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Expression of transforming growth factor-β receptor type I and type II in rat ventral prostate and Dunning R3327 PAP adenocarcinoma in response to castration and oestrogen treatment

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Abstract In the normal prostate, transforming growth factor-β1 (TGF-β1) inhibits epithelial cell growth and is associated with apoptosis. The role of TGF-β1 in prostate cancer remains, however, unclear. In this work, the expression of TGF-β receptor type I and II (TGFβ-RI and TGFβ-RII) in the Dunning R3327 PAP adenocarcinoma was studied, after castration and oestrogen treatment. Since castration induces apoptosis in the rat ventral prostate (VP) [21], but not in the Dunning R3327 PAP tumour [46], the TGF-β receptor levels in the tumour were compared to the receptor levels in the VP. Methods used were competitive reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry. In the VP, TGFβ-RI and TGFβ-RII expressions were increased after castration, indicating a negative regulation of TGFβ receptors by androgens. In the Dunning tumour, TGFβ-RI and TGFβ-RII levels were elevated and only TGFβ-RI showed a clear-cut increase after castration. The receptors were located in epithelial and smooth muscle cells in the VP and mainly in epithelial cells in the Dunning tumour. In conclusion, the elevated TGFβ receptor levels and the diminished androgen regulation of TGFβ-RII in the tumour distinguish the Dunning R3327 PAP tumour from the normal prostate and need to be further elucidated.

Key words TGF-β receptors · Prostate cancer · Competitive polymerase chain reaction · Immunohistochemistry

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Introduction

In the normal prostate, transforming growth factor-β1 (TGF-β1) has been shown to inhibit epithelial cell growth [14, 31], and to be associated with apoptosis [20, 21, 29]. TGF-β1 mRNA expression and receptor binding capacity are increased in parallel to the programmed cell death seen in the rat ventral prostate (VP) in response to castration [20, 21], and exogenous TGF-β1 may induce apoptosis in the VP also in the presence of physiological androgen levels [29]. Inhibition of epithelial cell growth and possible apoptosis-inducing effects have also been observed in some human prostatic tumour cell lines both in vitro [47] and in vivo [22]. Despite these observations, indicating that TGF-β1 is a prototypical epithelial cell inhibitor, the role of TGF-β in tumours remains unclear. The TGF-β levels in many cancers, including prostate cancer, are elevated [7, 34, 36, 39, 40]. Several Dunning rat prostate adenocarcinoma sublines express high levels of TGF-β1 mRNA [34, 36], and TGF-β has been suggested to be associated with tumour promotion and aggressiveness [35]. It was previously shown that the relative TGF-β1 mRNA expression in the Dunning R3327 PAP subline is about 6 times higher than in the rat VP [26]. Other striking differences between the Dunning R3327 PAP tumour and the VP are the lack of apoptosis [46] and TGF-β1 [26, 36] induction in the tumour in response to castration.

The effects of TGF-β are mediated by membrane-bound serine-threonine kinase proteins [2, 10, 25]. Two putative serine-threonine receptors, currently referred to as TGF-β receptor I (TGFβ-RI) and TGFβ-RII, bind TGF-βs with high affinity and have been identified in TGF-β-responding cells. Another widely distributed transmembrane TGF-β-binding protein is TGFβ-RIII [30]. TGFβ-RIII is a proteoglycan, which does not contain any obvious intracellular signalling motif and does not seem to be necessary for TGF-β signal transduction [27, 43]. Many TGF-β-resistant cell lines show no or low levels of TGF-β receptors [1, 19], and it has

been suggested that mutations or loss of TGFβ-RI or TGFβ-RII could contribute to tumour promotion in many human malignancies [11, 15, 28, 33, 37, 42]. Also the ratio between TGFβ-RII and TGFβ-RI appears to be important for the cellular response to TGF-β [9, 12, 32, 49]. It is, however, not known how the TGF-β receptors are expressed in cancerous prostatic tissues. In order to elucidate possible changes in the TGF-β receptor expression in the Dunning R3327 PAP adenocarcinoma, tumour TGFβ-RI and TGFβ-RII mRNA quantification and protein localisation were undertaken in the present study. Since castration induces apoptosis in the rat VP [21], but not in the Dunning R3327 PAP tumour [46], the TGF-β receptor levels in the tumour were compared to the receptor levels in the VP of the same animal, before and after castration. The effects of oestrogen on the TGF-β receptor mRNA levels in the Dunning tumour were also investigated, since combined castration and oestrogen treatment is known to reduce epithelial cell number in this tumour model [23], by an apoptotic mechanism [46].

