RESEARCH ARTICLE

Expression of Pituitary Tumor Transforming Gene 1 is an Independent Factor of Poor Prognosis in Localized or Locally Advanced Prostate Cancer Cases Receiving Hormone Therapy

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Abstract

We investigated the prognostic value of pituitary tumor transforming gene 1 (PTTG1) expression according to clinicopathological features among localized or locally advanced prostate cancer cases receiving hormone therapy. A retrospective study involved 64 patients receiving combined androgen blockade treatment was performed. PTTG1 expression was determined by immunohistochemical staining using initial needle biopsy specimens for diagnosis. Associations of PTTG1 with various clinicopathological features and disease-free survival were examined via uni- and multivariate analyses. No association between PTTG1 expression and clinical T stage, Gleason score, pretreatment PSA levels, risk groups was found (p =0.682, 0.184, 0.487, 0.571, respectively). Univariate analysis revealed that increased PTTG1 expression, T3 stage and high risk group were associated with increased risk of disease progression (p =0.000, 0.042, and 0.001), and high PSA level had a tendency to predict disease progression (p =0.056). Cox hazard ratio analysis showed that PTTG1 low expression (p =0.002), PTTG1 high expression (p =0.000) and high risk group (p =0.0147) were significantly related to decreased disease-free survival. In conclusion, PTTG1 expression determined by immunohistochemical staining in needle biopsy specimens for diagnosis is a negative prognostic factor for progression in localized or locally advanced prostate cancer receiving hormone therapy.

Keywords: Androgen deprivation therapy - DFS - prognosis - prostate cancer - pituitary tumor transforming gene 1

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Introduction

Prostate cancer represents a major global public health issue, and are the most frequently diagnosed tumor and the second largest cancer contributing to mortality in men in USA. It is estimated that 217,730 men will be diagnosed with and 32,050 men will die of cancer of the prostate in 2010 in USA (http://seer.cancer.gov/csr/ 1975¬_2007/ results_single/sect_01_table.01.pdf). In Asia, the incidence of prostate cancer has been increasing sharply for a decade, and prostate cancer has been an emerging threat to the health of aging men, though deficient of accurate epidemiological data in many Asian countries at present (Zhang et al., 2011).

According to therapeutic strategies, use of primary androgen deprivation therapy (PADT) has emerged as an option for men with clinically localized or locally advanced prostate cancer except for radical prostatectomy and radiotherapy in the world (Kawakami et al., 2006; Holmes Jr et al., 2007). In Japan, data on the treatment of prostate cancer shows that PADT is chosen to treat localized and locally advanced prostate cancer in an extremely high proportion of cases (Akaza et al., 2004). Data from Cancer of the Prostate Strategic Urologic Research Endeavour (CaPSURE) of the USA also shows an increase in recent years in the proportion of localized and locally advanced prostate cancer patients for whom PADT is being selected (Cooperberg et al., 2003). In 2002, Labrie et al. (2002) reported the efficacy of hormonal therapy for localized or locally advanced prostate cancer. In 2006, Akaza et al. (2006) further confirmed the usefulness of PADT for localized or locally advanced prostate cancer by analyzing the 10-year survival rates for men with localized or locally advanced prostate cancer treated with PADT or prostatectomy. However, some patients who receive PADT, if followed long enough, will develop evidence of resistance and progression. Thus, accurate pretreatment risk stratification is essential for both patient counseling and the design of adjuvant therapy. Several factors, such as volume of disease, risk category, and PSA velocity, have been assessed to be predictors of advanced prostate cancer progression after hormone therapy, but not for patients with localized or locally advanced prostate cancer receiving PADT (Kwak

