Tissue-specific and androgen-regulated expression of human prostate-specific transglutaminase

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Transglutaminases (TGases) are calcium-dependent enzymes catalysing the post-translational cross-linking of proteins. In the prostate at least two TGases are present, the ubiquitously expressed tissue-type TGase (TG_C), and a prostate-restricted TGase (TG_P). This paper deals with the molecular cloning and characterization of the cDNA encoding the human prostate TGase (hTG_P). For this purpose we have screened a human prostate cDNA library with a probe from the active-site region of TG_C. The largest isolated cDNA contained an open reading frame encoding a protein of 684 amino acids with a predicted molecular mass of 77 kDa as confirmed by *in vitro* transcription–translation and subsequent SDS/PAGE. The hTG_P gene was tissue-specifically expressed in the prostate, yielding an mRNA of approx. 3.5 kb. Furthermore, a 3-fold androgen-induced up-regulation of hTG_P mRNA expression has been demonstrated in the recently developed human prostate cancer cell line, PC346C. Other well established human prostate cancer cell lines, LNCaP and PC-3, showed no detectable hTG_P mRNA expression on a Northern blot. The gene coding for prostate TGase was assigned to chromosome 3.

INTRODUCTION

Transglutaminases (TGases, EC 2.3.2.13) are calcium-dependent enzymes that catalyse the acyl transfer reaction between peptide-bound glutamine residues and primary amine groups. This reaction results in the post-translational modification of proteins either by the incorporation of amines (such as polyamines) into proteins or by the cross-linking of proteins if the amine is a peptide-bound lysine. The cross-links are resistant to proteolytic enzyme degradation and thus contribute to the formation of highly insoluble macromolecules, which can be catabolized only by proteolysis of the protein chains [1,2].

Products of the enzymic actions of TGases can be found in most tissues [3] and body fluids [2,4,5]. A number of different TGases, with different structural properties and cellular origins, account for the formation of such reaction products. Well documented examples of TGases are plasma Factor XIIIa [6], keratinocyte TGase (TG_K) [7,8], epidermal TGase (TG_E) [9], tissue-type or cellular TGase (TG_C) [10,11] and prostatic TGase (TG_P) [5,12–14]. While the physiological functions of Factor XIIIa (blood clotting), TG_E and TG_K (squamous differentiation of the epidermis by formation of cornified cell envelopes) are evident, the roles of TG_C and TG_P are less well established. The ubiquitously expressed TG_C was suggested to be involved in processes associated with cell growth regulation, differentiation and programmed cell death [15–17]. This enzyme may take part in such diverse events as apoptotic body formation [3,18–20], extracellular matrix stabilization [2,21] and signal transduction [22]. TG_P was, until very recently, demonstrated only in rodents. In these animals, the enzyme is involved in the formation of copulatory plugs in the female genital tract after coitus [5], and may play a role in masking the antigenicity of the male gamete, thereby suppressing an immune response in the female genital tract against the sperm cells [23,24]. Isolation of the cDNA encoding rat prostate TGase (rTG_P) happened to be achieved when Ho et al. [12] attempted to clone the gene for DP1, an abundantly expressed rat dorsal prostate protein. DP1 occurred only in the dorsal rat prostate and the coagulating gland, where it comprised up to 25% of the total cellular protein, and its expression was found to be higher in intact than in castrated animals [12].

Also in the human prostate two types of TGases (TG_C and TG_P) might potentially be expressed. To isolate the human TGase similar to the rat DP1 enzyme, we have searched for human TG_P (hTG_P) cDNA clones in a human prostate cDNA library. Here we present the molecular cloning and characterization of hTG_P cDNA. In addition, this paper deals with chromosome assignment, organ specificity of hTG_P expression and androgenic regulation of hTG_P in human prostate cancer cells.

MATERIALS AND METHODS

Cell culture

LNCaP [25] and PC-3 [26] cells were cultured in RPMI medium supplemented with 7.5% (v/v) fetal-calf serum (FCS) and antibiotics. The PC346C cell line was recently established in our laboratory [27]. Cells were cultured in a Dulbecco's modified Eagle's medium (DMEM)/F12-based growth medium (GIBCO, Grand Island, NY, U.S.A.), essentially as described by Limon et al. [28], but dihydrotestosterone was replaced by the synthetic androgen R1881 (NEN, Boston, MA, U.S.A.) and with further supplementation of 2% (v/v) FCS. The growth of PC346C cells was stimulated by androgens [27]. To study androgen regulation

Abbreviations used: FCS, fetal calf serum; GPI, glycosylphosphatidylinositol; PEG, poly(ethylene glycol); PSA, prostate-specific antigen; TGase, transglutaminase; hTG_P and rTG_P, human and rat prostate TGase respectively; TG_C, human tissue-type TGase; TG_K, keratinocyte TGase; TG_E, epidermal TGase.

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The nucleotide sequence reported in this paper has been submitted to the Genbank®/EMBL Data Bank under the accession number U31905.

Table 1 Oligonucleotides used for isolation of cDNA clones and chromosomal localization of the hTG_P gene

Primer name*	Sequence †	Length	Orientation	Source	Position	Reference
λGT11.for	5-′GGTGGCGACGACTCCTGGAGCC-3′	22-mer	Sense	λGT11	–	
λGT11.rev	5′-GACACCAGACCAACTGGTAATG-3′	22-mer	Anti-sense	λGT11	–	
TRAGLU 1	5′-TA/TTGGCCAGTGCTGGGTTT-3′	19-mer	Sense	TG_C	955–973	[10]
TRAGLU 2	5′-TTC/GACCTCC/GGTGAAG/CACGA-3′	19-mer	Anti-sense	TG_C	1328–1310	[10]
TRAGLU R1	5′-GCTTTGGCCAGTGCTGGGTT-3′	20-mer	Sense	Rat DP1	820–839	[12]
TRAGLU R2	5′-CACCTCCGTGAACACAAATGT-3′	21-mer	Anti-sense	Rat DP1	1196–1176	[12]
HPSTG 1	5′-CTCATTCACATAGGTGTCCAC-3′	21-mer	Anti-sense	hTG_P	974–954	This paper
HPSTG 2	5′-TCATAGCACACATGGCCCT-3′	19-mer	Anti-sense	hTG_P	702–684	This paper