Materials and methods

Animal treatment and tissue preparation

Adult male Copenhagen × Fisher, F_1, rats (ALAB, Uppsala, Sweden) were subcutaneously transplanted with small pieces of the highly differentiated, androgen-sensitive Dunning R3327 PAP rat prostatic adenocarcinoma (originally obtained from Dr. N. Altman, Papanicolau Cancer Research Institute, Miami, FL), as previously described by Landström et al. [24]. 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 of 1–2 cm^3 (about 4 months after transplantation), the rats were randomly divided into two experimental groups. One group was castrated via scrotal incision, while the other remained intact and served as control. Among the castrated rats, a third group received daily subcutaneous injections of 50 μg oestradiol benzoate (Sigma, USA), as previously described [23, 24, 46]. Animals were sacrificed by decapitation at 1, 4, 7 or 14 days after castration and the VPs and Dunning tumours were quickly removed. Tissues were cut into small pieces, frozen in liquid nitrogen and stored at −70°C. Some pieces were fixed in 4% paraformaldehyde (PFA) in phosphate buffer saline (PBS) for 4 h, before being embedded in paraffin and subjected to immunohistochemistry (IHC).

Quantitative RT-PCR

RNA extraction

Total RNA was isolated from frozen rat VP and Dunning R3327 PAP adenocarcinoma tissues according to the guanidinium-acid-phenol extraction method by Chomczynski and Sacchi [5]. RNA concentrations were quantified using a UV spectrophotometer (Lambda 2, Perkin Elmer).

Internal and external standards

In the quantitative reverse transcriptase polymerase chain reaction (RT-PCR) method, RNA levels in a sample were calculated from RNA to internal standard (IS) ratios, as previously described [26]. These ratios were obtained by competitively amplifying the questioned RNA and the IS during the RT-PCR procedure. Standards for TGFβ-RI, TGFβ-RII and cyclophilin were synthesised according to the method of Celi et al. [3]. Briefly, complementary DNA (cDNA) fragments were amplified from rat VP, using the conventional sense PCR primers (s_c-primers) together with long antisense primers (a_l-primers, Table 1). The a_l-primers contained

Table 1 Oligonucleotides used as primers in the competitive RT-PCR method. Conventional sense and antisense primers (s_c- and a_c-primers) were used for competitive RT-PCR quantifications of TGFβ-RI, TGFβ-RII and cyclophilin mRNA in the rat VP and Dunning R3327 PAP adenocarcinoma. The long antisense primers (a_l) were used to synthesise internal standards for the competitive quantifications

Primer name	Nucleotide sequence	PCR products (lengths)
TGFβ-RI-s_c	5′-TACAGTGTTTCTGCCACCTCTGT-3′ (101–123 of human cDNA [10])	TGFβ-RI/-RI-IS (177 bp/166 bp)
TGFβ-RI-a_c	5′-CAGTTTTTGAAGAGGGTGCACATA-3′ (254–277, human cDNA [10])	TGFβ-RI (177 bp)
TGFβ-RI-a_l	5′-CAGTTTTTGAAGAGGGTGCACATACGAGG-AATTAAGTCAATTTCAGCTA-3′ (216–238, 254–277 of human cDNA [10])	TGFβ-RI-IS (163 bp)
TGFβ-RII-s_c	5′-GCATGAGCAACTGCAGCATCAC-3′ (200–240 human cDNA [25])	TGFβ RII/-RII-IS (215 bp/199 bp)
TGFβ-RII-a_c	5′-ACAGGAACACATGAAGAAAGTCTC-3′ (391–414 of human cDNA [25])	TGFβ-RII (215 bp)
TGFβ-RII-a_l	5′-ACAGGAACACATGAAGAAAGTCTCTCCTT-CATGACGCACGTGGGAGA-3′ (410–432 of rat cDNA [41]; 391–414 of human cDNA [25])	TGFβ-RII (199bp)
Cyclo-s_c	5′-TGCAGACAAGGTCCCAAAGACAG-3′ (4180–4200 of human exon 2[13])	Cyclophilin/cyclophilin-IS (362 bp/315 bp)
Cyclo-a_c	5′-CAAAGCGCTCCATGGCCTCCACA-3′ (6266–6288 of human exon 5 [13])	Cyclophilin (362 bp)
Cyclo-a_l	5′-CAAAGCGCTCCATGGCCTCCACACAGCCA-CTCAGTCTTGGCAGT-3′ (388–408 of rat cDNA [6]; 6266–6288 of human exon 5 [13])	Cyclophilin-IS (315 bp)

the conventional antisense (a_c) primer sequence at the 5′-end together with 3′-end nucleotides chosen to give a cDNA fragment shorter than the conventional PCR product (Table 1). The IS cDNA fragments were cloned into the pCR™II vector (Invitrogen BV, Leek, The Netherlands), sequenced (results not shown) and in vitro transcribed with SP6 RNA polymerase (Boehringer Mannheim) after linearisation with Ava 1. Resulting RNA standards were spectrophotometrically quantified and stored at −70°C until use. Cyclophilin, a protein equally expressed in most eukaryotic cells [6], was used as an external standard and amplified in parallel with TGFβ-RI and TGFβ-RII in all RT-PCR samples. The TGFβ-RI and TGFβ-RII mRNA levels were corrected for the corresponding cyclophilin levels in each RNA sample and displayed as relative values in the resulting figures.

