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Immunological characterization and activity of transglutaminases in human normal and malignant prostate and in prostate cancer cell lines

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Abstract Using biochemical assays, we compared enzyme activities with the immunoreactivity of antibodies against rat seminal transglutaminase (TGase), human erythrocyte TGase and guinea pig liver TGase in human normal prostate, primary prostatic carcinomas and prostatic carcinoma cell lines. Glandular cells of the epithelium were only exceptionally positive with the antibody against (rat) secretory TGase. Using the antibodies against tissue-type TGase, most immunoreactive cells were found in the basal cell layer of prostatic epithelium as well as in stroma (fibroblasts, endothelial cells), whereas immunoreactive glandular cells were sparse. In the case of benign prostatic hyperplasia, few, irregularly distributed secretory cells along with a small number of stromal cells were also immunoreactive with the tissue-type TGase antibody. In dedifferentiated carcinomas, immunoreactive cells were nearly completely absent. Of the prostate cancer cell lines, the LNCaP line showed neither TGase enzyme activity nor immunoreactivity, whereas the PC-3 cell line displayed significant enzyme activity and immunoreactivity. No hormone-dependent changes in either enzyme activity or immunoreactivity were recorded after in vitro treatment of the respective cell lines with estrogens, androgens and antiandrogens. As there is no correlation between androgen deprivation and TGase expression in nonmalignant and malignant human prostatic epithelial cells, TGase activity more likely indicates cellular lesions and consecutive repair mechanisms.

Key words Transglutaminases · Prostate cancer · Metastasis · Cellular wound repair

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Prostate cancer has now surpassed lung cancer in men as the most common cancer in the United States and its incidence and mortality are increasing [17]. In addition to parameters such as tumor cell ploidy, androgen dependence and proliferative activity, programmed cell death and metastatic potential are the essential determinants of malignancy. Of the enzymes related to these parameters of tumor aggressiveness, transglutaminases (EC 2.3.2.13) are the most multifarious in function in that they catalyze the formation of the very stable isopeptide bonds required in a number of biological responses. Of the latter, increased tissue stability [16], blood and semen clotting [38], wound-healing [37], formation of apoptotic bodies during programmed cell death [27], fixation of cells to extracellular matrix [2, 6] and stabilization of nuclear structure during growth arrest [9] are the most important [for reviews see 18, 21, 25].

Different TGases have been described, encoded by different cDNAs: keratinocyte TGase (type I) [28], tissue-type TGase (type II) [26] as well as plasma TGase (factor XIIIa) [22]. In rat, the cDNA of the seminal TGase derived from the coagulating gland [30] has been recently cloned [24] and was found to be closely related to a human prostate-derived cDNA encoding a putative secretory TGase (Grant FJ, personal communication).

In the present paper we have compared enzyme activities with the immunoreactivity of human prostate (normal, hyperplastic and tumor tissue as well as malignant cell lines with or without hormone treatment, respectively) as detected by antibodies directed against different TGase forms. Tissue-type TGase was the exclusive immunoreactive form in the intact gland accounting for all enzymic activity determined. As there was no correlation with an increase or decrease of immunoreactivities of the androgen receptor and 5α-reductase, respectively, tissue-type TGase is presumably expressed androgen independently. In addition to

tissue-type TGase at the epitheliostromal interface, factor XIIIa (blood serum TGase) was present in numerous stromal macrophages. Of the prostate cancer cell lines, LNCaP was unique, in that it is lacking both TGase immunoreactivity and enzyme activity. The apparent independence of TGase expression from hormonal stimuli, its expression in PC-3 and DU-145 and its absence in LNCaP cell lines, respectively, argue against a correlation with the degree of differentiation and metastatic potential and against hormone dependence.

Material and methods

Chemicals

[1,4-(*n*)-^{3}H]-putrescine dihydrochloride (specific activity 22.9 Ci/mmol) was purchased from Amersham-Buchler, Braunschweig, Germany. Putrescine dihydrochloride, phenylmethylsulfonylfluoride (PMSF), *N*,*N*-dimethylcasein (Trasylol) and monodansylcadaverine were obtained from Sigma, Munich, Germany. Protein A-Sepharose was purchased from Pharmacia, Freiburg, Germany.

Tissues

Fresh normal prostate and benign prostatic hytpertrophy (BPH) tissue was removed from surgical specimens obtained during total cystectomies (five cases), prostatectomies (three cases of prostate cancer) and transurethral resections. Samples were transported in Hank's balanced tissue medium to the Department of Anatomy (within 2 h). They were fixed in 2.5% paraformaldehyde in sodium cacodylate buffer (pH 7.3, 0.1 M) or Bouin's fixative, or shock-frozen in liquid nitrogen and used for cryostat sectioning. Some specimens were immediately homogenized and used for biochemical studies.

(Cysto)-prostatectomy specimens from a total of 27 patients (aged 48–72 years) with prostate cancer (18 cases, grades GI–GIII), pretreated for 3–4 months either with antiandrogens (300 mg/day cyproterone acetate, Androcur, $n = 8$), antiandrogen plus GnRH agonists (3×0.5 ml Suprefact, $n = 10$) or bladder cancer (untreated, $n = 9$), were fixed in Bouin's solution for 7 days, dehydrated and embedded in paraffin wax (courtesy of Prof. Dr. U. Tunn, Department of Urology, Municipal Clinics, Offenbach, Germany). Paraffin sections were cut at 5 µm thickness and were used for immunohistochemical reactions. In addition, autopsy specimens from prepubertal (one each of neonate, 1 year old, 5 years old) and (post) pubertal (two of 14 years old, one each of 15, 16, 17 and 19 years old) boys were received from the Department of Pathology, University of Marburg (courtesy of Prof. Dr. C. Thomas).