* GT11.for and GT11.rev represent the flanking GT11 primers used for PCR.
† TRAGLU 1 and TRAGLU 2 are degenerated primers as is indicated by a slash.

of gene expression, the cells were cultured in the absence and presence of 0.1 nM R1881 in growth medium containing dextran–charcoal-treated (DCC) serum. The cells were harvested from near-confluent cultures (after 7 days for LNCaP and 10 days for PC346C), and subjected to RNA isolation as described below.

Screening of a human prostate cDNA library

For the isolation of hTG_P, an oligo(dT) and random-primed λgt11 human prostate cDNA library (Clontech, Palo Alto, CA, U.S.A.) was screened with a random ^{32}P-labelled 380 bp probe from the active-site region of TG_C. This probe was obtained by PCR amplification with primer TRAGLU 1 and TRAGLU 2 (see Table 1 for list of primers) of this area from pSG5.hTG, a plasmid containing the entire cDNA sequence of human TG_C, which was kindly provided by Dr. P. J. A. Davies [11].

Hybond-N+ filters (Amersham, Aylesbury, Bucks., U.K.) were prehybridized with 100 μg/ml denatured salmon sperm DNA in poly(ethylene glycol) (PEG)-hybridization mix, composed of 0.25 M Na_2HPO_4,$2H_2O$ (pH 7.2), 0.25 M NaCl, 7% (w/v) SDS, 10% PEG 6000 and 1 mM EDTA, for 2 h. Hybridizations were carried out overnight at 50 °C, followed by two washes with 2×SSC/0.1% SDS and two washes with 1×SSC/0.1% SDS, each for 15 min at 50 °C (1×SSC: 0.15 M NaCl/0.015 M sodium citrate). Washed blots were covered with plastic film and exposed to RX medical X-ray film (Fuji) with intensifying screen for 4 days at −80 °C.

Positive plaques were picked up and rescreened at least twice until the selected plaques were completely purified. The inserts of these clones were amplified by PCR (see below), and directly sequenced with primer TRAGLU R2 (Table 1). The selected phage clone was purified and treated as described below under 'DNA sequencing'. Since this procedure resulted in the isolation of a clone containing only part of the hTG_P cDNA sequence (see the Results section), a 2.2 kb *Sac*I–*Hpa*I fragment of this clone was used as a probe to rescreen the same cDNA library under more stringent conditions with the purpose of isolating the entire hTG_P cDNA. Hybridizations and washes were performed at 65 °C instead of 50 °C. Selected recombinant phage clones were purified and treated as described below.

PCR amplification and primer removal

PCR amplifications of phage suspension aliquots, or of isolated DNA, were performed with superTaq polymerase (Sphaero Q, HT Biotechnology, U.K.) in the presence of 100 ng of each primer. All samples were first denatured at 94 °C for 5 min. Amplification followed in 35 cycles of 94 °C, 1 min; 55 °C, 1 min; 72 °C, 2 min; and a final extension at 72 °C for 10 min. PCR products were analysed by agarose gel electrophoresis. If necessary, primers were removed with the QIAquick PCR purification kit (Qiagen, Hilden, Germany).

DNA sequencing

DNA was isolated from the selected and purified recombinant phage clones following standard procedures [29] and the cDNA fragments were subcloned into the *Eco*RI site of pGEM-7Zf(+) (Promega, Madison, WI, U.S.A.) for sequencing. Plasmid isolations were performed with a Qiagen plasmid kit. Double-stranded DNA sequence analysis was performed by dideoxynucleotide chain termination using a T7 sequencing kit (Pharmacia, LKB Biotech, Uppsala, Sweden). Oligonucleotide primers used for walking primer sequencing were designed and synthesized on the basis of the obtained sequences (Pharmacia).

In vitro transcription–translation of hTG_P and TG_C cDNA

For *in vitro* transcription–translation studies the entire open reading frame of hTG_P cDNA was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen Corporation, San Diego, CA, U.S.A.). For comparison the entire 3.3 kb cDNA for TG_C [11] was subcloned into the *Eco*RI site of pcDNA3 (pcDNA3-TG_C). An empty pcDNA3 vector was used as a negative control. Subsequently, a one-tube, coupled transcription–translation was performed with the TNT® coupled reticulocyte lysate system from Promega using the T7 RNA promoter present in pcDNA3 for *in vitro* transcription of sense RNA. The reactions were performed on 1 μg of circular template DNA in a final volume of 25 μl according to the manufacturer's protocol. The transcription–translation was carried out at 30 °C for 90 min in the presence of ^{35}S-labelled methionine (Amersham, Aylesbury, Bucks., U.K.). Labelled products were separated by SDS/10%-PAGE. The gel was dried and the synthesized proteins were visualized by exposure to RX medical X-ray film (Fuji) for 4 h. Prestained markers (Novex, San Diego, CA, U.S.A.) were used as size standards.

Northern hybridization

Isolation of total RNA was carried out by the guanidine isothiocyanate/caesium chloride centrifugation method [29]. Total RNA (20 μg) was electrophoresed in a 1% agarose

formaldehyde gel and transferred to Hybond N+. RNA was fixed to the membrane by UV cross-linking. The blot was hybridized overnight at 60 °C with the 2.2 kb probe mentioned above in hybridization mix composed of 0.5 M Na_2HPO_4,$2H_2O$ (pH 7.2), 7% SDS, 1% BSA and 1 mM EDTA. The final wash step was in 0.3 × SSC/0.1% SDS at 60 °C for 30 min. The blot was exposed to RX medical X-ray film (Fuji) with intensifying screen for 4 days at −80 °C. The signals were quantified on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, U.S.A.).