Competitive RT-PCR

The competitive RT-PCR procedure was performed as previously described [26], with some modifications. Briefly, total RNA (100 ng) was mixed with the appropriate amount of IS in an RT volume of 15 µl. Each RNA sample was titrated with three amounts (double samples) of IS, ranging from 0.01–0.6 or 0.05–0.6 (TGFβ-RI), 0.05–0.9 or 0.5–5.0 (TGFβ-RII) to 70–700 amol (cyclophilin) in rat VP and Dunning R3327 tumour samples, respectively. After complete RT, each sample was divided into three PCR tubes, in order to amplify TGFβ-RI, TGFβ-RII and cyclophilin separately. The competitive PCR reactions were run for 30 cycles (95°C, 30 s; 59°C, 30 s; 72°C, 45 s) with corresponding conventional PCR primers (Table 1). The sense primers were all labelled with a fluorescent amidite (FluorePrime, Pharmacia Biotech AB, Sweden) at the 5′-end, according to the manufacturer's protocol, and the PCR products were analysed in an automatic laser fluorescence system (ABI PRISM 377 DNA sequencer, Perkin Elmer). The data were processed by the ABI PRISM™ GeneScan software (Perkin Elmer).

Antibodies

In the IHC experiments, two TGFβ-RI and three TGFβ-RII polyclonal antibodies were used. Antibodies against TGFβ-RI, V-22 (Santa Cruz Biotechnology, Santa Cruz, CA) and VPN [10] corresponded to amino acids 158–179 and antibodies against TGFβ-RII, C-16 (Santa Cruz), L-21 (Santa Cruz) and DRL [10] to amino acids 245–266 (L-21 and DRL) and 550–565 (C-16), respectively.

IHC

Cryostat sections (6 µm) of VP and Dunning R3327 tumour tissues were mounted on SuperFrost Plus microscopic slides (Mänzel-Gläser, Germany) and air dried for 10 min at room temperature. Sections were fixed in 4% PFA or in acetone before being rinsed 3 times in PBS. Paraffin sections (4 µm thick) were deparaffinated and rehydrated according to standard procedures, washed with PBS and heated in a microwave oven (Electrohelios, Sweden) at 600 W for 2 × 7.5 min and 1 × 5 min in 0.01 M citrate buffer, pH = 6.0, as described previously [38]. To quench endogenous peroxidase activity, slides were immersed in 1% H_2O_2 in methanol for 20 min. After another wash in PBS, unspecified binding was blocked in 5% normal goat serum (1% BSA in PBS) for 30 min prior to antibody incubations. Incubations with the primary antibodies were made overnight, in 4°C, with an antibody dilution of 1:400 (V-22, C-16 and L-21) or 1:1000 (VPN and DRL) in PBS. Immunoreactions were detected with biotinylated goat anti-rabbit antibodies followed by the ABC technique (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA) with AEC as the chromogen. The specificity of the IHC experiments was examined by preincubation of TGFβ-RI and TGFβ-RII antibodies with tenfold (w/w) excess of the V-22P and L-21P control peptides (Santa Cruz Biotechnology), respectively.

Statistical analysis

The statistical significance of differences between groups was analysed using the Mann-Whitney *U*-test. Data are presented as means together with SEM. *P*-values less than or equal to 0.05 were considered statistically significant.

Results

TGFβ-RI and TGFβ-RII mRNA expression

TGFβ-RI and TGFβ-RII mRNA levels in VP and Dunning R3327 PAP tumour tissues of endocrine-treated rats were quantified, using the competitive RT-PCR method, and compared to levels in intact control rats. The receptor mRNA levels were measured at days 1, 4, 7 and 14 in the castrated and at days 1 and 7 in the oestrogen-treated and castrated rats. Specificity of the RT-PCR reactions was initially determined by sequencing of the PCR products (results not shown).

In the rat VP, TGFβ-RI and TGFβ-RII mRNAs were induced after castration treatment, indicating a negative regulation of the TGF-β receptors by androgens. TGFβ-RI mRNA levels were significantly increased at day 4 ($P < 0.001$) and showed a peak value (4 times intact value, $P < 0.001$) at day 7 after castration (Fig. 1). TGFβ receptor type II showed an earlier and also more prolonged induction by androgen ablation, since the mRNA levels were induced at day 1 ($P < 0.05$) and the highest level (about 7 times intact value, $P < 0.001$) was measured at day 14 after castration (Fig. 1).

In the Dunning R3327 PAP tumour, the TGF-β type I receptor seemed to have preserved most of the negative regulation by androgens seen in the normal rat VP, although a slightly elevated intact mRNA value (3 times