Prostate-derived cells

Cells derived from BPH stroma ("P-21", fibroblast morphology, PSA negative) and epithelium ("P-22", epithelial morphology with tight junctions in between, PSA positive) were grown in vitro and serially passaged (more than 15 passages). Dr. D. Wolf, Department of Biochemistry, University of Munich, Germany, provided us with the LNCaP and the DU-145 prostate cancer cell lines. The PC-3 prostate cancer cell line was received from Dr. J. Romijn, Department of Urology, University of Rotterdam, The Netherlands. Cells were grown in 75-cm^2 Falcon flasks in RPMI 1640 medium supplemented with 10% fetal calf serum, 4 mM L-glutamine, 0.2% sodium bicarbonate, 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml amphotericin B. Culture medium was changed every 2nd day. For endocrine experiments, fetal calf serum was stripped using 0.25% (w/v) charcoal, 0.025% (w/v) dextran T30 (30 min, 56 °C). Hormones and antihormones (dihydrotestosterone, estradiol benzoate, flutamide and hydroxyflutamide) were dissolved in ethanol and added to the incubation media to give a final concentration of 10^{-5}–10^{-6} M, respectively. Hormone treatment was performed for 3–6 days. For biochemical studies, cells grown to confluence were washed with phosphate-buffered saline (PBS) and scraped from the flasks in homogenization buffer, composed of 100 mM TRIS/HCl buffer, pH 7.6, 5 mM dithioerythritol (DTE) and 3 mM ethylene glycol tetraacetic acid (EGTA) in bidistilled water containing Trasylol (50 KIU/ml buffer) and 1 mM phenylmethylsulfonyl fluoride (PMSF) using a rubber policeman and were further handled as described below. Immunohistochemical studies were performed using cells grown on cover slips.

Tissue preparation

Tissue samples were placed in ice-cold homogenization buffer (see above, 10 ml/g tissue), disrupted with an Ultraturrax and further homogenized with a Potter-Elvehjem homogenizer (ten strokes each). Cells were lysed by repeated freezing in liquid nitrogen and thawing. Homogenates were centrifuged at $1000 \times g$ for 10 min at 4 °C. The supernatants were used for protein determination [10], enzyme activity tests and Western blotting. For cell fractionation studies and immunoprecipitation, the $1000 \times g$ supernatant was centrifuged at $100\,000 \times g$ for 60 min at 4 °C.

Enzyme activity determinations

Transglutaminase activity was determined by quantitating the incorporation of [^{3}H]putrescine into dimethylated casein [19]. Unless otherwise indicated, 2 mM free Ca^{2+} was used in activity tests. Substrate solution consisted of 65 µl casein solution [1% (w/v) *N*,*N*-dimethylcasein, 15 mM $CaCl_2$ in homogenization buffer], and 65 µl putrescine solution (310 µM *n*-putrescine and 22.9 µCi [1,4(*n*)-^{3}H]-putrescine equaling 1 nmol/ml in homogenization buffer) was equilibrated for 5 min at 37 °C in a shaking water bath. The reaction was started by adding 65 µl ice-cold sample (2.5 mg protein/ml) to the prewarmed solution and the incubation was continued at 37 °C. Samples of 60 µl each were taken at 3, 6 and 9 min and transferred into 500 µl 10% trichloroacetic acid (TCA). After incubation for 30 min on ice, samples were centrifuged (2 min at $10\,000 \times g$). Pellets were washed twice with 5% (w/v) TCA and finally with 95% ethanol, dissolved in 400 µl 0.1 M NaOH solution and mixed with 4 ml scintillation cocktail (Rotiscint, Roth, Karlsruhe, Germany). Incorporated radioactivity was counted in a Wallac scintillation counter (Pharmacia-LKB, Freiburg, Germany). Controls were performed by measuring the TGase activity in the presence of an excess of EGTA (5 mM). The specific enzyme activity was defined as the amount of putrescine (labeled and unlabeled) that was incorporated into dimethylated casein per minute by 1 mg tissue protein present in the $100\,000 \times g$ supernatant. As tissue-type TGase is known to be inhibited by GTP at low Ca^{2+} concentrations [1, 7], the effect of GTP on TGase was tested as well. Enzyme activity was measured at a GTP concentration of 0.5 mM and Ca^{2+} concentrations of 7, 5, 2.5 and 0.5 mM.

Antibodies

A monoclonal antibody (CUB-7401) against guinea pig liver transglutaminase was kindly provided by Dr. P. Birckbichler from the Samuel Roberts Noble Foundation (Ardmore, Okla., USA). Dr. J. Hock from Behring AG, Marburg, kindly supplied us with his

monoclonal antibody against recombinant factor XIIIa. A polyclonal rabbit antibody against human erythrocyte TGase was prepared in our own laboratory. The antigen was prepared according to the method of Brenner and Wold [11]. Purified enzyme displayed a molecular weight of 80 ± 5 kDa and its catalytic properties were identical with commercial guinea pig liver transglutaminase. Antibodies were produced by intradermal injection into the dorsal skin of New Zealand rabbits and repeated booster injections 4 and 6 weeks later. Crude antiserum was characterized by Western blotting. In addition, a polyclonal rabbit antiserum against secretory transglutaminase from rat coagulating gland (courtesy of Dr. J. Seitz) was used both in immunohistochemistry and Western blotting experiments. To obtain evidence of the androgen dependence of the enzyme by colocalization of TGase with androgen receptor, antibodies against synthetic peptide fragments of the androgen receptor, kindly provided by Prof. G. Verhoeven, Leuven, Belgium, and Prof. P. Tuohimaa, Tampere, Finland, were used. Antibodies directed against human 5α-reductase isoenzymes 1 and 2, respectively, were prepared in our laboratory [15].

Western blot analysis

Western blotting was performed according to Towbin et al. [35], using the semi-dry technique. Briefly, samples were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) in a Mini-Protean II System (BioRad, Munich, Germany) and transblotted onto nitrocellulose sheets. Nitrocellulose membranes were incubated with the respective primary polyclonal or monoclonal antibodies, and signal detection was performed with swine anti-rabbit IgG followed by rabbit antiperoxidase-peroxidase (PAP) complex [33] or with peroxidase-labeled anti-mouse IgG, respectively. Visualization of peroxidase activity was performed with diaminobenzidine (DAB) [20]. In control experiments, the primary antibody was replaced by rabbit non-immune serum or by phosphate-buffered saline (PBS). All antibodies tested were monospecific on Western blots, if not otherwise stated.