A human multiple-tissue Northern blot (MTN II, Clontech) was used to determine the tissue specificity of hTG_P expression. Hybridization was performed with the same cDNA fragment as above using conditions recommended by the manufacturer. Washes were performed under high-stringency conditions (up to 0.1 × SSC/0.1% SDS at 63 °C for 30 min). The integrity of the blotted RNA was determined by hybridization with human *β*-actin cDNA.

Chromosomal localization of the gene encoding hTG_P

A human/hamster–human/mouse somatic cell hybrid panel (BIOS Research Laboratories, New Haven, CT, U.S.A.) was screened by PCR analysis with the HPSTG1–TRAGLU R1 primer set (Table 1). PCR reactions were performed on 50 ng of somatic cell hybrid DNAs as described above. An annealing temperature of 60 °C was used. The resulting products were analysed by agarose gel electrophoresis. The specificity of the reaction products was confirmed by blotting and subsequent hybridization with a ^{32}P random-labelled 2.2 kb *Sac*I–*Hpa*I hTG_P fragment. Hybridization and washes were as described above for Northern hybridization.

RESULTS

Isolation of cDNA clones

Screening under low-stringency conditions of approx. 1.2×10^6 p.f.u. of a *λ*gt11 human prostate cDNA library with a 380 bp fragment from the active-site region of TG_C yielded nine positive clones. Partial sequence analysis with primer TRAGLU R2 (Table 1) in the vicinity of the active site revealed that one clone (4.2) had high sequence similarity to rat DP1 [12]. The insert size was approx. 2.8 kb. The other clones appeared to be parts of TG_C cDNA. Sequence analysis of the entire insert of clone 4.2 showed an open reading frame encoding a protein of 574 amino acids. Comparison with the amino acid sequences of other TGases suggested that part of the 5′ end of the hTG_P cDNA was missing, probably due to a cloning artefact. To obtain further cDNA information, the same prostate cDNA library was rescreened under high-stringency conditions with the 2.2 kb *Sac*I–*Hpa*I fragment from clone 4.2 (see Figure 1 for restriction map). This resulted in a large number of positive plaques (about 500) of which 24 were isolated. As our main interest concerned the 5′ end of the hTG_P cDNA, a further selection was made by PCR with combinations of primer HPSTG 1 or HPSTG 2 and one of the flanking *λ*gt11 primers. In this way, three overlapping clones, clones 1.8, 1.12 and 1.17, were selected with inserts varying from 2.4 kb to 3.3 kb. The relative positions of the cDNAs of these clones are shown in Figure 1. *Eco*RI fragments of the three clones were sequenced. Whereas clone 4.2 appeared to lack a coding region for 130 amino acids at its N-terminus, both clone 1.8 and clone 1.17 contained the entire open reading frame of hTG_P cDNA. Further analysis showed a number of cloning artefacts (see Figure 1). Clone 1.17 contained amplifications of central parts of the hTG_P cDNA sequence at its 5′ and 3′ terminal ends, and 1.12 appeared to have a 5′ nucleotide stretch similar to that of clone 4.2 but situated more to the 5′-end.

cDNA sequence of hTG_P

Figure 2 shows the composite nucleotide sequence of the hTG_P cDNA as well as its deduced amino acid sequence. Its total length is 2983 bp with an open reading frame that corresponds to a protein of 684 amino acids with a predicted molecular mass of 77 kDa and a pI of 6.94.

Two start codons are present at the beginning of the open reading frame, preceded by an in-frame stop codon. Which one of the start codons is used for translational start cannot be deduced from the sequence. However, the DNA sequence surrounding the second methionine agrees well with the Kozak consensus sequence [30] with an A at position −3 and a G in position +4 (as the A of the ATG codon is designated +1). The 3′ non-coding region of hTG_P cDNA contains the variant polyadenylation signal, ATTAAA (Figure 2) [31].

The active-site amino acid sequence GQCWVF (residues 266–271) is present, suggesting that the clone encodes for an active TGase. Also other amino acids recently shown to be crucial for catalytic activity of TGases, but not part of the active centre, are present. These amino acids, Arg-37 (R), Arg-38 (R) [32,33], His-296 (H), His-327 (H) and Asp-350 (D) [34], occur in all human TGases. The amino acid sequence shows no typical Ca^{2+}-binding-site motif, like an EF-hand structure, but two regions enriched with acidic residues (residues 144–154 and 433–453) may reflect Ca^{2+}-binding sites. In addition, the putative hTG_P protein has seven potential N-glycosylation sites (sites are indicated in Figure 2).

In vitro transcription–translation of hTG_P and TG_C cDNA

To verify whether the isolated cDNA was able to produce a protein product and whether the size of the encoded protein corresponds to its predicted molecular mass, the entire coding region of hTG_P was cloned into pcDNA3 for *in vitro* transcription–translation. For this purpose, we first subcloned the 3′ *Eco*RI–*Eco*RI fragment of the largest clone, clone 1.17 (Figure 1), into the *Eco*RI site of pcDNA3. Subsequently, removal of the 3′ *Eco*RI site (including the 3′ artefact of clone 1.17) was achieved by cleavage with *Hpa*I (insert) and *Xba*I (vector). Because of the presence of a 5′ artefact in clone 1.17 (see above), we used the 5′ 184 bp *Eco*RI–*Eco*RI fragment from clone 1.8 for ligation to the *Eco*RI–*Hpa*I fragment of clone 1.17. In this way the entire coding region of hTG_P was cloned into pcDNA3 (pcDNA3-hTG_P) with only three nucleotides left in front of its start codon.