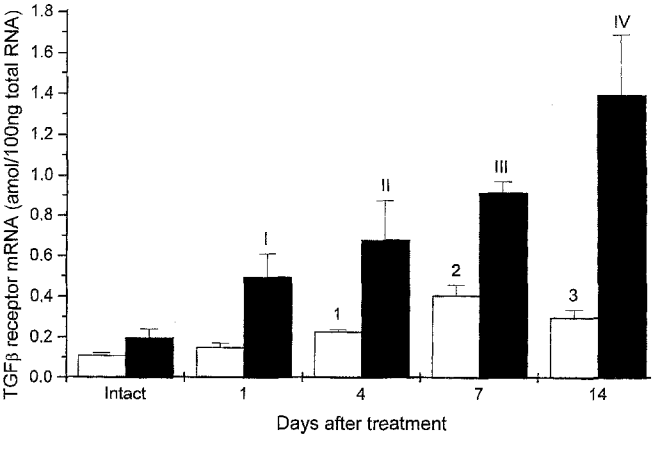

Fig. 1 Relative TGFβ-RI (*hollow bars*) and TGFβ-RII (*solid bars*) mRNA levels in the rat VP of intact and 1-, 4-, 7- or 14-day castrated rats, when quantified by competitive RT-PCR. The TGFβ-RI mRNA level was induced at day 4 and showed a peak value at day 7 ($^{1,2,3}P < 0.001$) after treatment. The TGFβ-RII mRNA level was induced at day 1 and showed a sustained induction at day 14 ($^{I,II}P < 0.05$, $^{III}P < 0.01$, $^{IV}P < 0.001$) after castration. Values are given as means ± SEM of five to eight rats in each group. Individual values were corrected for the corresponding cyclophilin mRNA levels

intact VP level, $P < 0.001$) was seen (Figs. 1, 2). Tumour TGFβ-RI mRNA level was significantly induced at day 4 ($P < 0.05$), as in the VP, and showed the highest value (2.2 times intact value, $P < 0.01$) at day 14 postcastration (Fig. 2). The TGF-β type II receptor mRNA expression did not, however, seem to be under as clear-cut negative androgen regulation as in the normal VP. TGFβ-RII mRNA expression in the intact tumour was elevated, 19 times intact VP level ($P < 0.001$, Figs. 1, 2), and showed no pronounced induction by castration treatment (Fig. 2). Not until day 14 was tumour TGFβ-RII mRNA level significantly increased by castration (1.5 times intact level, $P < 0.05$). This could be compared to the 2.5 ($P < 0.05$) times increase seen as early as day 1 and the 7 times increase seen at day 14 ($P < 0.001$) in the VP of the castrated animals. Oestrogen treatment did not further increase the TGFβ-RI mRNA levels in the Dunning tumour of the castrated rats (Figs. 2, 3). The TGFβ-RII mRNA levels, on the other hand, were decreased at day 1 ($P < 0.01$) and increased at day 7 ($P < 0.001$) after oestrogen treatment when compared to the intact group (Fig. 3).

Looking at the RII/RI ratios, it is clear that the ratios were markedly elevated in the Dunning R3327 PAP tumour, when compared to the ratios in the normal VP (Table 2). The intact RII/RI ratio in the tumour was 9.4 and intact RII/RI ratio in the VP was 1.7 ($P < 0.001$, Table 2). Castration treatment resulted in a significantly increased RII/RI ratio in the VP at day 14 after treatment ($P < 0.01$, Table 2). This effect could not be seen in the Dunning tumour, although the ratios were constantly elevated in the tumour, ranging from 7.9 to 10, also after castration treatment. Oestrogen treatment, on the other hand, resulted in RII/RI alterations in the tumour (Fig. 3). The RII/RI ratio was decreased at day 1 ($P < 0.01$) and increased at day 7 ($P < 0.05$) after oestrogen treatment, when compared to the RII/RI ratio in the intact group (Table 2).

TGFβ-RI and TGFβ-RII protein localisation

The TGFβ-RI and TGFβ-RII protein localisations in the VP and the Dunning R3327 PAP adenocarcinoma were studied using the IHC technique. It was shown that TGFβ-RI and TGFβ-RII were expressed in epithelial and stromal cells in both the VP and in the Dunning

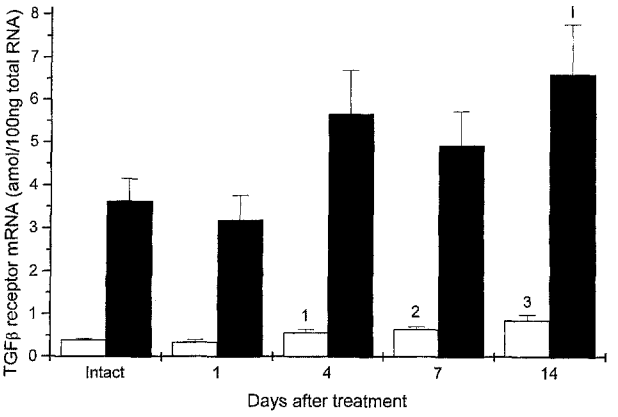

Fig. 2 Relative TGFβ-RI (*hollow bars*) and TGFβ-RII (*solid bars*) mRNA levels in the Dunning R3327 PAP adenocarcinoma of intact and 1-, 4-, 7- or 14-day castrated rats, when quantified by competitive RT-PCR. The TGFβ-RI mRNA level was induced at day 4 ($^{1}P < 0.05$, $^{2,3}P < 0.01$), as in the rat VP, but the TGFβ-RII mRNA level was not induced until day 14 ($^{1}P < 0.05$) after castration. Values are given as means ± SEM of five to eight rats in each group. Individual values were corrected for the corresponding cyclophilin mRNA levels