Immunoprecipitation

For immunoprecipitation, the 100000 × *g* supernatant of cell homogenates was adjusted to 0.5% Triton X-100 and 0.05% SDS. Precipitation was attempted by incubation of 200 µl homogenate with 5 µl antiserum against human tissue-type TGase or pre-immune serum, respectively (12 h at 4 °C), and 10 µl protein A-Sepharose for 2 h at room temperature. After extensive washing [25 mM sodium phosphate, pH 7.5, 3 mM ethylene diaminetetraacetic acid (EDTA), 3 mM DTE, 130 mM NaCl, 1% Triton X-100, 0.1% SDS], complexes were separated from the matrix by boiling the protein A-Sepharose in 40 µl 166 mM DTE and 20 µl SDS-PAGE sample buffer (187 mM TRIS/HCl, pH 6.8, 6% SDS, 30% glycerol, 20 mM DTE, 0.05% bromphenol blue) for 15 min, followed by centrifugation (10000 × *g*, 10 min, room temperature (RT)) and were further analyzed by Western blotting.

Immunohistochemistry

Cryostat or paraffin sections from solid tissue cut at 5–7 µm were mounted on chromalum-gelatin-coated slides. Cryostat sections were briefly fixed with ice-cold acetone, followed for 10 s in ice-cold methanol and washed for 10 min in PBS. Cells were grown on 0.5-cm-round cover slips and fixed as described above. The respective mono- and polyclonal antibodies were applied in a moist chamber at room temperature for 1 h (or 24 h at 4 °C, respectively). After extensive washing with PBS, the immunoperoxidase staining was performed as described for Western blotting.

Results

Enzyme activity

TGase activity was determined by measuring the amount of putrescine incorporated into TCA-precipitable material after incubation of tissue and cell homogenates with *N*,*N*-dimethylated casein and labeled putrescine. Specific enzyme activity of TGase decreased in prostatic tissues and cancer cell lines in the following order (Fig. 1): PC-3 (androgen-insensitive prostate cancer cell line) 1400 µU/mg (equaling 1400 pmol putrescine/min per milligram protein); DU-145 (androgen-insensitive prostate cancer cell line) 400 µU/mg; P-22, P-21 (BPH-derived epithelial and stromal cell lines) 350 and 200 µU/mg, benign prostatic hyperplasia tissue 20 µU/mg. No determinations were performed in primary carcinoma as it is difficult to obtain pure cancer tissue. In the LNCaP cell line (androgen-sensitive prostate cancer cell line), no activity could be detected at all. In no case did hormonal treatment of any of the cell lines (with dihydrotestosterone, estrogens or antiandrogens) result in a change in TGase activity (not shown).

To identify the subcellular localization of the enzyme, cell fractionation studies were performed. As shown in Fig. 2 for PC-3 cells, TGase activity was present in the soluble fraction of prostatic tissue. Less than 4% of total enzyme activity of homogenates was found in the 1000 and 100000 × *g* pellets (experiments with fresh prostatic tissue gave comparable results; not shown). To discriminate between secretory and tissue-type transglutaminase, GTP-inhibition experiments were conducted. At low calcium concentrations (0.5 mM), GTP is known as a reversible, noncompetitive inhibitor of the tissue-type TGase. In contrast, no

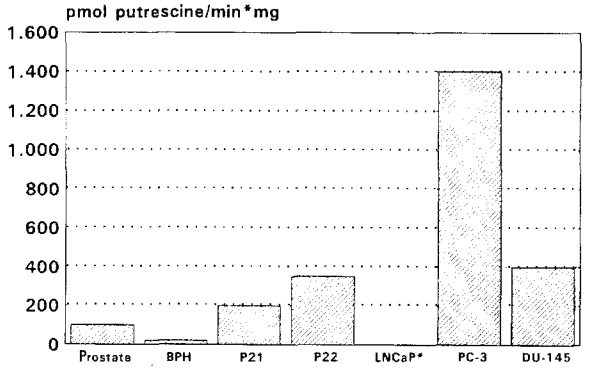

Fig. 1 Quantitative determination of TGase activity/mg protein in homogenates of prostatic tissues and cells by measuring incorporation of [^{3}H]putrescine into dimethylated caseine (four experiments, triplicate determinations). Activity is highest in the androgen-independent, fast-growing PC-3 cell line. Note that TGase activity is low in BPH tissue due to surgical treatment, whereas P21 and P22 were derived from surviving cells derived from BPH tissue. * Not detectable

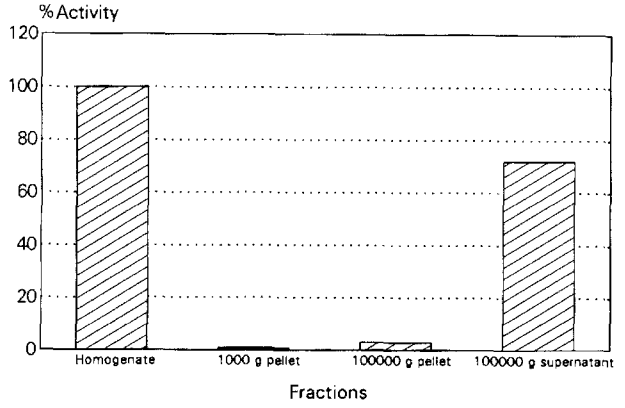

Fig. 2 Determination of relative TGase activity in different fractions of PC-3 prostate cancer homogenates. Most of the TGase activity is recovered in the 100 000 × *g* supernatant, indicating that the enzyme is present preferentially in the soluble nonparticulate form. The total recovery of the enzyme (supernatant and pellet) activity is less than 100%

