Upregulation of PTTG1 is associated with poor prognosis in prostate cancer

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Abbreviations:
ERG, V-Ets Avian erythroblastosis virus E26 oncogene homolog; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; PTTG1, pituitary tumor-transforming gene 1 protein; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; TMA, tissue micro array

Pituitary tumor-transforming gene 1 (PTTG1) is a regulator of chromosome stability. PTTG1 overexpression had been associated with tumor aggressiveness in several cancer types. To examine its prognostic utility in prostate cancer, a tissue microarray including 12,427 tumors with clinical and molecular data was analyzed by immunohistochemistry. PTTG1 immunostaining was largely absent in normal prostate epithelial cells. In cancers, staining was considered weak in 5.4%, moderate in 5.6% and strong in 0.8%. Strong staining was linked to advanced pT stage, high classical and quantitative Gleason grade, high Ki67-labeling index (all \( P < 0.0001 \)) and lymph node metastasis (\( P = 0.0083 \)). The prognostic impact of PTTG1 expression was independent of established preoperative and postoperative prognostic features. Comparison with molecular features revealed that PTTG1 upregulation was associated with nine of 12 common genomic deletions (\( P < 0.05 \)), p53 alterations and high androgen receptor levels (\( P < 0.001 \) each), but was unrelated to the TMPRSS2:ERG fusion status. In conclusion, these data identify PTTG1 as a strong and independent prognostic feature in prostate cancer. PTTG1 measurement, either alone or in combination with other biomarkers might be instrumental for determining prostate cancer aggressiveness.

KEYWORDS
ERG, prostate cancer, PTTG1, TMA

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INTRODUCTION

Prostate cancer is the most common cancer type among men in developed countries.1 Prostate cancer exhibits a highly variable disease course ranging from incidentally discovered biologically insignificant tumors to rapidly progressing cancer with overt metastasis. Reflecting the broad heterogeneity of tumor aggressiveness, treatment strategies vary from conservative approaches like ‘watch and wait’ or active surveillance to radical operation. Inadequate treatment, that is, under- or over-treatment, may have severe implications on quality of life, morbidity and mortality.2,3 Prognostic markers to assess the ‘aggressiveness’ of any individual tumor at time of diagnosis are of particular importance to assure optimal treatment decisions and to minimize unnecessary or delayed therapy.

Pituitary tumor-transforming gene 1 (PTTG1; securin) is an ubiquitously expressed regulator of sister-chromatid separation also acting as a transcription factor.4 In various tumor types, including gastrointestinal carcinomas (colorectal, esophageal, gastric and hepatocellular carcinoma5–9), urological tumors (renal cell carcinoma, bladder cancer and testicular tumors9–11) and gynecologic tumors (breast cancer, endometrial cancer12,13) overexpression of PTTG1 is associated with unfavorable tumor phenotype and adverse prognosis.1,5–9 For instance, a dramatic reduction of survival time from 9 to 1.8 years was found for adenocortical carcinomas with low compared to high PTTG1 levels.14 Interaction with p53, stimulation of c-MYC expression through direct promoter interaction and induction of chromosomal instability via either regulation of sister-chromatid separation or inhibition of DNA damage repair mechanisms have been proposed as possible cause for the oncogenic effects of PTTG1 overexpression.15–18 Cell culture experiments have consistently found increased cell proliferation in cases of PTTG1 activation.19–21 Several data suggest a role of PPTG1 in prostate cancer. Lin et al. demonstrated higher expression of PTTG1 in prostate cancers as compared to normal epithelial cells.22 In an immunohistochemical study on 64 prostate cancers, Cao et al. described elevated PTTG1 expression levels to be linked to poor survival.23 Zhang et al. described high level PTTG1 expression in prostate cancer metastases.24 Moreover, regulation of PTTG1 via androgen receptor has been demonstrated through an androgen response element in the −851 to −836 region of the PTTG1 promoter region24 and PTTG1 overexpression was also described in prostate cancer tumor cell lines.24–27

To better understand the potential clinical relevance of immunohistochemical PTTG1 measurement in prostate cancer, we took advantage of our large prostate cancer tissue microarray (TMA) and evaluated PTTG1 in more than 12,000 prostate cancers and compared PTTG1 expression with various clinical, pathological and molecular parameters.