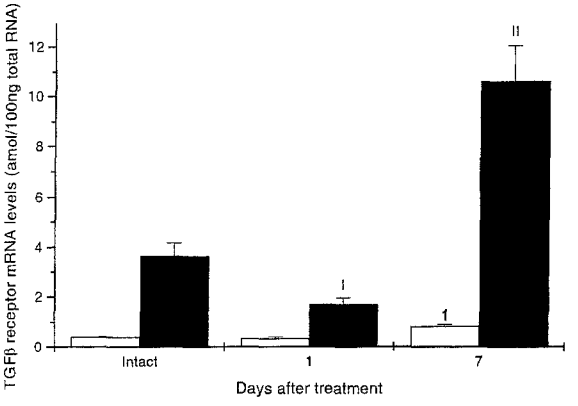

Fig. 3 Relative TGFβ-RI (*hollow bars*) and TGFβ-RII (*solid bars*) mRNA levels in the Dunning R3327 PAP adenocarcinoma of intact and 1- and 7-day castrated plus oestrogen-treated rats, when quantified by competitive RT-PCR. The TGFβ-RI mRNA level was increased at day 7 ($^{I}P < 0.01$) and the TGFβ-RII mRNA level was decreased at day 1 ($^{I}P < 0.01$) and increased at day 7 ($^{II}P < 0.001$) after combined castration and oestrogen treatment. The TGFβ-RII mRNA levels were significantly different from levels in the day 1 and 7 castration only groups (results not shown). Values are given as means ± SEM of five to eight rats in each group. Individual values were corrected for the corresponding cyclophilin mRNA levels

Table 2 TGFβ-RII to TGFβ-RI mRNA ratio in rat VP and Dunning R3327 PAP adenocarcinoma before and after castration or combined castration and oestrogen treatment. Values are shown as means ± SEM for five to eight rats in each group

	Intact	Day 1	Day 4	Day 7	Day 14
VP					
Castration	1.7 ± 0.25	3.3 ± 0.74	3.0 ± 0.88	3.0 ± 0.64	4.0 ± 0.56*
Dunning R3327 PAP					
Castration	9.4 ± 0.83[a]	9.9 ± 0.81[b]	10 ± 0.52[c]	7.9 ± 0.89[d]	8.3 ± 1.4[e]
Castration plus oestrogen		6.0 ± 0.50*		13 ± 1.4**	

*$P < 0.01$ and **$P < 0.05$ when compared to the corresponding intact groups
[a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.001$, [d]$P < 0.05$ and [e]$P < 0.01$ when compared to the corresponding VP levels

PAP tumour, although the expression patterns were not entirely homogeneous. Difficulties in determining the relative locations of TGFβ-RI and TGFβ-RII in the different cell types arise, since the antibodies and fixation procedures used resulted in somewhat heterogeneous staining patterns (Tables 3, 4). Fixation of cryosections in PFA gave a better agreement between antibody-staining patterns than acetone fixation, and seemed to be the most reliable procedure.

In the VP of intact animals, TGFβ-RI protein was mainly located in the epithelial cells and in the smooth muscle cells (SMC) lining the glandular epithelium and the blood vessels (Table 3, Fig. 4a). The TGFβ-RII staining in the intact VP was more pronounced in the SMC than in the luminal epithelial cells, when detected with the L-21 and C-16 antibodies (Table 4, Figs. 4d, 5a). The DRL antibody, on the other hand, showed an intense immunostaining of both epithelial cells and of SMC (Table 4). The C-16 antibody gave a particularly strong staining of the basal epithelial cells (Table 4, Fig. 5a).

Also in the intact Dunning R3327 PAP tumour, the TGFβ-RI and TGFβ-RII proteins were observed in the epithelium and in the stroma (Tables 3, 4, Figs. 4c,f, 5c). The basal epithelial cells also in the tumour showed intense TGFβ-RII staining when detected with the C-16 antibody (Table 4, Fig. 5c). This strong TGFβ-RII staining of the basal cells in the tumour was also detected with the L-21 antibody (Table 4, Fig. 4f). Neither castration alone (7 days) nor combined with oestrogen treatment (1 or 7 days) resulted in any obvious change in TGFβ-RI or TGFβ-RII localisation in the VP or in the Dunning R3327 PAP adenocarcinoma (results not shown).

The specificity of the ICH experiments was verified using preblocked primary antibodies. Immunodetections with preblocked antibodies showed no staining of epithelial or stromal cells in the VP or Dunning R3327 PAP tumour tissues (Figs. 4b,e, 5b,d).