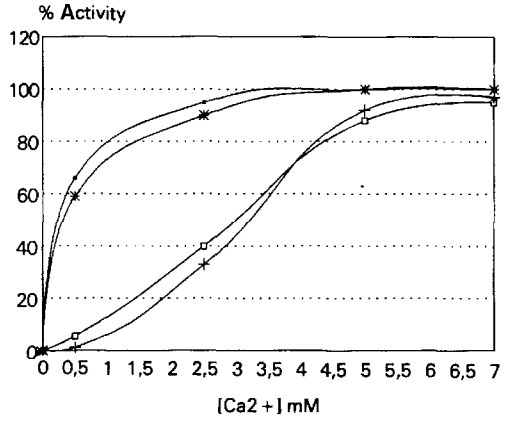

Fig. 3 TGase activity determinations of homogenates from human prostate and PC-3 cells at different calcium concentrations using GTP as a specific inhibitor of tissue-type TGase. At calcium concentrations in the range of 0.5 mM, activity is significantly inhibited in the presence of 0.5 mM GTP (maximum TGase activity is 100% at free [Ca^{2+}] more than 2 mM) -■- PC-3(−); -+- PC-3(+); -×- prostate (—); -□- prostate (+)

inhibition of secretory TGase from the secretion of rat dorsal prostate (and rat coagulating gland) can be achieved by addition of GTP. Incubation of supernatants from homogenates of prostatic tissue and of PC-3 cells with 0.5 mM GTP in the presence of decreasing levels of calcium (Fig. 3) showed the characteristic inhibition pattern of tissue-type TGase enzyme activity in both samples.

Western blotting and immunoprecipitation

Using the monoclonal antibody against guinea pig liver TGase (kindly provided by Dr. P. Birckbichler), a protein band at 80 kDa was immunoreactive in all prostatic tissues and cells except for the LNCaP line (Fig. 4). When the polyclonal antibody against tissue-type TGase from human red cells was used, a nearly identical staining pattern was achieved (Fig. 5). This latter antibody, however, did not cross-react with the guinea pig TGase (75 kDa, cf. Fig. 4). The finding that both antibodies react with the same protein, i.e., a human tissue-type TGase, could be further corroborated by immunoprecipitation experiments, where the tissue-type TGase was precipitated with the polyclonal rabbit antibody against human red-cell TGase. The precipitated protein was stained in Western blots with the monoclonal antibody against guinea pig liver TGase (Fig. 6). No precipitable antigen was present in the LNCaP homogenate.

An antibody against the secretory TGase (65 kDa) from rat coagulating gland, which is cross-reactive with

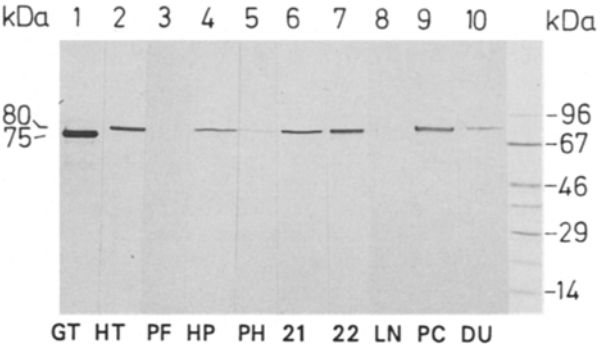

Fig. 4 Western blot analysis of homogenates of prostatic tissues and cells using a monoclonal antibody against tissue-type TGase. A band cross-reactive with the authentic 75-kDa antigen from guinea pig liver (*GT*) is seen at 80 kDa with human red-cell TGase (*HT*), human prostate (*HP*), benign prostatic hyperplasia (*PH*), prostatic stromal cell line P-21 (*21*), prostaic epithelial cell line P-22 (*22*) and prostate cancer cell lines PC-3 (*PC*) and DU-145 (*DU*). No immunoreaction is seen with the human blood serum factor XIIIa (*PF*) and the androgen-dependent LNCaP prostate cancer cell line (*LN*)

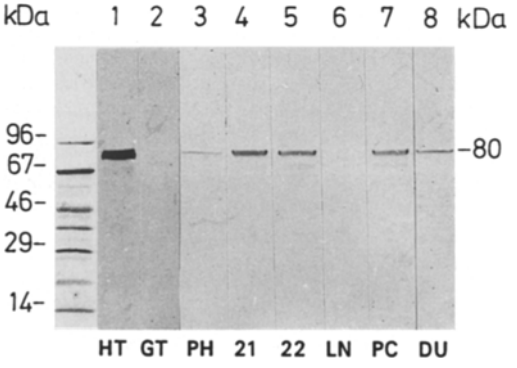

Fig. 5 Western blot analysis of prostatic tissues and cell lines using a polyclonal antibody against human tissue-type (red-cell) TGase. Abbreviations as in Fig. 4. The antibody is not cross-reactive with the guinea pig liver TGase. No reaction is seen in the LNCaP line

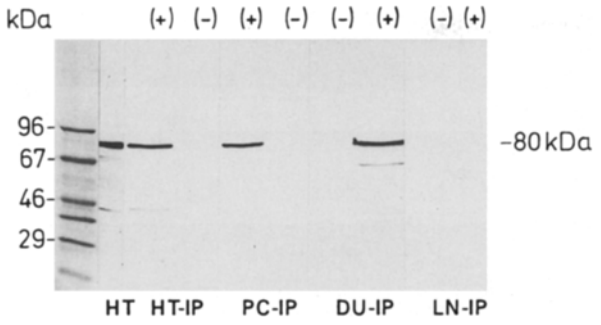

Fig. 6 Western blot analysis of immunoprecipitated TGase (purified or in cell homogenates). The antigen was immunoprecipitated using a polyclonal antibody against human tissue-type TGase [+] or pre-immune serum [−], respectively, and protein A-Sepharose. Immune complexes (*x-IP*; *x* = abbreviations as in Fig. 4) were separated on SDS-polyacrylamide gels, transferred to nitrocellulose paper and stained with the monoclonal antibody against guinea pig tissue-type TGase