MATERIAL AND METHODS

Patients

Specimens from radical prostatectomies from 12,427 patients, operated between 1992 and 2012 at the Department of Urology and the Prostate Cancer Center Martini Clinic at the University Medical Center Hamburg-Eppendorf were available. Histopathological data was retrieved from the patient files, including tumor stage, prostate-specific antigen (PSA) serum levels, Gleason grade, nodal stage and stage of the resection margin. In addition to the classical Gleason categories, ‘quantitative’ Gleason grading was performed as described before.28 In brief, for every prostatectomy specimen, the percentages of Gleason 3, 4 and 5 patterns were recorded in cancerous tissues as part of the regular process of Gleason grading. Gleason 3+4 and 4+3 cancers were subdivided according to the percentage of Gleason 4 patterns in eight subgroups: 3+4 ≤ 5% Gleason 4, 3+4 6–10%, 3+4 11–20%, 3+4 21–30%, 3+4 31–49%, 4+3 50–60%, 4+3 61–80% and 4+3 > 80% Gleason 4. Additional groups were defined by the presence of a tertiary Gleason 5 pattern, including 3+4 Tert.5 and 4+3 Tert.5. From 11,623 patients, follow-up data were available with a median follow-up period of 49 months (Table 1). PSA recurrence was defined as the time point at which postoperative serum PSA was at least 0.2 ng/mL and increasing at subsequent measurements. The TMA manufacturing process has been described in detail before.29 In short, a single 0.6 mm tissue core was taken from one donor tissue block of each patient. The donor block was merely selected for high tumor cell content, but not for a particular tumor focus or Gleason pattern in order to avoid a potential selection bias towards focal but potentially non-representative tumor areas. Twenty-seven TMA blocks were constructed, each containing between 144 and 522 tumor samples. A multitude of molecular markers have been previously analyzed on this particular tumor cohort, that is, earlier sections of the same TMA, before. The results have been compiled into an associated molecular database that contains data on ERG expression in 10,678,30 ERG break-apart fluorescence in situ hybridization (FISH) analysis in 7099 (expanded from31), Ki67-labeling index in 4426 (expanded from32), p53 immunohistochemical data in 10,040 (expanded from33), androgen receptor expression in 7971 cases (expanded from31) and deletion status of 3p14 (FOXP1) in 7201 cases (expanded from36), PTEN (10q23) in 6803 cases (expanded from37), 12p13 (CDKN1B) in 6187 cases (expanded from38), 8p21 in 7,001 cases (expanded from39), 5q13 in 6962 cases (unpublished), 5q21 (CHD1) in 8074 (expanded from34), 6q15 (MAP3K7) in 6069 cases (expanded from35), 8p21 in 7,001 cases (expanded from39), PTEN (10q23) in 6803 cases (expanded from37), 12p13 (CDKN1B) in 6187 cases (expanded from38), 12q24 in 7435 cases (expanded from39), 13q14 in 7499 cases (expanded from40),
Table 1 Composition of the prostate prognosis tissue microarray

