on the other hand, most likely originated from decreased expression of TGFβ-RII in the tumour cells. Despite the very low TGFβ-RII protein levels in the AT-3 and MatLyLu tumours in vivo, these tumours contained TGFβ-RII mRNA levels that were comparable to the levels in the other tumours. Furthermore, when the AT-3 cells were grown in vitro they expressed TGFβ-RII protein levels that were comparable to the levels in the AT-1 and AT-2 cells. These results indicate that the AT-3 cells have the ability to express substantial levels of the TGFβ-RII protein, and that the discrepancy in TGFβ-RII mRNA and protein expression in the AT-3 and MatLyLu tumours in vivo may be caused by post-transcriptional downregulation. If post-transcriptional downregulation is the mechanism behind the low expression of TGFβ-RII seen in human prostate cancer [15, 18, 19, 47], it may be therapeutically possible to increase TGFβ-RII levels in the tumour cells. The regulation of TGFβ-RII expression therefore needs to be thoroughly investigated.

TGFβ-RI and TGFβ-RII have been shown to work as a heteromeric complex [48], and different TGF-β1 effects are believed to be mediated by divergent post-receptor signalling pathways in prostate cancer cells [13], as well as in other cells [2]. However, although several studies have shown that TGFβ-RII is needed for induction of TGF-β1 growth inhibition [4, 17, 36, 42, 50], other TGF-β1 effects such as modulation of extracellular matrix components can be mediated by TGFβ-RI only [4]. Therefore, it is possible that loss of TGFβ-RII expression with prostate cancer progression is part of the mechanism behind the reduced sensitivity to TGF-β1 growth inhibition in the anaplastic Dunning tumours. Furthermore, in the absence of TGFβ-RII, TGF-β1 may enhance the metastatic potential of the tumour cells not only by stimulating angiogenesis and suppressing immune responses, but also by inducing possible TGFβ-RI-mediated effects, such as stimulation of proteolytic activity and cell motility and invasion.

In conclusion, high expression of TGF-β1 and TGFβ-RI and low expression of TGFβ-RII may contribute to prostate tumour progression and metastasis in the Dunning prostatic adenocarcinoma model. However, despite the finding of low TGFβ-RII protein levels in the Dunning AT-3 and MatLyLu tumours in vivo, the AT-3 cells expressed high TGFβ-RII levels in vitro, and TGF-β1 has been shown to bind to TGFβ-RII and to inhibit growth of the MatLyLu cells in vitro under certain conditions [28]. These results indicate that there may be factors that influence tumour sensitivity to TGF-β1 growth inhibition by regulating the TGFβ-RII protein level in the tumour cells. Further studies are therefore needed to clarify the mechanism behind acquisition of TGF-β1 resistance in prostate tumour progression, and to elucidate the importance of the TGFβ system in prostate tumourgenesis.

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