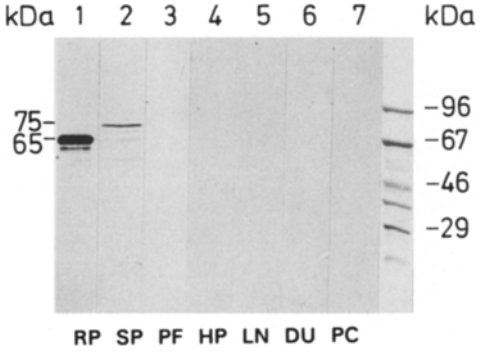

Fig. 7 Western blot analysis of prostatic tissue and cells using a polyclonal antibody against secretory TGase from rat dorsal prostate and coagulating gland. Abbreviations as in Fig. 4. *RP* rat dorsal prostate, *SP* human seminal plasma. The antiserum recognizes the authentic antigen at 65 kDa in rat dorsal prostate (*RP*) and a slightly larger molecule at 75 kDa in human seminal plasma (*SP*). No cross-reactivity is seen with any of the prostatic tissues and cells

an isoform in human seminal plasma (75 kDa, Seitz, unpublished, Fig. 7), did not stain a corresponding band in prostate homogenates or prostate cancer cells. A weak reaction of prostate homogenates, but not of cancer cell homogenates, was achieved with the factor XIIIa antibody (results not shown). This was interpreted to indicate the presence of factor XIIIa containing cells, such as macrophages, in the tissue.

Immunohistochemistry

In pubertal glands of two 14-year-old boys, prostatic epithelium reacted with the antiserum against red-cell TGase and somewhat less with the monoclonal tissue-type antibody. In contrast, no immunostaining was observed in infantile prostate tissue (5-year-old boy) with any of the antisera used (not shown). In the adult prostate (Figs. 8a, b, 9a–d), only a few glandular and some basal epithelial cells were immunoreactive with red-cell TGase (Fig. 8a). In addition to the former, subepithelial stromal cells and vascular endothelium were clearly immunoreactive (Fig. 8b) with the monoclonal antibody against tissue-type transglutaminase. The antibody against factor XIIIa selectively stained stromal macrophages in both normal (not shown) and carcinomatous prostate (Fig. 8c) tissue.

To identify the potential hormonal dependence of TGase immunoreactivity in the human prostate, sequential sections were stained with an antibody against the androgen receptor, human 5α-reductase isoenzyme 1 and human 5α-reductase isoenzyme 2, respectively. Immunoreactivities of individual cells for androgen receptor (Fig. 9a), 5α-reductase-1 (Fig. 9b) and 5α-reductase-2 (Fig. 9c) were correlated with TGase immunoreactivity (Fig. 9d) present in these cells. There was no obvious correlation in a sense that cells expressing strong TGase immunoreactivity would show specific alterations of androgen receptor or reductase immunoreactivity or display any other structural deficits.

An additional approach was to correlate TGase immunoreactivity with prostate cancer marker expression (PSA) in specimens removed from patients treated with antiandrogen for 4 months. Only very few tumor specimens showed some immunoreactivity for red-cell TGase (Fig. 10a) or, still less intense, tissue-type TGase (Fig. 10c). In metaplastic foci, immunoreactivity for tissue-type TGase was completely absent (Fig. 10b). In contrast to the very sporadic and irregular TGase immunoreactivity of cancer cells, distribution of immunoreactive PSA (Fig. 10d) was fairly homogeneous in most neoplastic acini of antiandrogen-treated specimens, although at clearly reduced levels.

In prostate cancer cell lines, a positive immunoreaction with tissue-type TGase was achieved with PC-3 and DU-145 cells (not shown), while no reaction was seen in LNCaP cells with any of the TGase antibodies used. Hormonal treatment of the cancer cell lines did not result in changes of distribution or intensity of the immunoreaction in either PC-3 or DU-145 cells. No immunoreaction for TGase was induced in the LNCaP cell line by hormonal treatment.

Discussion

In the present study, three forms of TGase have been considered in the human prostate as potentially contributing to the total enzyme activity of organ homogenates, namely (1) tissue-type TGase, (2) secretory TGase and (3) factor XIIIa. As deduced from the calcium-dependent GTP sensitivity of the enzyme activity and the immunoreactivity with mono- and polyclonal antibodies against tissue-type TGase, repectively (shown by immunoprecipitation, Western