Figure 3 shows that both pcDNA3-hTG_P and pcDNA3-TG_C produced a single protein. The molecular mass of the hTG_P protein was established to be approx. 77 kDa which is in agreement with its predicted molecular mass. The TG_C construct yielded a protein of approx. 85 kDa which size is somewhat larger than its predicted molecular mass of 77 kDa. This observation is in agreement with the *in vitro* translation results of Gentile et al. [10]. The control plasmid produced no protein bands.

Expression of hTG_P in the prostate and in prostate-derived cell lines

To determine the expression pattern of hTG_P a human multiple-tissue Northern blot with poly(A)$^+$ RNA from eight different tissues including prostate was hybridized under high stringency conditions with a 2.2 kb *Sac*I–*Hpa*I fragment from clone 4.2. As

Figure 1 Restriction map and relative positions of cDNA clones for hTG_P

Black bars represent coding regions. Cloning artefacts are indicated by broken black bars. Clones 4.2 and 1.12 contained at their 5′ ends the same artificial nucleotide sequence but at different positions. Clone 1.17 contained amplifications of central parts of the hTG_P cDNA at its terminal ends. Clone 1.8 had only three nucleotides in front of its start codons.

shown in Figure 4 hTG_P mRNA was exclusively expressed in the prostate. A single mRNA was detected with a length of approx. 3.5 kb. Prolonged exposure of the Northern blot did not result in a positive signal in either of the other tissues tested. The hTG_P mRNA could be clearly distinguished from the approx. 4 kb TG_C mRNA as was determined by Northern-blot analysis of total RNA from benign prostate hyperplasia tissue (results not shown).

Next it was investigated whether hTG_P was also expressed in prostate cancer cell lines. For this purpose we performed Northern-blot analysis of total RNA from the well-known human prostate carcinoma cell line LNCaP as well as from a prostate cancer cell line, PC346C, recently developed in our laboratory [27] (see the Materials and methods section). While no hTG_P mRNA could be detected in LNCaP, PC346C expressed one mRNA of the same length (3.5 kb) as has been found in prostatic tissue. The Northern blot results for LNCaP and PC346C are shown in Figure 5. We could not detect hTG_P mRNA in the androgen-independent cell line PC-3 [26] (results not shown).

Androgen-regulated expression of hTG_P

To investigate androgen regulation of hTG_P expression, we used the androgen-responsive prostate cancer cell lines LNCaP and PC346C. Both cell lines were grown in the presence or absence of 0.1 nM R1881. Northern-blot analysis of total RNA from treated and untreated cells, using the 2.2 kb *Sac*I–*Hpa*I fragment from clone 4.2 as probe, showed that in PC346C cells the amount of hTG_P mRNA was 3-fold increased in the presence of R1881, demonstrating that the hTG_P expression can be up-regulated by androgen. However, even after induction with R1881, no hTG_P mRNA could be visualized in the LNCaP cells (Figure 5). The same blot was also hybridized with a probe for TG_C, but neither in LNCaP nor in PC346C cells was TG_C expression detected.

Chromosomal localization of the hTG_P gene

Chromosomal localization of the gene encoding hTG_P was performed by screening by PCR a panel of 20 human/hamster and human/mouse somatic-cell-hybrid cell lines that contain each of the human chromosomes in one or more of the hybrid DNAs (Figure 6). For this approach the primer pair HPSTG1–TRAGLU R1 was used (Table 1).

PCR analysis on chromosomal DNA isolated from PC346C cells (results not shown) resulted in a product of 250 bp, while PCR on hTG_P cDNA would have yielded a product of 150 bp (see Table 1). This difference in size is most probably due to the presence of an intron of approx. 100 bp in this part of the gene. Comparison with the organization of other TGase genes in that region is in favour of this assumption (see Kim et al. [35]).

Figure 2 Nucleotide and deduced amino acid sequence of hTG_P cDNA

The deduced amino acid sequence is numbered from the first methionine. The active-site sequence GQCWVF is bold-typed and the active-centre cysteine is marked with an asterisk. A putative polyadenylation signal is double-underlined. An in-frame stop codon preceding the start codons is indicated by a closed circle. Two putative Ca^{2+}-binding regions are underlined. Potential N-glycosylation signals (N-X-S/T) are indicated by closed triangles. The sequence deviates at several positions from the sequence recently published by Grant et al. [54]. A remarkable difference was found at positions 338–342 of the deduced amino acid sequence. In all four isolated clones we here found an insertion of the five amino acids DLPKG, which is also present at this position in all other TGases. Other amino acid substitutions were found at positions K313E, R466Q and F584N. In addition, the 23 nucleotides at the 3′-terminal end were found to be different (similar in clones 4.2, 1.12 and 1.17).

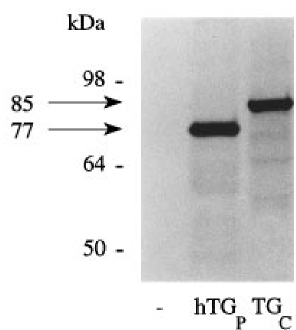

Figure 3 ***In vitro* transcription–translation of hTG_P and TG_C cDNA**

The entire open reading frames of the cDNAs encoding hTG_P (pcDNA3-hTG_P) and TG_C (pcDNA3-TG_C) were subcloned into the eukaryotic expression vector pcDNA3. One μg of each vector was used for a one-tube *in vitro* transcription–translation reaction. As a control pcDNA3 without insert (−) was used. Protein products were separated by SDS/10%-PAGE. On the left-hand side the molecular mass of the translated proteins and of the molecular mass standards are indicated.