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et al., 2002; Chung et al., 2008; Abouassaly et al., 2009). Consequently, there is a great need for markers that we can apply to biopsy specimens to accurately predict the risk of disease progression in patients with prostate cancer receiving PADT and allow appropriate treatment planning. Pituitary tumor transforming gene 1 (PTTG1) was first isolated from rat pituitary tumor cells in 1997 and has been identified an oncogene because PTTG1 overexpression induces cellular transformation in vitro and tumor formation in nude mice. As human securin, PTTG1 participates in mitotic spindle checkpoint pathway and inhibits sister chromatin separation ensure chromosomal stability (Pei and Melmed, 1997; Zou et al., 1999). In contrast to restricted normal tissue expression, PTTG1 is abundantly expressed in a wide variety of tumors, and is associated with metastasis and a poor clinical outcome with several types of tumors, suggesting that PTTG1 may play a role in tumorigenesis (Vlotides et al., 2007). PTTG1 has been also identified as one of the key 'signature genes' to predict metastasis in prostate cancer (Ramaswamy et al., 2003). Zhu et al. (2006) detected PTTG1 protein expression in a high percentage of prostate cancer tissues in prostate tissue samples by immunohistochemistry, and proved that ectopic PTTG1 gene expression promoted prostate cancer cell proliferation and tumorigenesis both in vitro and in nude mice, and down-regulation of PTTG1 led to suppression of tumor cell growth, suggesting that PTTG1 may be a potential prognostic marker and a therapeutic target for prostate cancer.

However, no study was performed to evalue the relation between expression of PTTG1 and prostate cancer progrssion in patients who receiving hormone therapy. In the present study, we retrospectively determined whether PTTG1 overexpression on diagnostic prostate needle biopsy specimens obtained from patients with localized or locally advanced prostate cancer could be a useful marker in predicting progression after hormone therapy.

Materials and Methods

Patients

The subjects were 64 patients who attended Chinese PLA General Hospital and received a diagnosis of T2N0M0 or T3N0M0 prostate cancer between June 2003 and January 2010. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Chinese People's Liberation Army General Hospital. Written informed consent was obtained from all participants. meeting the following strict criterions: treated by continuous combined androgen blockade (CAB) without radical prostatectomy or radiation for various reasons, including high risk of surgical complications, advanced age and patient preference; being good responsive to PADT, and PSA dropped to undetectable level (< 0.2 ng/ml) after three months; appropriate follow-up data and biopsy tissues being available.

Follow up

During the first 6 months after treatment, PSA levels were examined monthly. After that, PSA levels were examined every 3 months. Bone scan and Transrectal Ultrasound were performed annually. When indicated, nuclear magnetic resonance or computed tomography of the lungs and abdomen were also performed. Progression was considered in one of the following circumstances: (a) PSA measurement > 0.2 ng/ml, and the judgment of PSA recurrence assumed elevation of PSA level on three consecutive occasions; (b) radiological or histological evidence of local progression or metastasis. Follow-up was terminated upon disease progression of the patient or by June 30, 2010. The study performance was approved by the Ethics Committee of Chinese People's Liberation Army General Hospital.

Immunohistochemical staining

Immunohistochemical staining was performed using the single core that had a highest Gleason score (GS) as a result of a systematic sextant needle biopsy. PTTG expression in prostate biopsy specimens was detected by the two-step immunohistochemical staining method. Formalin-fixed, paraffin-embedded tissue sections (4μm) were deparaffinized in xylene and rehydrated in a graded series of ethanol. For antigen retrieval, slides were exposed to citrate buffer (10 mmol/l, pH 6.0) and heated for 30 minutes in microwave oven and allowed to cool at room temperature for 20 minutes. The slides were then incubated for 30 minutes in PBS with 0.3% hydrogen peroxide to block endogenous peroxidase activity and washed again with PBS. Subsequently, the slides were incubated with primary antibodies diluted 1:100 in PBS-1% bovine serum albumin (BSA) for 60 minutes at room temperature. Primary antibody was rabbit polyclonal anti-PTTG antibody. The EnVision method was used for staining. TBS buffer was used instead of primary antibody as the negative control, and colon cancer tissues were used as the positive control. All tissues were stained at the same time to avoid false positive and false negative staining results.

Immunohistochemical evaluation

Both the extent and intensity of immunostaining were considered when scoring PTTG 1 protein expression according to Hao et al. (Hao et al., 2000). The intensity of positive staining was scored as 0, negative; 1, weak; 2, moderate; 3, strong. The percentage of PTTG1 reactive cells was assessed counting 100 tumor cells in serial sections, and scored as 0, <5%; 1, >5-25 %; 2, >25-50 %; 3, >50-75 %; 4, >75 % of the prostate cancer cell. The final score was determined by multiplying the intensity score and the extent score, yielding a range from 0 to 12. Scores 9-12 were defined as high expression, 5-8 as low expression and 0-4 as negative expression. The sorces were assessed in dependently by two skilled pathologists. Discrepant cases were reviewed at a multihead microscope and a consensus reached. All specimens were evaluated without knowledge of the patients' clinical information.