| Study cohort on TMA (%) | Biochemical relapse among categories (%) |
|-------------------------|------------------------------------------|
| (n = 12 427)            |                                          |
| Follow-up (months)      |                                          |
| n                       | 11 623 (24.8%)                           |
|                         | (93.5%)                                  |
| Mean                    | 61.4                                     |
| Median                  | 49                                       |
| Age (years)             |                                          |
| ≤50                     | 334 (2.7%)                               |
|                         | 61 (18.3%)                               |
| 51–59                   | 3061 (24.8%)                             |
|                         | 690 (22.5%)                              |
| 60–69                   | 7188 (58.2%)                             |
|                         | 1676 (23.3%)                             |
| ≥70                     | 1761 (14.3%)                             |
|                         | 454 (25.8%)                              |
| Pretreatment PSA (ng/mL)|                                          |
| <4                      | 1585 (12.9%)                             |
|                         | 247 (15.6%)                              |
| 4–10                    | 7480 (60.9%)                             |
|                         | 1403 (18.8%)                             |
| 10–20                   | 2412 (19.6%)                             |
|                         | 794 (32.9%)                              |
| >20                     | 812 (6.6%)                               |
|                         | 425 (52.3%)                              |
| pT stage (AJCC 2002)    |                                          |
| pT2                     | 8187 (66.2%)                             |
|                         | 1019 (12.4%)                             |
| pT3a                    | 2660 (21.5%)                             |
|                         | 919 (34.5%)                              |
| pT3b-4                  | 1528 (12.3%)                             |
|                         | 941 (61.7%)                              |
| Gleason grade           |                                          |
| ≤3+3                    | 2848 (22.9%)                             |
|                         | 235 (8.3%)                               |
| 3+4                     | 6679 (53.8%)                             |
|                         | 1258 (18.8%)                             |
| 3+4, 21–30%             | 1327 (10.7%)                             |
|                         | 283 (21.3%)                              |
| 3+4, 2–10%              | 703 (5.7%)                               |
|                         | 218 (31%)                                |
| 3+4, 31–49%             | 579 (4.7%)                               |
|                         | 200 (34.5%)                              |
| 3+4, Tert 5             | 433 (3.5%)                               |
|                         | 114 (26.3%)                              |
| 4+3                     | 1210 (9.7%)                              |
|                         | 580 (47.9%)                              |
| 4+3, Tert 5             | 646 (5.2%)                               |
|                         | 333 (51.5%)                              |
| ≥4+4                    | 596 (4.8%)                               |
|                         | 360 (60.4%)                              |
| qGleason grade          |                                          |
| ≤3+3                    | 2848 (22.9%)                             |
|                         | 235 (8.3%)                               |
| 3+4, ≤5%                | 1621 (13.1%)                             |
|                         | 171 (10.5%)                              |
| 3+4, 6–10%              | 1636 (13.2%)                             |
|                         | 262 (16%)                                |
| 3+4, 11–20%             | 1327 (10.7%)                             |
|                         | 283 (21.3%)                              |
| 3+4, 21–30%             | 703 (5.7%)                               |
|                         | 218 (31%)                                |
| 3+4, 31–49%             | 579 (4.7%)                               |
|                         | 200 (34.5%)                              |
| 3+4, Tert 5             | 433 (3.5%)                               |
|                         | 114 (26.3%)                              |
| 4+3, 50–60%             | 482 (3.9%)                               |
|                         | 199 (41.3%)                              |
| 4+3, 61–100%            | 541 (4.4%)                               |
|                         | 282 (52.1%)                              |
| 4+3, Tert 5             | 646 (5.2%)                               |
|                         | 333 (51.5%)                              |
| ≥4+4                    | 596 (4.8%)                               |
|                         | 360 (60.4%)                              |
| pN stage                |                                          |
| pN0                     | 6970 (91%)                               |
|                         | 1819 (26.1%)                             |
| pN+                     | 693 (9%)                                 |
|                         | 458 (66.1%)                              |
| Surgical margin         |                                          |
| Negative                | 9990 (81.9%)                             |
|                         | 1872 (18.7%)                             |
| Positive                | 2211 (18.1%)                             |
|                         | 1009 (45.6%)                             |

Percentage in the column ‘Study cohort on TMA’ refers to the fraction of samples across each category. Percentage in column ‘Biochemical relapse among categories’ refers to the fraction of samples with biochemical relapse within each parameter in the different categories. Numbers do not always add up to 12 427 in the different categories because of cases with missing data. Abbreviations: AJCC, American Joint Committee on Cancer; PSA, prostate-specific antigen; TMA, tissue micro array.

17p13 (TP53) in 8307 cases (expanded from33) and 18q21 in 7032 cases (expanded from41). The use of archived diagnostic left-over tissues for manufacturing of tissue microarrays and their analysis for research purposes as well as patient data analysis has been approved by local laws (HmbKHG, §12,1) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). The whole study has been carried out in compliance with the Helsinki Declaration.

**Generation of PTTG1 expressing control cells**

Human cervical carcinoma (HeLa) cells were cultured in Dulbecco's Modified Eagles Medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S) at 37°C and 5% CO₂. A construct encoding for PTTG1 (Origene cat. #RC211038) was transfected into competent Escherichia coli cells (One ShotTM Top10, ThermoFisher Scientific, Waltham, MA). After 24 h, amplified plasmid was extracted (cat #740579, Macherey-Nagel, Düren, Germany) and transfected into 3 × 10⁶ HeLa cells (JetPEI DNA Transfection reagent, Polyplus-transfection S.A., Illkirch, France). Cells were grown for another 24 h, harvested, pelleted, fixed in 4% buffered formalin overnight and embedded in a paraffin block. Non-transfected HeLa cells were used as negative controls.