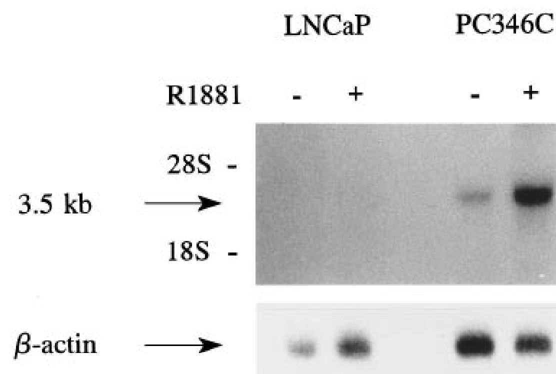

Figure 5 Androgen-regulated expression of hTG_P mRNA

Northern-blot analysis of PC346C and LNCaP grown in the presence (+) or absence (−) of 0.1 nM of the synthetic androgen R1881 and further treated as described in the Materials and methods section. Each lane contains 20 μg of total RNA. The blot was hybridized under high-stringency conditions (see the Materials and methods section) with a random ^{32}P-labelled 2.2 kb *Sac*I–*Hpa*I fragment from clone 4.2. Hybridization with a β-actin probe was performed as a control.

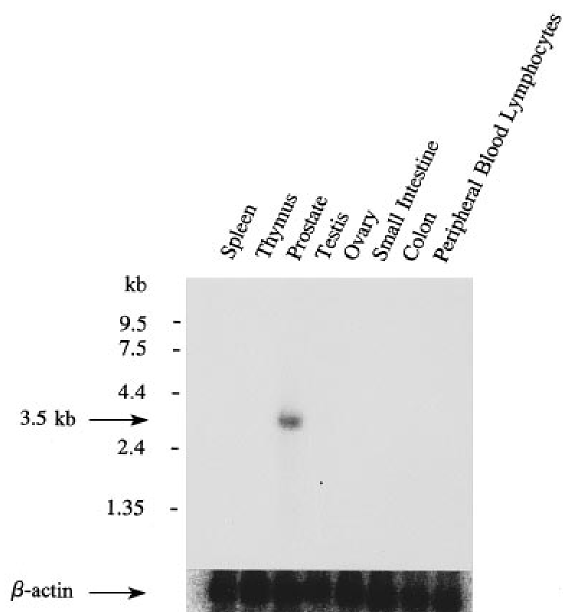

Figure 4 Northern-blot analysis of hTG_P expression in human tissues

A human multiple-tissue Northern blot was hybridized under high-stringency conditions (see the Materials and methods section) with a random ^{32}P-labelled 2.2 kb *Sac*I–*Hpa*I fragment from clone 4.2. Each lane contained 2 μg of poly(A^+) RNA. The integrity of the blot was determined by hybridization with human β-actin cDNA.

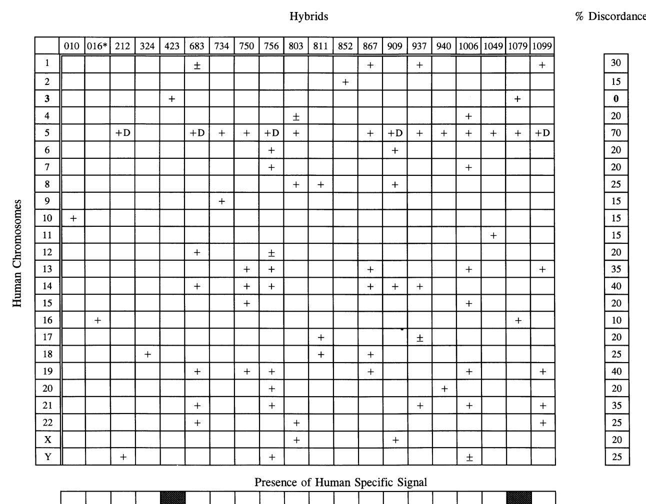

Figure 6 Assignment of the hTG_P gene to human chromosome 3

A panel of human/hamster and human/mouse somatic cell hybrid DNAs was screened by PCR with the HPSTG1–TRAGLU R1 primer set (see Table 1). PCR products generated by this primer pair were separated on a 1% agarose gel (results not shown). DNA was amplified from somatic hybrid cell lines (cell line designations are indicated at the top of the Figure), as well as human, hamster and mouse genomic DNA. The Figure shows correlation between the presence of human chromosomes in the somatic cell line panel (as described by the manufacturer), and the presence or absence of a specific PCR product. On the left-hand side the human chromosome number is indicated. The somatic cell lines in which a specific PCR product could be detected are indicated in the lower bar by the shaded areas. Percentage discordance is shown at the right. Assignment of the hTG_P gene to chromosome 3 had a discordance of 0%, while all other human chromosomes were excluded by $\geqslant 10\%$ discordance. Symbols: an asterisk indicates human/mouse somatic cell hybrid, all others are human/hamster hybrids; D, multiple deletions; $+$, $> 30\%$ of the cells contain the given chromosome; $\pm$, 5–30% of the cells contain the given chromosome.

As presented in Figure 6 only the presence or absence of human chromosome 3 is concordant with the presence of a PCR product, whereas at least two discordancies were observed for all other chromosomes. The authenticity of the obtained 250 bp products was proven by hybridization with the 2.2 kb *Sac*I–*Hpa*I fragment from clone 4.2. No cross-reactivity was found with hamster or mouse chromosomal DNA present in the DNA hybrids.