Discussion

Transforming growth factor-β1 has previously been shown to inhibit epithelial cell growth [14, 31] and also to be associated with the castration-induced apoptosis in

Table 3 TGFβ-RI protein localisation in intact VP and Dunning R3327 PAP adenocarcinoma. TGFβ-RI was detected with the V-22 and VPN antibodies on PFA and acetone (*A*)-fixed cryostat sections in luminal and basal epithelial cells, in periglandular and perivascular smooth muscle cells (SMC), in endothelial cells and in fibroblasts. The table shows relative staining intensities of the different cell types, indicating negative (–) to intense (+++) staining. Parentheses indicate a weak, but possibly positive, staining

Cells	VP				Dunning R3327 PAP			
	V-22		VPN		V-22		VPN	
	PFA	A	PFA	A	PFA	A	PFA	A
Epithelial								
Luminal	++	++	++	–	+	+	+	–
Basal	+	(+)	+	–	+	+	+	–
SMC								
Periglandular	++	(+)	+	–	(+)	–	(+)	–
Perivascular	++	(+)	+	–	+	–	(+)	–
Endothelial	+	–	+	–	(+)	–	(+)	–
Fibroblasts	+	–	+	–	(+)	–	(+)	–

Table 4 TGFβ-RII protein localisation in intact VP and Dunning R3327 PAP adenocarcinoma. TGFβ-RII was detected with the C-16, L-21 and DRL antibodies on PFA- and acetone (*A*)-fixed cryostat sections in luminal and basal epithelial cells, in periglandular and perivascular smooth muscle cells (SMC), in endothelial cells and in fibroblasts. The table shows relative staining intensities of the different cell types, indicating negative (–) to intense (+++) staining. Parentheses indicate a weak, but possibly positive, staining

Cells	VP						Dunning R3327 PAP					
	C-16		L-21		DRL		C-16		L-21		DRL	
	PFA	A	PFA	A	PFA	A	PFA	A	PFA	A	PFA	A
Epithelial												
Luminal	+	+	+	+	+++	++	(+)	(+)	+	+	++	++
Basal	+++	+++	+	+	+++	++	+++	+++	+++	+++	++	++
SMC												
Periglandular	++	+	+++	+++	++	++	(+)	(+)	+	+	++	+
Perivascular	++	+	+++	+++	++	++	(+)	(+)	++	++	++	+
Endothelial	+	+	+	+	+	+	(+)	(+)	+	+	+	+
Fibroblasts	+	+	+	+	+	+	(+)	(+)	+	+	+	+