Statistical analysis

The parameters investigated were T stage, GS, pretreatment PSA level, risk groups and the status of PTTG1 expression. The correlation between PTTG1

Table 1. Clinicopathologic Features of PTTG1 Expression in Prostate Cancer

Factors	PTTG high expression (n=17) No. (%)	PTTG low expression (n=27) No. (%)	PTTG negative expression (n=20) No. (%)	p-value*
Age (y)				0.926
≤76	8(12.5)	18 (28.1)	10 (15.6)	
>76	9 (14.1)	9 (14.1)	10(15.6)	
PSA ng/ml				0.487
<20	11(17.2)	20 (31.2)	11(17.2)	
≥20	6(9.4)	7 (10.9)	9(14.1)	
T stage				0.682
T2	13(20.3)	19(29.7)	14(21.9)	
T3	4(6.2)	8(12.5)	6(9.4)	
Gleason score				0.184
<7	5 (7.8)	14(21.9)	8(12.5)	
7	5 (7.8)	8 (12.5)	9(14.1)	
>7	7 (10.9)	5(7.8)	3(4.7)	
Risk group				0.571
Low or moderate	4(6.3)	14(21.9)	7(10.9)	
High	13(20.3)	13(20.3)	13(20.3)	
Disease progression				0.006
No	0(0)	5(7.8)	7(10.9)	
Yes	17 (26.6)	22(34.4)	13(20.3)	

PTTG1, The pituitary tumor transforming gene 1; GS, Gleason score; PSA, prostate-specific antigen; y, years; *Spearman correlation test, two sided. Risk group

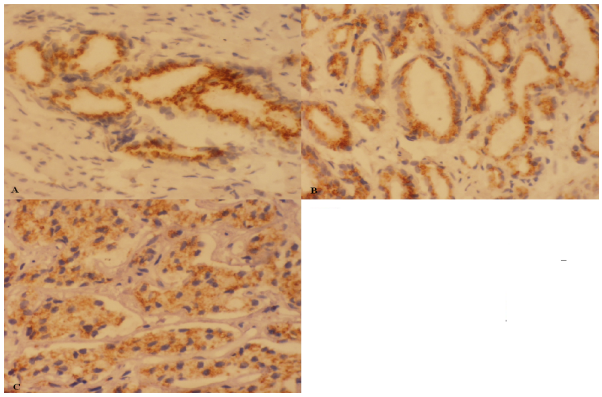

Figure 1. PTTG1 Expression in Prostate Cancer of Gleason Score <7 (A), 7 (B) and >7 (C) (40×10) Respectively

expression and clinicopathological parameters were evaluated using Spearman correlation test. Survival curves were generated using the method of Kaplan and Meier and the significance of differences was assessed with the log-rank test. For univariate and multivariate analyses, Cox proportional hazard analysis was used to assess the independence of parameters to predict the disease free survival after hormone therapy. All P-values < 0.05 were considered as statistically significant. All analyses were performed with the SPSS 13.0 for Windows software.

Results

Clinical characteristic

Mean patient age was 75.1 years (range, 61-85 years; median, 76 years). Mean follow-up time of was 51.0 months (range, 3.6-171.4 months; median, 40.1 months). Mean pretreatment PSA were 22.6 (range, 1.0-240.0 ng/ml; median, 14.80 ng/ml). Table 1 summarizes the