DISCUSSION

In this paper, we describe the molecular characterization of the cDNA encoding a prostate TGase, hTG_P. The composite cDNA sequence consists of 2983 nucleotides, containing an open reading frame encoding a protein of 684 amino acids. The deduced amino acid sequence contains significant similarity with other members of the human TGase family (Figure 7). Especially in the region of the active site these sequences display a high degree of

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HTGASEP    MMDASKELQ...................................................    9
HTGASEK    ---GPRSDVGRWGGNPLQPPTTPSPEPEPEPDGRSRRGGGRSFWARCCGCCSCRNAADDD   60
HFXIIIA    -...........................................................    1
HTGASEC    -AEELVLERCDLELE.............................................   15
HTGASE3    -AA.LGVQSINWQKA.............................................   14

HTGASEP    ......................................................VLHIDF   15
HTGASEK    WGPEPSDSRGRGSSSGTRRPGSRGSDSRRPVSRGSGVNAAGDGTIREGMLVVNG-DLLSS  120
HFXIIIA    .....SETSRTAFGGRRAVPPNNSNAAEDDLPTVELQGVVPRGVNLQEFLNVTS-HLFKE   56
HTGASEC    ............................................................   15
HTGASE3    ............................................................   14
                                ▼▼
HTGASEP    LNQDNAVSHHTWEFQTSSPVFRRGQVFHLRLVLN.QPLQSYHQLK.LEFSTGPNPSIAKH   73
HTGASEK    RSDQ-RRE---D-YEYDELIV----P--ML-L-S.RTYE-S.DRIT--LLI-N--EVG-G  178
HFXIIIA    RWDT-K-D---DKYENNKLIV----S-YVQIDFS.R-YDPRRD-FRV-YVI-RY-QEN-G  115
HTGASEC    ...T-GRD---ADLCREKL-V----P-W-T-HFEGRNY-ASVDSLTFSVV---A--QEAG   72
HTGASE3    ...F-RQA---DK-SSQELIL----N-QVLMIM-KGLG..SNERLEFIDT---Y--ESAM   69

HTGASEP    TLVVLDPRTPSDHYN.WQATLQNESGKEVTVAVTSSPNAILGKYQLNVKTGNHILKSEE.  131
HTGASEK    -H.-II-VGKGG.SGG-K-QVVKA--QNLNLR-HT-----I--F-FT-R-QSDAGEFQLP  236
HFXIIIA    -Y.IPV-IVSELQSGK-G-KIVMREDRS-RLSIQ---KC-V--FRMY-AVWTPYGVLRTS  174
HTGASEC    -KARF.-LRDAVEEGD-T--VVDQQDCTLSLQL-TPA--PI-L-R-SLEASTGYQG...S  128
HTGASE3    -KA-F.-LSNGSS.GG-S-V--ASN-NTL-ISIS-PAS-PI-R-TMALQIFSQGGI...S  124

HTGASEP    ....NILYLLFNPWCKEDMVFMPDEDERKEYILNDTGCHYVGAARSIKCKPWNFGQFEKN  187
HTGASEK    FDPR-EI-I------P--I-YVDH--W-Q--V--ES-RI-Y-TEAQ-GERT--Y---DHG  296
HFXIIIA    RNPETDT-I------ED-A-YLDN-K--E--V---I-VIFY-EVND--TRS-SY----DG  234
HTGASEC    SFVLGHFI----A--PA-A-YLDS-E--Q--V-TQQ-FI-Q-S-KF--NI-------QDG  188
HTGASE3    SVKLGTFI------LNV-S---GNHA--E--VQE-A-IIF--STNR-GMIG-------ED  184

HTGASEP    VLDCCISLLTE.......SSLKPTDRRDPVLVCRAMCAMMSFEKGQGVLIGNWTGDYEGG  240
HTGASEK    ---A-LYI-DR.......RGMPYGG-G---N-S-VIS--VNSLDDN-------S---SR-  349
HFXIIIA    I--T-LYVMDR.......AQMDLSG-GN-IK-S-VGS--VNAKDDE---V-S-DNI-AY-  287
HTGASEC    I--I-LI--DVNPKFLKNAGRDCSR-SS--Y-G-VGSG-VNCNDD----L-R-DNN-GD-  248
HTGASE3    I-SI-L-I-DRSLNFRRDAATDVAS-N--KY-G-VLS--INSNDDN---A---S-T-T--  244

                                     ▼▼▼▼▼▼                        ▼
HTGASEP    TAPYKWTGSAPILQQYYNTKQA.VCFGQCWVFAGILTTVLRALGIPARSVTGFDSAHDTE  299
HTGASEK    -N-SA-V--VE--LS-LR-GYS.-PY--------VT-----C--LAT-T--N-N-----D  408
HFXIIIA    VP-SA----VD--LE-RSSENP.-RY--------VFN-F--C------I--NYF----ND  346
HTGASEC    VS-MS-I--VD--RRWK-HGCQR-KY-------AVAC----C----T-V--NYN----QN  308
HTGASE3    RD-RS-D--VE--KNWKKSGFSP-RY--------T-N-A--S----S-VI-N-N-----D  304

                                      ▼                      ▼
HTGASEP    RNLTVDTYVNENGKKITSMTHDSVWNFHVWTDAWMKRPDLPKGYDGWQAVDATPQERSQG  359
HTGASEK    TS--M-I-FD--M-PLEHLN----------N-C--------S-F----V-------T-S-  468
HFXIIIA    A--QM-IFLE-D-NVNSKL-K-----Y-C-NE---T-----V-FG------S----N-D-  406
HTGASEC    S--LIEYFR--F-EIQGDKSEMI.----C-VES--T----QP--E----L-P----K-E-  367
HTGASE3    ---S--V-YDPM-NPLDKGSDSV.------NEG-FV-S--GPP-G---VL----------  363

HTGASEP    VFCCGPSPLTAIRKGDIFIVYDTRFVFSEVNGDRLIWLVKMVNGQEELHVISMETTSIGK  419
HTGASEK    I-----CSVES-KN-LVYMK---P-I-A---S-KVY-QRQDDGSFKIVY-...-EKA--T  525
HFXIIIA    MYR---ASVQ--KH-HVCFQF-AP---A---S-LIYITA-KDGTHVVEN-...DA-H---  463
HTGASEC    TY----V-VR--KE--LSTK--AP---A---A-VVD-IQQDD.-SVHKSINRSLIV..-L  424
HTGASE3    --Q---ASVIGV-E--VQLNF-MP-I-A---A--IT--YDNTT-KQWKNSVNSH-I..-R  421