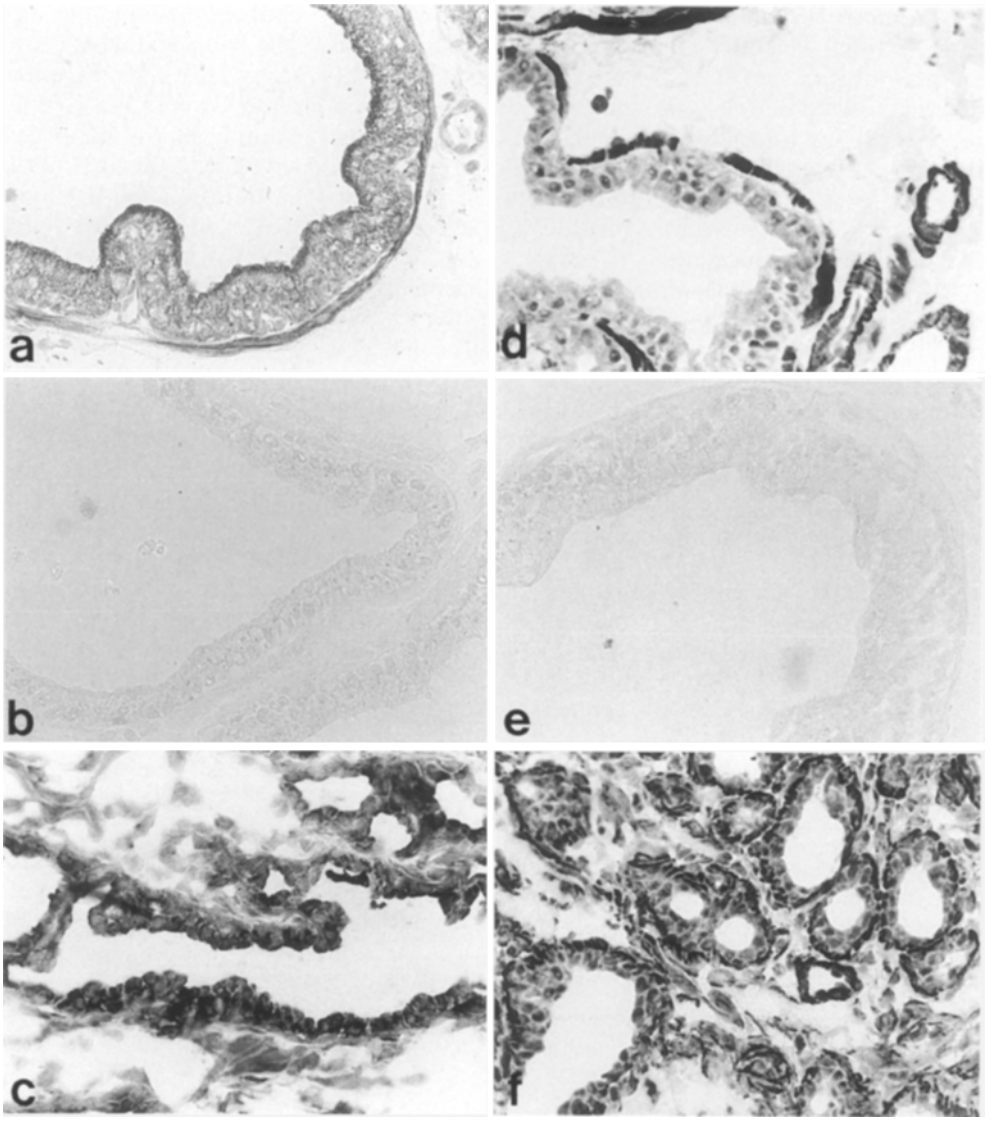

Fig. 4a–f Immunohistochemical staining of TGFβ-RI with the V-22 antibody (**a–c**) and TGFβ-RII with the L-21 antibody (**d–f**), in the rat VP and Dunning R3327 PAP adenocarcinoma, × 400. TGFβ-RI and TGFβ-RII antibody incubations showed receptor localisation in epithelial cells and SMC in the VP (**a, d**) and mainly in epithelial cells in the Dunning R3327 PAP tumor (**c, f**). Basal epithelial cells in the tumour showed a particularly strong staining of TGFβ-RII (**f**). Control sections (**b, e**), incubated with preblocked antibodies, were unstained

the rat VP [20, 21, 29]. By contrast, neither apoptotic index [46] nor TGF-β1 expression [26] is increased in the Dunning R3327 PAP adenocarcinoma in response to castration. In the present work, we have studied the expression of TGFβ-RI and TGFβ-RII in the Dunning R3327 PAP adenocarcinoma after castration, in order to elucidate possible receptor changes in the tumour, when compared to the VP. The TGF-β receptor expression in response to combined castration and oestrogen treatment was also studied, since this treatment has been shown to reduce epithelial cell number in the Dunning tumour [23], by an apoptotic mechanism [46].

By using competitive RT-PCR and IHC techniques, we have detected TGF-β receptor mRNA and protein in the rat VP and in the Dunning R3327 PAP tumour. In the VP, TGFβ-RI and TGFβ-RII expression were induced by castration. These results are consistent with results recently reported by Kim et al. [18] and indicate a negative regulation of TGF-β receptor expression in the normal prostate by androgens, in a manner similar to regulation of TGF-β1 [21, 26]. The androgen regulation of the TGFβ-RI and TGFβ-RII expression in the Dunning tumour was found to be different from the regulation in the VP. TGFβ-RI and, especially, TGFβ-RII mRNA levels were elevated in the tumour, and only TGFβ-RI showed a clear-cut induction after castration treatment. High levels of TGFβ-RII have previously been associated with reduced malignancy and epithelial cell death and low levels with tumour promotion and TGF-β1 resistance in gastric, breast and colon carcinomas [16, 28, 33, 37, 42]. Referring to these studies, the elevated TGFβ-RII expression found in the Dunning tumour might indicate that the tumour has preserved the ability to respond to TGF-β, although the TGF-β inhibitory effect is overridden by some other growth stimulatory factors. Speculatively, it is, however, tempting to suggest that the high TGFβ-RII level in the Dunning tumour is indirectly caused by receptor defects, resulting in increased TGF-β1 and TGFβ receptor expressions because of lack of TGF-β responses. An ob-