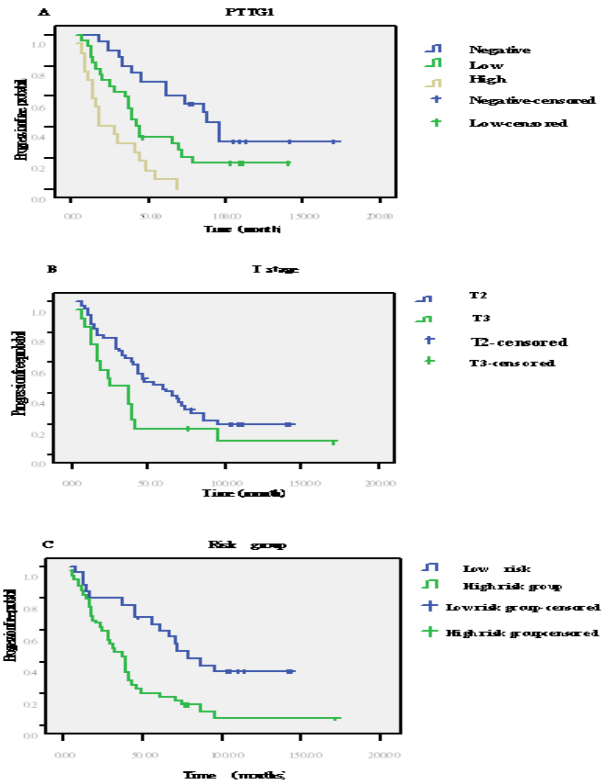

Figure 2. Prognostic Value of PTTG1 Expression or T Stage. (A) Kaplan-Meier plots of disease free probability of each group of different PTTG1 expression. Statistically differences were observed among high, low and negative groups (log rank, p=0.000). (B) Kaplan-Meier plots of disease free probability of each group of different T stage. Statistically differences were observed between T2 and T3 groups (log rank, p=0.042). (C) Kaplan-Meier plots of disease free probability of each group of different risk group. Statistically differences were observed between low and high groups (log rank, p=0.001)

clinicopathological characteristics of these patients in a contingency table. Among the patients, 38 subjects were treated with antiandrogent plus luteinizing hormone-releasing hormone agonist, while 26 subjects received antiandrogent plus bilateral orchiectomy.

Immunohistochemical staining

In prostate cancer cells, PTTG1 was expressed mainly in perinuclear granular particles in the cytoplasm which were rough and dark yellow. With regard to subcellular localization, PTTG1 staining was observed in the cytoplasm of tumor cells. In a small number of cells, PTTG1 was expressed in the nuclei, which was observed mainly in poorly differentiated tumors. PTTG1 reactivity was not detected in histologically normal epithelial cells in areas adjacent to the tumor (Figure 1).

Among the 64 prostate carcinoma specimens, 17 (26.5%) were high expression, 27 (42.2%) low expression, and 20 (31.3%) negative for PTTG1 immunoreactivity. The pretreatment PSA levels were dichotomised into < 20 vs ≥ 20 ng/ml. Gleason scores of biopsy specimens were stratified into 3 groups: Gleason Score < 7, Gleason score 7, or Gleason score > 7. High-risk patients were difined as having a PSA level ≥ 20 ng/mL, stage T3 disease, or a Gleason score ≥ 8. The low-risk category included all other patients. No meaningful association between PTTG1 expression and GS groups, clinical T stage, PSA level,

Table 2. Results of Univariate and Multivariate Analysis

Factors	Univariate analysis		Multivariate ananlysis	
	HR (95%CI)	p value	HR (95% CI)	p value
IHC PTTG expression				
Neg	reference	0	reference	0
low	2.142 (1.071-4.286)	0.031	3.724 (1.641-8.451)	0.002
high	5.426 (2.501-11.774)	0	8.045 (3.202-20.210)	0
Risk group(low:high)	2.805(1.526-5.158)	0.001	4.062(1.324-12.462)	0.014
Age (≤76:>76)	0.920(0.533-1.588)	0.764	0.910(0.506-1.637)	0.752
PSAg (<20:≥20)	1.743(0.980-3.101)	0.056	0.857(0.394-1.863)	0.696
Gleason score <7	reference	0.095	reference	0.529
Gleason score =7	0.850(0.444-1.629)	0.625	0.921 (0.462-1.837)	0.816
Gleason score >7	1.808(0.916-3.570)	0.088	0.625(0.273-1.430)	0.266
T(2:3)	1.857 (1.022-3.372)	0.042	1.423(0.594-3.407)	0.429

HR, Hazard Ratio; CI, confidence interval; Cox proportional hazard model and single parameter analysis was used to determine the prognostic significance of age group, GS group, T stage (T3/T2), pretreatment PSA level, risk group and PTTG1 expression; All were used as categorical variables