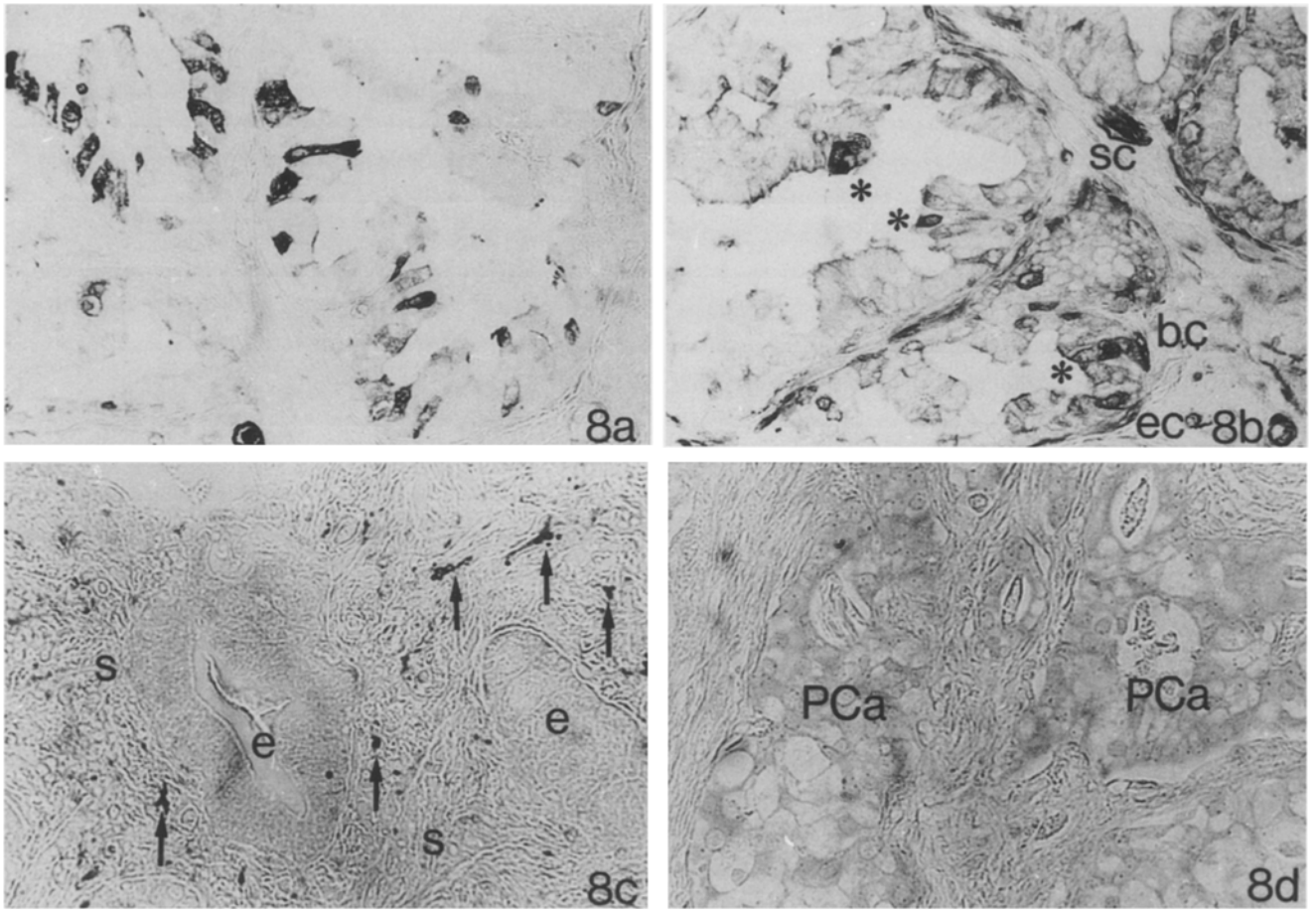

Fig. 8a–d Immunoreactivity of human prostatic tissue with antibodies against different transglutaminases (unlabeled antibody-enzyme method). **a** Normal prostate section incubated with an antibody against red-cell TGase; few epithelial cells are stained, whereas stromal cells are largely negative. **b** *Same* specimen, stained with a monoclonal antibody against tissue-type TGase showing only few immunoreactive secretory cells (*asterisk*), some positive basal cells (*bc*) and stromal smooth muscle (*sc*) and endothelial cells (*ec*). **c** Prostate cancer specimens containing several polymorphic macrophages immunoreactive for factor XIIIa (*arrows*). No reaction of epithelium (*e*) and stroma (*s*) is visible. **d** Prostate cancer (*PC*) specimen stained with the antiserum against rat seminal TGase derived from coagulating gland; only nonspecific background is seen, × 400

blotting and immunohistochemistry), tissue-type TGase was by far the most prevalent form in the human prostate. Immunoreactivity was present in the basal cell layer and a few secretory cells (in Bouin-fixed specimens) of the epithelium and also in the stroma. This distribution pattern is in line with recent findings on transglutaminase in rat prostate, at both the protein [39] and the RNA [4] levels. In human organs, tissue transglutaminase is usually more prevalent in mesenchymally derived cells such as endothelium, smooth and cardiac muscle, fibroblasts, mesangial cells and others [34]. There were slight differences in the distribution pattern of immunoreactive cells depending on the fixation used, as in Bouin-fixed specimens more immunoreactive cells were seen. As suggested by Thomázy and Fésüs [34], tissue-type TGase is preferentially expressed in epithelia during certain steps of differentiation. This also seems to apply for the prostatic epithelium, where TGase immunoreactivity was generally present in pubertal specimens. Prostatic epithelial maturation is an androgen-regulated process [3]. It is not imperative, however, that TGase expression in pubertal specimens is under direct androgen influence, but instead may be due to processes common in tissue maturation. Fésüs et al. [18] have regarded testosterone as an apoptosis-preventing factor whose deficiency would result in programmed cell death of prostatic epithelium with concomitant transglutaminase activation.In contrast to that assumption, in human prostate tissue-type TGase is obviously only transiently expressed in epithelium of pubertal glands, when testosterone levels have started to rise.

In our antiandrogen-treated prostate cancer specimens, in contrast, no altered expression of tissue-type TGase was monitored. Also, in the prostate cancer cell lines no increased TGase levels were seen after antiandrogen or estrogen treatment. Hence, hormonal regulation of tissue-type TGase is unlikely in prostate cancer