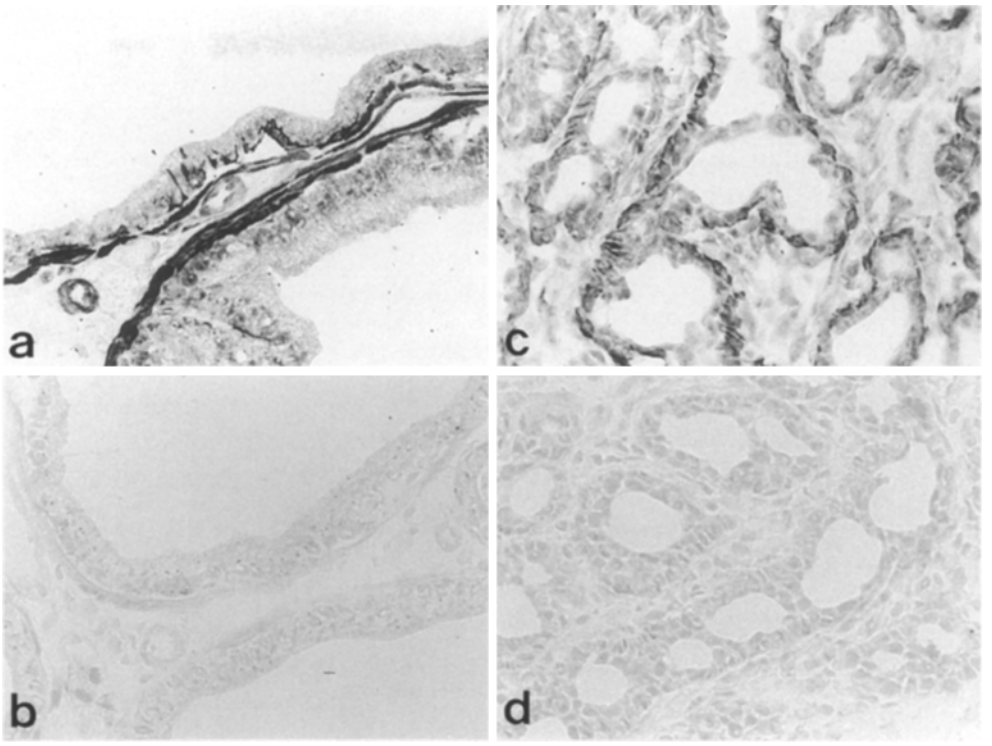

Fig. 5a–d Immunohistochemical staining of TGFβ-RII with the C-16 antibody in the rat VP and Dunning R3327 PAP adenocarcinoma, × 400. The TGFβ-RII antibody incubation showed receptor localisation in epithelial cells and SMC in the VP (**a**) and mainly in epithelial cells in the Dunning R3327 PAP tumor (**c**). Basal epithelial cells in the VP and the tumour showed a particularly strong staining. Control sections (**b, d**), incubated with preblocked antibodies, were unstained

vious defect would be some aberration in the TGF-β receptor expressions, making the tumour cells unresponsive for TGF-β1 inhibition of epithelial cell proliferation, but not necessarily abolish possible growth-promoting effects mediated by TGF-β1. Steiner and Barrack [35] have demonstrated that Dunning R3327 MATLyLu cells, when transfected with a TGF-β1-overproducing vector, produce larger, less necrotic and more metastatic tumours than control MATLyLu cells in vivo, although they are growth inhibited in vitro. It was suggested that these tumour-promoting effects originated in the ability of TGF-β1 to suppress the immune system, stimulate angiogenesis and enhance the invasive potential due to ECM modulation. It is, however, not known how TGF-β mediates all these different effects. TGFβ-RI and TGFβ-RII have been shown to signal as a heteromeric complex consisting of two TGFβ-RI and two TGFβ-RII molecules [4, 44, 48]. The possibility that other complex compositions exist can, however, not be ruled out, since the ratio between TGFβ-RII and TGFβ-RI has been demonstrated to affect the cellular response to TGF-β in some systems [9, 12, 32, 49].

The significantly increased RII/RI ratio in the VP at day 14 after castration might indicate a selection for cells with high TGFβ-RII levels, during the castration-induced involution of the prostate. Two antibodies against TGFβ-RII showed a particularly strong IHC staining of basal epithelial cells, and since these cells do not go into apoptosis in response to castration [8, 45], the high RII/RI ratio possibly reflects a selection for basal epithelial cells in the VP in response to castration. If this is true, the lack of apoptosis induction in the Dunning R3327 PAP tumour in response to castration might be associated with the elevated RII/RI ratios in the tumour. In contrast to castration treatment alone, combined castration and oestrogen treatment increased the RII/RI ratio in the Dunning tumour at day 7 after castration. This is in line with the previous reasoning about RII/RI ratio and apoptosis susceptibility, since the oestrogen treatment might, due to its ability to induce apoptosis at day 1 after treatment [46], contribute to a further selection for cells with high RII/RI ratios. The decrease in RII/RI ratio seen at day 1 after combined castration and oestrogen treatment might possibly, by an at present unknown mechanism, make some tumour cells enter apoptosis.

In a recent study, the TGFβ-RII was reported to be mainly located in the epithelial cells in PFA-fixed VP [18]. This TGF-β receptor location is consistent with the location we find in PFA-fixed VP and Dunning R3327 PAP tumour (results not shown). In the cryostat sections in this study, however, intense TGFβ-RI and TGFβ-RII immunostaining was found both in the epithelium and in the SMC. This staining pattern is logical, since previous in vitro studies have shown that not only primary cultures of prostatic epithelial cells [14], but also primary cultures of prostatic SMC [17], are growth inhibited by TGF-β1. Taken together, these findings indicate auto- and paracrine TGF-β1 effects in the normal prostate, since the TGF-β1 mRNA expression has previously been shown to take place in the epithelial cells [26]. They also indicate stromal-epithelial interactions in the mediation of TGF-β effects in prostatic tissues. On the basis of this present

work, it is, however, not possible to state the relative TGFβ-RI and TGFβ-RII protein expression between the different cell types in the prostate, since the antibodies used did not give entirely homogeneous results.

In conclusion, we have detected TGFβ-RI and TGFβ-RII mRNAs and proteins in the rat VP and in the Dunning R3327 PAP adenocarcinoma. In the VP, TGFβ-RI and TGFβ-RII mRNA levels were increased after castration, indicating a negative regulation of TGF-β receptors by androgens. In the Dunning tumour, TGFβ-RI, TGFβ-RII and RII/RI mRNA levels were elevated and only TGFβ-RI showed a clear-cut increase after castration. The receptors were located in epithelial cells and SMC in the VP and mainly in epithelial cells in the Dunning tumour. The elevated TGF-β receptor and RII/RI levels and the diminished androgen regulation of TGFβ-RII in the tumour distinguish the Dunning R3327 PAP tumour from the normal prostate and need to be further elucidated.

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