**Immunohistochemistry**

Tissue micro array sections were freshly cut and immunostained within one day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121°C in pH 7.8 Tris-EDTA-Citrate buffer. Primary PTTG1 antibody (rabbit; polyclonal; 1:50; Sigma Aldrich HPA008890, St. Louis, MO, USA) was applied at 37°C for 60 min. Bound antibody was subsequently visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer’s directions. Specificity of the primary anti-PTTG1 antibody used in our study had been in-house validated in HeLa cells transfected with PTTG1 cDNA (Origene cat. #RC211038) (Fig. S2) and externally in the Human Protein Atlas by means of immunohistochemistry and protein mapping. The staining pattern observed in our study was typically cytoplasmic accompanied by membranous staining, and in rare cases also nuclear. Because these findings are consistent with the staining pattern described in the Human Tissue Atlas, we consider our staining as suitable to detect PTTG1. Accordingly, all kind of staining (membranous/ cytoplasmic and nuclear) was considered for analysis. The staining intensity (0, 1+, 2+ and 3+) and the fraction of positive tumor cells were separately recorded for each tumor containing tissue spot. A final score was built of these two parameters according to the following criteria as previously reported:31,43 a negative score was given when complete absence of staining was observed. A weak score when staining demonstrated intensity of 1+ in ≤ 70% of the tumor cells or a staining intensity.
of 2+ in ≤ 30% of the tumor cells. Of note, such weak staining is clearly discernable from negative staining as it involves staining intensities of 1+ to 2+ with different fractions of stained cells. A moderate score when a staining demonstrated intensity 1+ in > 70% of tumor cells, a staining intensity of 2+ in > 30% but in ≤ 70% of the tumor cells or a staining intensity of 3+ in ≤ 30% of the tumor cells; and a strong score when staining demonstrated intensity of 2+ in > 70% of the tumor cells or a staining intensity of 3+ in > 30% of the tumor cells.

PTTG1 staining (weak, moderate or strong) and negative PTTG1. In this analysis, positive PTTG1 expression was associated with a high risk of PSA recurrence (P < 0.0001; Fig. 2a).

PTTG1 and TMPRSS2:ERG fusion status

For the tumors with informative PTTG1 immunostaining, data on TMPRSS2:ERG fusion status (obtained by FISH) were available in 5361 cases and for ERG immunopexpression in 7967 cases. Both FISH and immunohistochemistry (IHC) data were concordant in 4992 of 5274 cases (94.7%), for which both analyses had been successfully performed. A statistically significant difference of PTTG1 expression was not observed between tumors with and without ERG protein expression or positive ERG fusion status (Fig. S1). A subgroup analysis evaluating the impact of PTTG1 on PSA recurrence revealed a prognostic role of PTTG1 expression in both ERG negative (P < 0.0001, Fig. 2b) and ERG positive cancers (P = 0.0014, Fig. 2c)

PTTG1 and chromosomal deletions

For all 12 analyzed chromosomal regions, PTTG1 immunostaining was stronger and more frequent in case of deletion (Fig. 3). These differences reached statistical significance in nine of 12 deletions (P < 0.05 each).

PTTG1 and Ki67-labeling index, p53 status and androgen receptor expression

Presence and intensity of PTTG1 immunostaining was significantly linked to increased Ki67-labeling index (Table S1; P < 0.0001). This also applied for any analyzed subgroup with Gleason >3+3 (P < 0.0001 for Gleason 3+4, and 4+3; P = 0.0028 for Gleason ≥4+4). PTTG1 immunostaining was also associated with p53 expression levels (P < 0.001; Fig. 4). Frequency of PTTG1 staining was 10.9% in the cohort of p53 negative tumors and increased to 19.3% and 29.8% the cohorts of p53 low and p53 high cancers. The analysis of all cancers, as well as of ERG positive and negative subgroups revealed a marked raise of PTTG1 levels with increasing androgen receptor expression (P < 0.0001 each; Fig. 5).