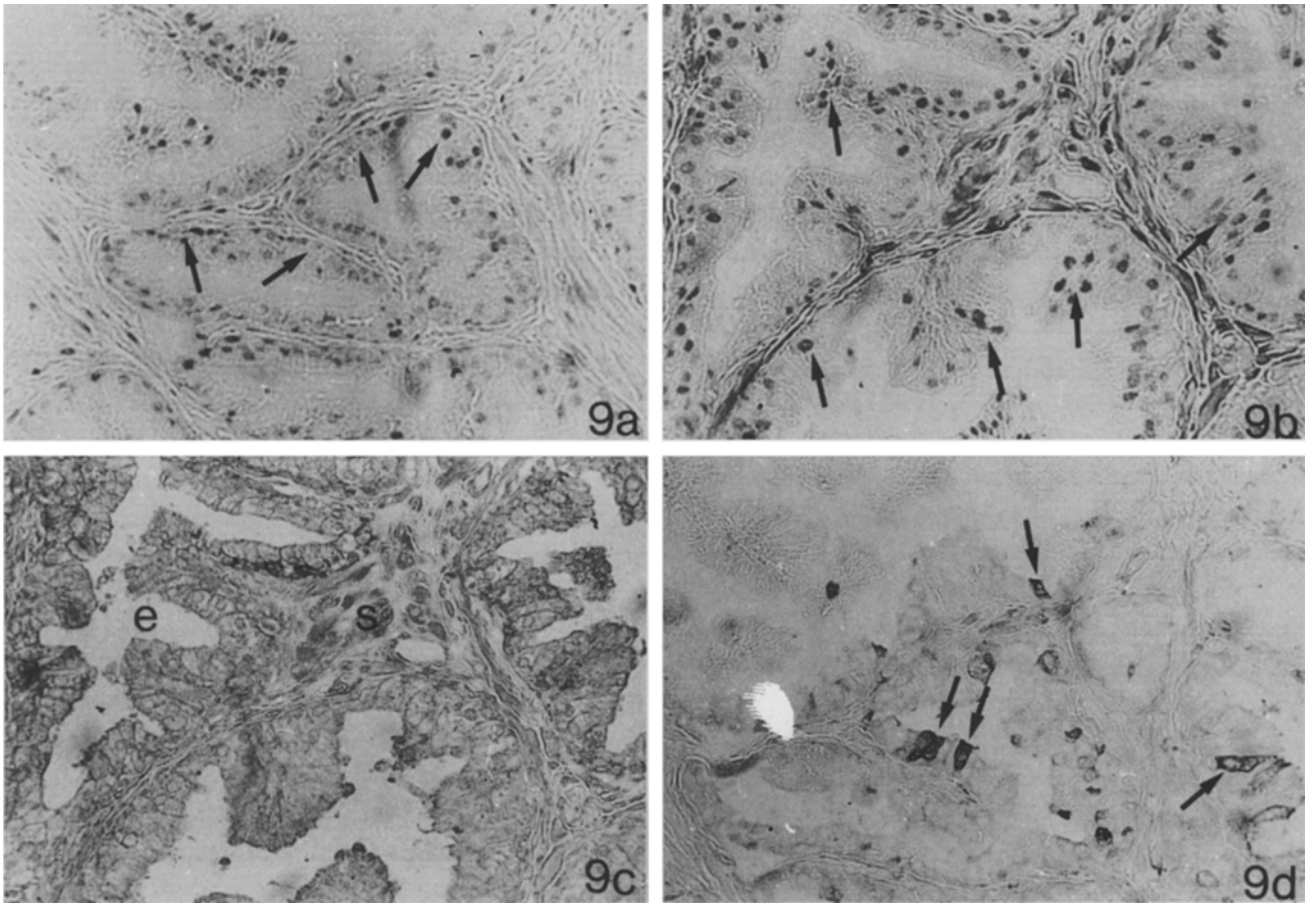

Fig. 9a–d Correlation of TGase and androgen marker immunoreactivity in intact human prostatic tissue (formaldehyde-fixed, paraffin-embedded). **a** Visualization of androgen receptor immunoreactivity; preferential staining of epithelial nuclei. Note the differences in staining intensity (arrows). **b** Same specimen processed for 5α-reductase-1 immunoreactivity. *Arrows* indicate strongly stained nuclei in epithelium. Stromal immunoreaction is clearly stronger than with the androgen receptor antibody. **c** Sequential section processed for 5α-reductase-2, showing a more or less generalized immunoreaction of both epithelial (*e*) and stromal (*s*) cells. **d** Sequential section processed for TGase immunoreactivity using the polyclonal rabbit antibody. Only few epithelial cells are stained (*arrows*). No correlation with androgen receptor and 5α-reductase expression has been found

as well as in the respective immunoreactive cell lines. This assumption corresponds well with the above-mentioned findings in prostates from antiandrogen-treated patients, where TGase immunoreactivity remained unchanged, whereas immunoreactivities of prostatic secretory proteins (acid phosphatase and PSA) were greatly diminished. In previous studies in rat ventral prostate [4], we found a constitutive expression of tissue-type TGase, which could not be influenced by hormonal manipulations. All these findings clearly argue against a hormonal regulation of prostatic tissue-type TGase.

Loss of tissue-type TGase has been related to a number of tumor-associated functions such as malignant transformation [8], proliferation [9], loss of polarized structure [5], cell-cell and cell-matrix contacts [2, 6, 31] and receptor-mediated endo- and exocytosis [12]. The question therefore was whether or not tissue-type transglutaminase was present in human prostatic cancer. In most solid tissue specimens, prostate cancer cells were devoid of TGase activity. The same applied for (squamous) metaplastic cells. Findings in the in vitro cell lines likewise argue against a relationship of transglutaminase expression, tumor dedifferentiation and hormone sensitivity in prostate cancer.

Interestingly, tissue-type TGase was expressed in both of the androgen-independent cell lines (PC-3, DU-145) and was absent in the androgen-dependent LNCaP line. It is not clear whether or not the deficiency in TGase expression in LNCaP is due to a defect on the DNA or RNA level. The LNCaP line, however, has a clearly higher differentiation level than, e.g., the PC-3 line, in that the former still contains the (mutated) androgen receptor and secretory proteins (acid phosphatase, prostate-specific antigen).

Tissue-type TGase exists in both membrane-bound and cytosolic forms [23]. In prostatic tissue, the cytosolic localization is predominant in both epithelium and stroma. In rat dorsal prostate we have