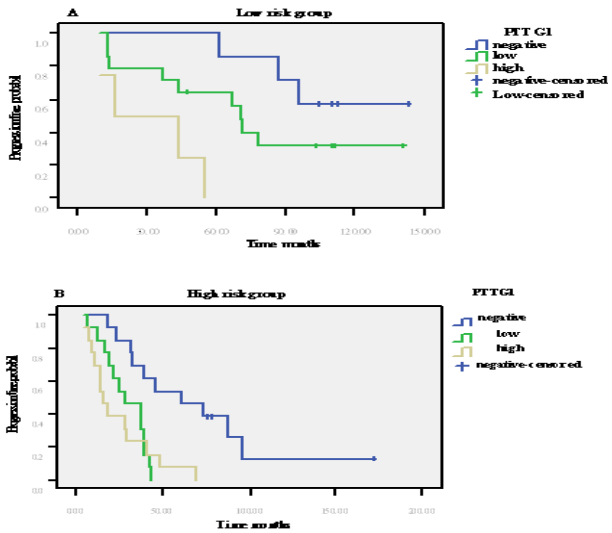

Figure 3. Prognostic Value of PTTG1 Expression Stratified by Risk Group. (A) Kaplan-Meier plots of disease free probability of each group of different PTTG1 expression in low risk group. Statistically differences were observed among high, low and negative expression (log rank, p=0.000). (B) Kaplan-Meier plots of disease free probability of each group of different PTTG1 expression in high risk group. Statistically differences were observed among high, low and negative expression (log rank, p=0.000)

risk groups. PTTG1 expression in relation to clinical and pathologic features is summarized in Table 1.

Univariate Analysis

Univariate Cox proportional hazards regression analysis was used to evaluate high and low PTTG1 expression (p=0.000), high risk group (p=0.001) and T3 stage (p=0.042) as prognostic predictors of a shorter time to disease progression after CAB. Although PSA level has a tendency to predict disease progression, this finding did not achieve statistical significance (p= 0.056). Age and Gleason score provided no prognostic value in this set of patients (Table 2). The predictive value of PTTG1 expression, risk group and T stage were evaluated using Kaplan-Meier actuarial analysis (Figure 2). The mean PFS time for the high PTTG1 expression patients was 25.3 (95% confidence interval (CI), 16.1-34.6) months, whereas that for the patients with low PTTG1 expression was 53.4 (95% CI, 36.8-70.1) months, negative expression 94.0 (95% CI, 68.7-119.4) months. The mean PFS time of patients in high-risk group was 40.7 (95% CI, 28.7-52.7) months, whereas in low-risk group 81.1 (95% CI, 61.3-100.9) months. The mean PFS time of patients with T2 was 62.7 (95% CI, 49.3-76.2) months, whereas the mean PFS time of T3 was 41.3 (95% CI, 47.2-74.5) months.

Multivariate Analysis

To determine the smallest number of parameters that could jointly predict disease progression in our cohort of patients, the multivariate Cox proportional hazard model and stepwise selection analysis was used. When all parameters with prognostic factors (i.e. age group, T stage, GS group, risk group, pretreatment PSA level and PTTG1 expression) were included in the model, high risk group (p=0.0147, hazard ratio=4.062), and PTTG1 low expression (p=0.002, hazard ratio=3.724), and PTTG1 high expression (p=0.000, hazard ratio=8.045) reached statistical significance in predicting decreased PFS (Table 2).

To demonstrate the joint effects of PTTG1 expression and risk group on disease progression, Kaplan-Meier analysis was performed. As shown in Figure 3, in both low and high risk subgroups patients with high PTTG1 expression had a worse prognosis than patients with low or negative PTTG1 expression (p=0.000). Thus, the highest probability of disease progression was found in patients with high PTTG1 expression and high-risk group, whereas individuals with negative PTTG1 expression and low-risk group had the lowest probability of progression.

Discussion

In the present study, we show for the first time that PTTG1 overexpression in prostate cancer is statistically associated with decreased PFS after CAB therapy both in univariate and multivariate analysis, even though is not associated with the Gleason score, PSA level and clinical T stage. Several factors which were typically used so far to predict outcome of curative therapeutic strategies, such as Gleason score and PSA level, losted their prognostic value for patients with localized or locally advanced prostate cancer receiving PADT in this cohort. Moreover,

prostate cancer patients with different risk group could be further classified based on the PTTG1 expression in their prostate cancer specimens to predict disease progression more accurately.