Multivariate analysis

To assess the prognostic effect of PTTG1 expression in prostate cancer, four different types of multivariable analyses were
performed as previously described.\textsuperscript{44,45} Postoperative parameters (pT, pN, surgical margin status, preoperative PSA value, Gleason grade) were incorporated in Scenario 1. Scenario 2 included the same postoperatively available parameters except pN. The lymph node status was excluded because the surgical indication and extent of surgery is not standardized for prostate cancer, which may introduce a bias towards high grade cancers. Scenarios 3 and 4 intended to represent the preoperative situation to the best possible extent: clinical tumor stage (cT stage), preoperative PSA and prostatectomy Gleason grade were incorporated in Scenario 3. Since a postoperative determination of the Gleason grade is ‘better’ than the preoperatively determined Gleason grade (subjected to sampling errors and consequently under-grading in more than one third of cases\textsuperscript{46}), this parameter was replaced by the original preoperative biopsy Gleason grade in Scenario 4. PTTG1 expression yielded independent prognostic information in all four scenarios ($P < 0.0274$; Table S2).

\textbf{DISCUSSION}

Our study reveals a strong and prognostic impact of elevated PTTG1 expression in prostate cancer, which was independent of established histopathological parameters. Detectable PTTG1 staining was observed in 11.8% of prostate cancers. This was lower than found by Cao \textit{et al.} and Zhu \textit{et al.} who described PTTG1 expression in 84% of prostate cancers.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{example_images}
\caption{Examples of pituitary tumor-transforming gene 1 protein (PTTG1) immunostainings in (a) normal prostate glands and cancer spots with (b) lack of staining (c), weak staining, (d) moderate staining, and (e) strong staining.}
\end{figure}
64 cancers and 83% of 41 cancers. Reasons for these discrepancies may include the use of different antibodies, staining protocols or the scoring criteria to detect positivity in examined slides. Higher expression levels of PTTG1 in many prostate cancers compared to adjacent non-neoplastic prostate epithelial cells suggests a role of PTTG1 in prostate cancer development. These findings are in line with previous studies showing higher expression in prostate cancer than in normal prostatic epithelium and also with earlier functional data. Various studies had suggested an oncogenic effect of PTTG1 by showing increased cell proliferation and prolonged cell survival in case of PTTG1 overexpression and suppressed cell growth after PTTG1 downregulation/knockout in prostate cancer cell lines. The striking association of high PTTG1 expression with unfavorable tumor phenotype and patient outcome seen in this study argues for a significant in vivo effect of PTTG1 activation in the progression of prostate cancer. This is also supported by Cao et al. who described a significant association between high PTTG1 expression and decreased survival in a cohort of 64 prostate cancers.

Results from various other tumor entities parallel the observations on PTTG1 in prostate cancer. Immunohistochemical studies described increased PTTG1 staining in the cytoplasm of tumor cells whereas the adjacent non-neoplastic tissue was negative or only weakly or focally positive. Additional nuclear staining of tumor cells was described in some studies. PTTG1 overexpression was linked to poor prognosis in various tumors, such as renal cell carcinoma, adrenocortical carcinoma, laryngeal cancer, oral squamous cell