HTGASEP    NISTKAVGQDRRRDITYEYKYPEGSSEERQVMDHAFLLLSSEREHRRPVKENFLH..MSV  477
HTGASEK    L-V---ISSNM-E----L--H----DA--KAVET-AAHG-KP..NVYANRGSAEDVA-Q-  583
HFXIIIA    L-V--QI-G-GMM---DT--FQ--QE---LALET-LMYGAKKPLNTEG-MKSRSNVD-DF  523
HTGASEC    K----S--R-E-E---HT-----------EAFTR-NH......LNKLAEKEETGMA-RI  477
HTGASE3    Y-------SNA-M-V-DK-------DQ----FQK-LG......KLKPNTPFAATSSMGLE  475

HTGASEP    QSDDVLL.GNSVNFTVILKRKTAALQNVNILGSFELQLYTGKKMAKLC.DLNKTSQIQGQ  535
HTGASEK    EAQ-AVM.-QDLMVS-M-INHSSSRRT-KLHLYLSVTF---VSGTIFK.ETK-EVELAPG  641
HFXIIIA    EVENAV-.-KDFKLSITFRNNSHNRYTITAYL-ANITF---VPK-EFK.KETFDVTLEPL  581
HTGASEC    RVGQSMNM-SDFDVFAHITNN--EEYVCRL-LCARTVS-N-ILGPECGTKYLLNLTLEPF  537
HTGASE3    TEEQEPSIIGKLKVAGM-AVGKEVNLVLLLKNLSRDTKTVTVN-TAWTIIY-G-LVHEVW  535

HTGASEP    VSEVTLTLDSKTYINSLAILDDEPVIRGFIIAEIVESKEIMASEVFTSFQYPEFSIELPN  595
HTGASEK    A-DRVTMPVA..-KEYRPH-V-QGAMLLNVSGHVK--GQVL-KQHTFRLRT-DL-LT-LG  699
HFXIIIA    SFKKEAV-IQ..AGEYMGQ-LEQASLHF-VT-R-N-TRDVL-KQKS-VLTI--II-KVRG  639
HTGASEC    SEKSVPLCILYEK..YRDC-TESNL-KVRALLVEPVINSYLLA-RDLYLEN--IK-RILG  595
HTGASE3    KDSA-MS--PEEE..AEHPIKISYAQYERYLKSDNMIRITAVCK-PDESEVVVERDIILD  593

HTGASEP    TGRIGQLLVCNCIFKNTLAIPLTDVKFSLESLGISSLQTSDHGTVQPGET..IQSQIKCT  653
HTGASEK    AAVV--ECEVQIV---P-PVT--N-V-R--GS-LQRPKILNV-DIGGN--..VTLRQSFV  757
HFXIIIA    -QVV-SDMTVTIQ-T-P-KET-RN-WVH-DGP-VTRPMKKMFREIR-NS-..V-WEEV-R  697
HTGASEC    EPKQKRK--AEVSLQ-P-PVA-EGCT-TV-GA-LTEE-KTVEIPDPVEAGEEVKVRMDLV  655
HTGASE3    NPTLTLEVLNEARVRKPVNVQMLFSNPLD-PVRDCV-MVEGS-LLLGNLKIDVPTLGPKE  653

HTGASEP    PIKTGPKKFIVKLSSKQVKEINAQKIVLITK                               684
HTGASEK    -VRP--RQL-AS-D-P-LSQVHGVIQ-DVAPAPGDGGFFSDAGGDSHLGETIPMASRGGA  817
HFXIIIA    -WVS-HR-L-ASM--DSLRHVYGELD-Q-QRRPSM                           732
HTGASEC    -LHM-LH-LV-NFE-DKL-AVKGFRN-I-GPA                              687
HTGASE3    RSRVRFDILPSRSGT--LLADFSCNKFPAI-AMLSIDVAE                      693
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Figure 7 Comparison of the deduced amino acid sequence of hTG_P with other human TGases

HTGASEP, K, C, 3 and HFXIIIA represent hTG_P, TG_K [55], TG_C [10], TG_E [9], and plasma Factor XIIIa [56], respectively. Bold-type residues represent amino acids present in all sequences. Hyphens indicate identities with the amino acids of hTG_P. Dots represent gaps introduced for optimal alignment. Closed triangles indicate amino acids, mentioned in the text, important for the catalytic function of TGases.

similarity not only concerning the deduced primary amino acid sequences, but also the hydrophobicity directly C-terminal to the active site [6,7]. The amino acid sequences of hTG_P and rTG_P share an identity of 53 %. The overall identity of hTG_P with the other TGases is approx. 30 %. The degree of similarity is very low C-terminal to the putative Ca^{2+}-binding site at positions 433–453. Essential amino acids are present in the deduced amino acid sequence, such as the active-site cysteine which is an absolute prerequisite for TGase activity as well as the amino acids directly surrounding this cysteine [34]. Also, other amino acids which are away from the active centre in the primary sequence but are required for catalytic function of TGases, because of their putative influence on the protein conformation, are present [32–34]. Together, these results suggest that we have isolated a cDNA encoding an active prostatic TGase.

We assigned the gene encoding hTG_P unambiguously to chromosome 3. No other human genes encoding TGases known so far are localized on this chromosome. The other TGase genes are assigned to chromosome bands 6p24-p25, Factor XIIIa [36], 14q11.2-q13, TG_K [37], and TG_C as well as TG_E to 20q11.2-20q12 [38,39]. While this paper was in preparation, a report by Gentile et al. confirmed our results by sublocalizing the hTG_P gene to chromosome band 3p21.33-p22 [40].