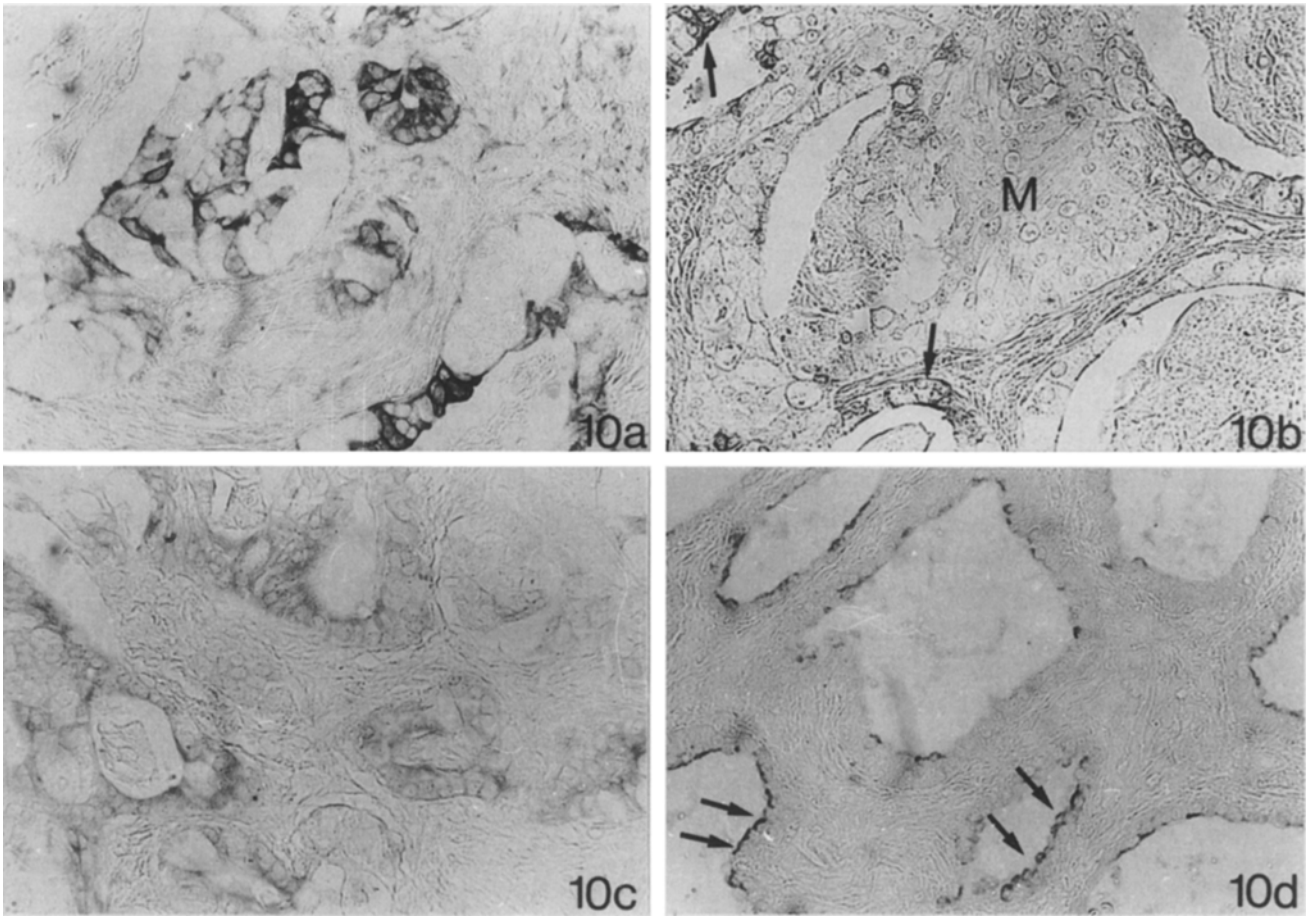

Fig. 10a–d TGase and tumor marker expression in neoplastic prostate tissue. **a** Prostate cancer (grade GIII, 4 months treatment with cyproterone acetate plus GnRH-agonist). A number of epithelial cells show tissue-type TGase immunoreactivity. **b** Same specimen, metaplastic area (*M*) free of TGase immunoreactivity. A fewer cancer cells show slight TGase immunoreactivity (*arrow*). **c** Same specimen stained for red-cell TGase. Immunoreaction of cancer cells is in the background range. **d** Same specimen, hyperplastic glands show some residual PSA (*arrows*) immunoreactivity (nonreleased secretory material?)

immunoelectron microscopically localized secretory TGase exclusively in the cytoplasm [32], the cisternae of endoplasmic reticulum being completely unlabeled.

There are a number of reports [29, 40] on metastasizing carcinomas with low TGase activity on a nonactive, perhaps particle-bound form of the enzyme that can be stimulated by mild proteolysis. In prostatic cell lines we found no such inactive particulate form. As already mentioned, in the relatively highly differentiated LNCaP line neither enzymic nor immunological TGase activity could be detected. Studies are under way to scrutinize the lack of TGase-mRNA in this line, using induction with retinoic acid or sodium butyrate [13, 14]. In preliminary experiments, TGase activity in PC-3 and DU-145 cells was induced by sodium butyrate and retinoic acid independent of any hormonal influence. This points to a more general function of the enzyme, perhaps in cellular wound repair.

Secretory transglutaminase could not uneqivocally be demonstrated in our specimens due to the fact that our antibody was directed against a rat prostatic secretory TGase. The presence of a secretory TGase in human prostate has been inferred from DNA sequence comparison studies (Grant FJ, personal communication), showing great homology between a cDNA derived from human prostate mRNA and the recently cloned cDNA encoding the secretory TGase from rat dorsal prostate. The latter form was found to show major differences from other forms of TGases [24]. A third TGase contributing to the total enzyme activity in human prostate is factor XIIIa, which was restricted to stromal macrophages, i.e., its activity was not present in prostatic epithelium, fibroblasts or smooth muscle cells. The number of immunoreactive macrophages varied over a broad range in the specimens studied. No clear-cut relationship to malignant or nonmalignant prostatic tissue was observed. There have been similar reports for other carcinomas, e.g., mammary cancer [36].

In summary, the present report does not support the contention of androgen-dependent regulation of tissue-type TGase either during programmed cell death in normal prostate or of expression in prostate cancer inversely correlated with metastatic behavior of the carcinoma. The secretory prostatic TGase, the cDNA of which has been recently cloned, could not unequivocally be demonstrated at the immunological level. Factor XIIIa TGase is present to some extent in prostatic stromal macrophages. The functional significance of tissue-type TGase in prostate and in tumor biology of prostate cancer remains to be assessed.

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