| Parameter                  | n evaluable | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | P value |
|----------------------------|-------------|--------------|----------|--------------|------------|---------|
| **All cancers**            | 9123        | 88.2         | 5.4      | 5.6          | 0.8        |         |
| **Tumor stage**            |             |              |          |              |            | <0.0001 |
| pT2                       | 5877        | 90.4         | 4.2      | 4.7          | 0.7        |         |
| pT3a                      | 2079        | 85.7         | 7.2      | 6.4          | 0.7        |         |
| pT3b-pT4                  | 1167        | 82.0         | 8.0      | 8.8          | 1.2        |         |
| **Gleason grade**         |             |              |          |              |            | <0.0001 |
| ≤3+3                      | 1890        | 92.6         | 2.2      | 4.7          | 0.6        |         |
| 3+4                       | 5052        | 89.1         | 4.9      | 5.2          | 0.8        |         |
| 3+4 Tert.5                | 325         | 85.5         | 7.4      | 5.8          | 1.2        |         |
| 4+3                       | 951         | 83.4         | 8.4      | 7.7          | 0.5        |         |
| 4+3 Tert.5                | 518         | 82.8         | 10.4     | 5.8          | 1.0        |         |
| 8                         | 95          | 81.1         | 6.3      | 8.4          | 4.2        |         |
| 9–10                      | 318         | 77.4         | 10.7     | 11.0         | 0.9        |         |
| **Gleason grade quant**   |             |              |          |              |            |         |
| ≤3+3                      | 1750        | 92.6         | 2.2      | 4.7          | 0.6        |         |
| 3+4 ≤5%                   | 1191        | 91.3         | 3.9      | 4.1          | 0.1        |         |
| 3+4 6–10%                 | 1198        | 90.4         | 4.2      | 4.5          | 0.9        |         |
| 3+4 11–20%                | 970         | 87.5         | 6.2      | 5.6          | 0.6        |         |
| 3+4 21–30%                | 523         | 87.9         | 5.2      | 6.2          | 0.7        |         |
| 3+4 31–49%                | 391         | 85.7         | 6.8      | 7.0          | 0.4        |         |
| 3+4 Tert.5                | 278         | 85.5         | 7.4      | 5.8          | 1.2        |         |
| 4+3 50–60%                | 330         | 85.1         | 7.5      | 7.2          | 0.3        |         |
| 4+3 61–80%                | 297         | 82.7         | 9.2      | 7.5          | 0.6        |         |
| 4+3 >80%                  | 72          | 76.6         | 14.9     | 7.4          | 1.1        |         |
| 4+3 Tert.5                | 429         | 82.8         | 10.4     | 5.8          | 1.0        |         |
| 8                         | 69          | 81.2         | 7.1      | 8.2          | 3.5        |         |
| 9–10                      | 216         | 78.3         | 11.2     | 9.4          | 1.1        |         |
| **Lymph node metastasis** |             |              |          |              |            | 0.0083  |
| N0                        | 5257        | 87.2         | 5.7      | 6.4          | 0.7        |         |
| N+                        | 524         | 81.7         | 8.8      | 8.6          | 1.0        |         |
| **Preop. PSA level (ng/mL)** |         |              |          |              |            | 0.0499  |
| <4                        | 1082        | 87.3         | 4.4      | 7.4          | 0.8        |         |
| 4–10                      | 5515        | 88.8         | 5.3      | 5.1          | 0.8        |         |
| 10–20                     | 1833        | 88.0         | 5.5      | 5.9          | 0.6        |         |
| >20                       | 665         | 86.3         | 7.5      | 5.3          | 0.9        |         |
| **Surgical margin**       |             |              |          |              |            | 0.0961  |
| Negative                  | 7267        | 88.6         | 5.3      | 5.4          | 0.7        |         |
| Positive                  | 1857        | 86.6         | 5.8      | 6.7          | 0.9        |         |

Abbreviations: PSA, prostate-specific antigen; PTTG1, pituitary tumor-transforming gene 1 protein.
carcinoma, gastric cancer and esophageal cancer.\textsuperscript{7,9,14,50,52–54} Observations made on cultured tumor cell lines derived from multiple different tumor types were also supportive of an oncogenic role of PTTG1.\textsuperscript{19,55–57} Overall, the available data suggest that PTTG1 is an oncogenic protein with a broad and important role across many different tumor types.

Molecular analyses of >10 000 cancers represent an ideal application of the TMA technology developed by our group more than 20 years ago. The method enables a highly standardized high throughput analysis of tissues.\textsuperscript{43} The random selection of one cancer containing tissue sample per patient measuring 0.6 mm in diameter even assures identical tissue quantities analyzed by patient and avoids a selection bias potentially induced by pathologists selecting tumor areas that are ‘representative’ based on their perception. The only study comparing molecular info obtained on a TMA versus large sections with clinical outcome information was conducted by Torhorst et al. in 2001.\textsuperscript{58} In that study, TMA

![Figure 2](image_url)

**Figure 2** Pituitary tumor-transforming gene 1 protein (PTTG1) expression and biochemical recurrence in (a) all cancers, (b) ERG fusion negative cancers, and (c) ERG fusion positive cancers.

![Figure 3](image_url)

**Figure 3** Pituitary tumor-transforming gene 1 protein (PTTG1) immunostaining and genomic deletions in all prostate cancers.

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data from each of four TMA spots per patient provided markedly better prognostic information than obtained from corresponding large sections despite of a lower rate of positivity in TMA spots. Because the use of multiple samples per patient induced a bias towards higher positivity rates in patients with more interpretable spots in this and in other studies, we strongly advocate the use of one sample per patient. This approach enabled us to reproduce all analyzed clinically relevant correlations between molecular and clinical tumor features such as HER2, vimentin and Ki67.59–62 Since numerous studies can be performed with one set of TMAs, this approach results in a considerable database that further supports the understanding of new data.