In vitro transcription–translation of hTG_P cDNA demonstrated that the core protein without any post-translational modifications behaves as a protein of approx. 77 kDa on SDS/PAGE, which is slightly larger than the 65 kDa rat DP1 [12,41]. Results of Seitz and co-workers [42,43] suggested that a protein of about the same molecular mass, with cross-reactivity to an antibody against rTG_P, is present in human semen.

The putative hTG_P protein contains seven N-glycosylation consensus sequences distributed over the entire protein. Four of these are at identical sites in hTG_P and rTG_P. However, this does not necessarily result in glycosylation at these sites. Both TG_C and Factor XIIIa contain several potential N-glycosylation sites, but no glycosylation has been observed [1,6]. In the rat, TG_P was reported to be glycosylated [12,13] and to contain a glycosylphosphatidylinositol (GPI) anchor which may protect the protein from autoaggregation [13]. Until now, the identified GPI-anchored proteins were demonstrated to contain a hydrophobic sequence at their C-terminal sequence [44]. Based upon these results one would not expect a GPI-anchor for either rTG_P or hTG_P, because of the lack of a hydrophobic stretch of amino acids at their C-terminus. It cannot be excluded, however, that for the attachment of the GPI-anchor to rTG_P other sequence determinants might be used. It would be of interest to know whether hTG_P is also glycosylated and has a protective GPI-anchor.

Androgens are essential for the growth and maintenance of prostate tissue and control the formation of prostatic secretions. Immunohistochemical and mRNA studies have shown that RatDP1 or rTG_P is androgen-regulated [12,45]. The expression of rTG_P was demonstrated to be restricted to the dorsal lobe of the prostate and the coagulation gland. Expression of the hTG_P gene leads to an mRNA of approx. 3.5 kb both in prostatic tissue and in the androgen-responsive prostate cancer cell line PC346C. The androgen-independent prostate cancer cell line PC-3 (results not shown) as well as the androgen-responsive prostate carcinoma cell line LNCaP showed no expression of hTG_P mRNA as determined by Northern-blot hybridization. As far as the tissues tested are concerned, hTG_P is exclusively expressed in the prostate. Presently we are examining a more extended number of tissues to confirm prostate-specificity of hTG_P expression. Androgen-dependent expression of hTG_P was observed in PC346C cells. The level increased 3-fold in the presence of 0.1 nM of the synthetic androgen R1881. The question remains open as to whether the androgen-up-regulated expression of hTG_P is due to a direct regulation at the transcriptional level or is a consequence of stabilization of the mRNA. Indirect evidence obtained by Ho et al. [12] suggests that the latter is the case for rTG_P and that androgen acts through prolongation of the rTG_P mRNA half-life. At present, only a few human genes have been described, for example the prostate-specific genes human glandular kallikrein-1 and prostate-specific antigen (PSA), as being under androgenic control at the transcriptional level. Both human glandular kallikrein-1 and PSA contain androgen-responsive elements in their promoters [46,47]. Kinetic studies of the androgen-regulated expression of hTG_P mRNA and isolation and characterization of the promoter region of hTG_P may elucidate at which level androgens regulate its expression. Additionally, comparison of the promoter regions of genes specifically expressed in the prostate may give some clues to tissue-specific regulatory elements.

Unlike hTG_P, TG_C is up-regulated by androgen withdrawal in the rat ventral prostate [48]. However, this may be an indirect consequence of the induction of the apoptotic pathway by removal of androgens from the circulation.

In contrast to rTG_P, for which there is a very obvious function in the formation of the copulatory plug [5], little is known about the physiological role of hTG_P. Most likely the function of hTG_P is in semen. However, in contrast to PSA, for example, which is also excreted by the prostate, the deduced protein sequence does not contain an obvious signal sequence for excretion [46]. The same is true for rTG_P [12]. For this protein evidence has been obtained that secretion might occur via an alternative pathway [45], which is also suggested for two other TGases, Factor XIIIa and TG_C [2]. Some effort has been made to obtain evidence for the presence and function of hTG_P in human semen. A report of Lilja and Laurell [49] suggests that, in contrast to rat seminal clotting, TGase activity may not be essential for human semen coagulation. They hypothesized that, besides disulphide bridges in particular, non-covalent protein interactions are important for the clot structure. In addition, they did not find TGase activity in human semen, while Porta et al. [50] demonstrated TGase activity in human seminal plasma of normal individuals. Others found only TGase activity in infertile patients with semen having prolonged liquefaction time [42,43]. Besides a role of hTG_P in coagulation, it may function in suppressing the sperm surface antigenicity, thereby preventing an immunological response in the female genital tract. Suppression of antigenicity through the involvement of the cross-linking activity of TGase has been reported for rat and rabbit, as well as for human sperm [23,24,51]. At last, an intracellular role of hTG_P cannot be excluded.

Presently, prostate cancer is the most common cancer in the male population of the U.S.A. and Europe. A main problem in the treatment of clinically localized prostate cancer is the presence of micrometastasis at the time of radical prostatectomy [52]. Monitoring the presence of prostate cancer cells in tissues and blood before and after treatment may offer the possibility to optimize treatment methods [53]. Given its prostate-restricted expression, as suggested by our results, hTG_P may represent a valuable alternative to PSA as a marker for detection of prostate cancer cells, for instance by reverse-transcription PCR analysis.

We are interested in prostatic TGases and their possible role in the regulation of human prostate tumour growth and metastasis (TG_C) and their usefulness as differentiation or tumour markers (hTG_P) in prostate cancer. The isolation of the cDNA for hTG_P and some first clues to the specificity and regulation of its expression may help us to discriminate between the prostatic TGases, and to get more insight into the putative function(s) of

the hTG_P protein. Currently, antisera against the C-terminal region of either hTG_P or TG_C are being generated to enable us to discriminate at the protein level as well.

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