With regard to PTTG1 expression in patients with prostate cancer, Zhu et al. (2006) detected in a higher percentage of prostate cancer tissues (34/41, 82.9%) than we did (68.7%, 44/64). The reason for the little different results seem to be that the disease stage of the specimens used for comparison varied and that there were differences in the procedure for evaluation of PTTG1 expression, including the condition of the antigen, type of antibody used, and method of restoration of the antigen. Although the tissue examined was only the partial biopsy specimen obtained at the diagnosis, our results indicate that PTTG1 expression can be fully detected even by IHC using a biopsy specimen. Since PTTG1 expression was observed in virtually most of prostate cancers, one thing can be said from our results that detection of PTTG1 expression using the biopsy specimen obtained at diagnosis could help to identify patients with aggressive disease who require aggressive therapy, being treated with more intensive therapy, such as CAB combined with HDR-brachytherapy, intensity-moderated radiotherapy, EBRT, or some forms of chemotherapy.

The pituitary tumor transforming gene 1 (PTTG1) is a multifunctional gene encoding a 23-kDa, 202-amino acid securin-like protein that induces cell transformation in NIH3T3 cells and tumor formation in nude mice (Zhang et al., 1999). Overexpression of PTTG1 enhances the cell proliferation, migration, invasion, and/or tumorigenicity and the induction of aneuploidy of cells derived from cancers of the human pituitary, kidney, esophagus, breast, head, and neck, liver, thyroid, lung, etc (Solbach et al., 2004; Zhou et al., 2005; Kim et al., 2006; Malik and Kakar, 2006; Rehfeld et al., 2006; Solbach et al., 2006; Wuttig et al., 2009; Liang et al., 2011). PTTG1 overexpression correlates with metastasis and poor overall survival in various human cancers (Heaney et al., 2000; Malik and Kakar, 2006; Wuttig et al., 2009), and decreasing PTTG expression through PTTG siRNA inhibits tumor growth in ovarian (El-Naggar et al., 2007), hepatic (Cho-Rok et al., 2006). And lung (Kakar and Malik, 2006) cancer cell lines, indicating that PTTG1 may be a new therapeutic target for cancer treatment.

The most intresting point is that PTTG1 is related to endocrine response. PTTG1 expression can not only be androgen upregulated in castrated rat prostate and human prostate cancer cell LNCaP (Zhu et al., 2006), but also be induced by estrogen through an estrogen-response element in the PTTG1 promoter region in prolactinoma (Heaney et al., 1999). Androgen pathways and estrogen signaling all have been showed to play important roles in prostate cancer development and progression (Bonkhoff and Berges, 2009; Celhay et al., 2010). PTTG1 has also been identified as one of new candidate genes associated with endocrine therapy resistance in breast cancer (Ghayad et al., 2009). In our present study, subjects with PTTG1 overexpression had a shorter time to tumor progression than that with low PTTG1 expression. These results suggest that disruption of PTTG1 may be one of the major factors contributing to androgen deprivation therapy resistance, and inhibtition of this gene may be a potential therapeutic target in the suppression of prostate cancer progression. We hypothesize that PTTG1 overexpression may be associated with advanced disease that responds poorly to hormone therapy, just as Rb loss was (Sharma et al., 2007). Further studies are necessary to clarify the role of PTTG1 in development and progression of prostate cancer. It might be interesting to invastigate whether PTTG1-transfected hormone sensitive prostate cancer cell line (i.e. LNCaP) could survive under androgen deprivation circumstance.

The cohort of our study is restricted to 64 patients with localized or locally advanced prostate cancer. Despite its limited size, the strength of this cohort is its restriction to T2-T3 tumor without lymph node and distant metastasis and undetectable PSA level after continuous CAB therapy in first 3 months, because thus disease progression indeed reflects tumor aggressiveness rather than being the result of enlargement of the metastasis tumor, and biochemical failure reflects transformation to androgen independent prostate cancer rather than being the result of a bad response to PADT.

There are two limitations of the study. The first is that the subjects are good responsive to hormonal therapy and patiensts with nonmetastasis, thus it is not clear whether the present results are applicable to poor responders or patients with metastasis. The second is that the detection approach, immunohistochemistry is semiquantitative. But immunohistochemistry approach is convenient and economically efficient, widely applied and much easier to be implemented into clinical practice.

In summary, this paper introduces that PTTG1 immunostaining in patients with prostate cancer may be a useful approach to predicting PFS after combined androgen blockade treatment in Chinese patients with localized or locally advanced disease and may identify those patients who may benefit from novel aggressive therapeutic strategies.

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