For this study, data utilized from previous studies include the TMPRSS2:ERG fusion because this is the most common molecular alteration in prostate cancer, 12 different chromosomal

**Figure 4**  Pituitary tumor-transforming gene 1 protein (PTTG1) immunostaining and P53 expression.

**Figure 5**  Pituitary tumor-transforming gene 1 protein (PTTG1) immunostaining and androgen receptor expression.

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deletions because these represent the next most common genomic alterations in this cancer type as well as immunohistochemical data on androgen receptor, p53 and Ki67 because of suggested interactions of PTTG1 with these proteins and the pivotal role of tumor cell proliferation. The fact that high PTTG1 expression was significantly linked to 9 of 12 analyzed chromosomal deletions might be related to the known role of PTTG1 in DNA replication by regulating sister-chromatid separation during mitosis. Several authors have earlier proposed that PTTG1 might trigger genetic instability.\textsuperscript{15,16} The marked increase of immunohistochemically detectable p53 protein in cancers with elevated PTTG1 levels provides in vivo evidence for a functional interaction of these proteins as suggested by in vitro data.\textsuperscript{18} The significant increase of PTTG1 protein levels depending on the cellular androgen receptor levels is consistent with androgen dependency of PTTG1 which might be caused by an androgen response element in the PTTG1 promoter region.\textsuperscript{24} c-MYC promoter stimulation is another potential oncogenic mechanism of PTTG1.\textsuperscript{17} Since c-MYC is a strong positive regulator of cell proliferation, the increased mitotic rate in PTTG1 positive tumors could hypothetically be explained by a PTTG1/c-MYC interaction.

It is of note that PTTG1 expression was unrelated to the TMPRSS2:ERG fusions status. TMPRSS2:ERG fusions occur in about 50% of prostate cancers and leads to a permanent overexpression of the transcription factor ERG. ERG expression does not directly impact patient prognosis but results in a dysregulation of more than 1600 genes in affected prostate epithelial cells.\textsuperscript{63} As a consequence, the expression levels of many proteins differ between ERG positive and negative cancers and also the prognostic impact of many tumor relevant proteins differ between these subgroups.\textsuperscript{45,64,65} That the prognostic impact of PTTG1 expression did not markedly vary between ERG positive and ERG negative cancers argues for the practical applicability of measuring PTTG1 protein for prognosis assessment.

Elevated PTTG1 expression was an independent prognostic marker irrespective of which pre- or postoperatively available established prognostic features were used for comparison. Also the striking link of PTTG1 expression and features of aggressive prostate cancer such as advanced tumor stage, high Gleason grade and high tumor cell proliferation underscores the biologic significance of PTTG1 in this tumor. We consider molecular prognostic features all the more important since several statistically strong and clinically well-established prognostic parameters suffer from significant shortcomings in clinical praxis. pT stage and nodal status are lacking during the preoperative therapeutic decision making. Even in a postoperative setting, the likelihood of detecting lymph node metastases largely depends on the extent of the surgical procedure and the amount of effort taken to search for metastases in the pathology laboratory.\textsuperscript{66}

The Gleason Grade, the most powerful preoperatively available prognostic marker, suffers from substantial inter-observer variability reaching up to 40% in individual biopsies.\textsuperscript{67} In contrast, PTTG1 levels can easily be examined in cancer tissue from core needle biopsies, facilitating its incorporation in a preoperative multiparametric prostate cancer algorithm to estimate tumor behavior.

Only a single 0.6 mm tissue spot per tumor has been analyzed in this study. Given that prostate cancers are typically multifocal and heterogeneous, it is likely that the true PTTG1 positivity rate is higher than the 12% detected in our study. However, the tumor content of a 0.6 mm tissue spot fairly resembles that of a core needle biopsy, which makes our TMA a suitable model for PTTG1 analysis in presurgical settings.

In summary, overexpression of PTTG1 is a strong and independent prognostic marker in prostate cancer. PTTG1 analysis, either alone or in combination with other prognostic biomarkers might be of clinical utility in the assessment of prostate cancer aggressiveness.

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DISCLOSURE STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

CF, SY, RS, Ji, EB and GS designed the study and drafted the manuscript. HHu, HHe, MF, AHa, MG and TS have a part in study design. CF, SY, FB and MTC performed IHC analysis and scoring. CG, DH, AML and WW participated in pathology data analysis. CHM, KMK and RS performed statistical analysis. SK, DD, SS, SM, PL, SW and TSC participated in data interpretation, and helped to draft the manuscript. All authors read and approved the final manuscript.

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