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ORIGINAL ARTICLE

Prostate Cancer

Combined tests of prostate specific antigen and testosterone will improve diagnosis and monitoring the progression of prostate cancer

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Prostate-specific antigen (PSA) testing has been widely used to screen men for prostate cancer (PCa) and to monitor PCa progression. However, more studies have shown that around 15% of men with low or normal PSA levels have PCa. In this study, we aimed to investigate the relationship of androgen and PSA levels and to better understand the reason that some PCa patients have low serum PSA values. The *in vitro* data demonstrated that cultured LNCaP cells ceased to produce PSA after androgen withdrawal and resumed PSA production after androgen was re-added. The *in vivo* experiment results showed that 48% of PCa xenografts carrying mice have serum PSA level lower than 4 ng ml⁻¹. The serum PSA levels increased significantly with rises in testosterone (T) levels 1 week after T pellet implantation. These data indicated that the androgen is a key factor controlling the production of PSA. Low serum PSA levels in mice with PCa xenografts are associated with low serum T levels. Raising serum T levels in tumor caring mice will also significantly increase serum PSA level. This may have clinical implications when screening PSA in men, who have occult PCa.

Asian Journal of Andrology (2015) 17, 807–810; doi: 10.4103/1008-682X.148721; published online: 17 February 2015

Keywords: prostate cancer; prostate specific antigen; testosterone

INTRODUCTION

Prostate-specific antigen (PSA) is a glycoprotein produced almost exclusively by prostate epithelial cells, which have androgen receptor (AR). The production of PSA is responsive to androgens and testosterone (T) is the primary and main form of androgens. Since the serum level of PSA is often elevated in men with prostate cancer (PCa), PSA test has been widely used to screen men for PCa and to monitor the progression in patients of PCa. There is no specific normal or abnormal serum level of PSA though most doctors considered PSA levels of 4.0 ng ml⁻¹ and lower to be within the normal range. However, more recent studies have shown that around 15% of men with PSA levels below 4.0 ng ml⁻¹ have PCa.^{1–3}

In order to better understand why a significant amount of men with low PSA levels have PCa, in this study, we aimed to investigate the relationship of T levels and PSA production in both *in vitro* and *in vivo* experiments.

MATERIALS AND METHODS

Cell culture

Prostate cancer LNCaP cells (ATCC, CRL-1740) were cultured in RPMI-1640 medium (Gibco, 61870036) supplemented with 10% fetal bovine serum (FBS) (Hyclone, SH30910.03HI) and 1X Antibiotics-Antimycotic (Gibco, 15240062) in incubator (37°C, 5% CO₂ atmosphere).

Testosterone treatment and prostate specific antigen production assay *in vitro*

Twenty-four well culture plates were coated with 200 µl per well of 50 µg ml⁻¹ poly-D-lysine (in PBS) for 30 min at 37°C, and washed twice with distilled water. LNCaP cells were seeded 15,000 per well (each sample in triples) in RPMI-1640 medium supplemented with 10% FBS. After 48 h, medium was changed to fresh serum free medium (date was designed as day 0). Serum free medium was changed and collected for PSA test every day until day 4. Different concentrations of T or dihydrotestosterone (DHT) (0, 0.5, 1, 2, 4, 8, and 16 ng ml⁻¹) were added to different wells on day 4 after medium change. Twenty-four hours later, PSA levels in medium from each well were tested with PSA Elisa kit (MyBiosource, MBS494521). T and DHT levels in 10% FBS supplemented medium and serum free medium were also tested by Elisa (T-Rocky Mountain Diagnostic, AA E-1300. DHT-MyBiosource MBS366006).

LNCaP tumor xenografts development on nude mice

Male nude/nude athymic mice (Jackson Lab, 007850) were used in this experiment for LNCaP cell tumor xenografts development. Cultured LNCaP cells (passages 20 to 50) were detached and separated by 0.25% trypsin-EDTA solution (Gibco, 25300) and washed by serum free medium. Five million cells in 200 µl serum-free medium were inoculated subcutaneously to each mouse (5–7 weeks old) and tumor development was checked every 2 days.

Testosterone level manipulation on nude mice

When tumor size reaches 5 mm × 5 mm (4–11 weeks after cell inoculation), the mice with LNCaP tumor xenografts were divided into two groups: control group and T pellet implantation group. Mice in the T pellet implantation group were implanted subcutaneously with a 2 mg T pellet (Testopel, Bartor Pharmacal). Eight normal control male nude mice (14–16 weeks old) without cell inoculation were also implanted with 2 mg T pellet.

Mice serum testosterone and prostate specific antigen tests

Blood samples from all the mice were collected before and 1 week after implanting 2 mg T pellet. Serum PSA and T levels were tested by Elisa.

Statistical analysis

Statistical significance was determined on the basis of the *t* test. All data are shown as mean ± standard deviation (s.d.). Tumor volume was calculated by the formula $W^2 \times L/2$.

RESULTS

Testosterone treatment and prostate specific antigen production assay in vitro

Testosterone and DHT Elisa results demonstrated that 10% FBS supplemented medium contains 0.1 ng ml⁻¹ of T and 0.007 ng ml⁻¹ of DHT. No T or DHT were detected in serum-free medium. The *in vitro* data demonstrated cultured LNCaP cells decreased PSA production after the cells were shifted to serum-free culture conditions. Furthermore, these cells ceased PSA production after consecutive serum-free medium changes in 4 days. When T was added to the fresh serum-free medium on day 4, PSA production resumed and was detected on day 5 (Figure 1). DHT had the same effects on the PSA production in LNCaP cells (figure not shown).

Prostate specific antigen levels in LNCaP tumor xenografts caring mice

Serum samples from 25 LNCaP tumor xenografts caring mice were tested for PSA levels. The results indicated that 48% of these mice had PSA levels lower than 4 ng ml⁻¹, especially in mice with smaller tumor volume (Table 1), though the relative ratio of tumor volume to body weight is much higher in these tumor caring mice than in PCa patients. It was also notable that serum average T levels of these mice was 0.83 ng ml⁻¹ (range 0.32–2.3 ng ml⁻¹), which mimics the serum T level in hypogonadal men (normal serum T level in adult men is between 2.4 and 9.5 ng ml⁻¹ - Mayo Clinic).

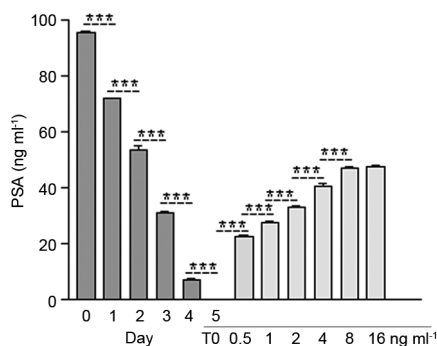


Figure 1: LNCaP cell were seeded in 24 well poly-lysine coated culture plates in RPMI-1640 medium supplemented with 10% FBS for 48 h. Medium was changed to fresh serum free medium (day 0), serum free medium was changed and collected for PSA test every day till day 4. Different concentrations of T were added to different wells on day 4 and PSA level in each well was tested on day 5. ****P* < 0.001. T: testosterone; PSA: prostate specific antigen; FBS: fetal bovine serum.

Effect of testosterone levels on serum prostate specific antigen levels in LNCaP tumor xenografts caring mice

Normal male nude mice (14–16 weeks old) had an average serum T level of 1.01 ng ml⁻¹ (*n* = 8, range 0.12–1.59 ng ml⁻¹, s.d. 0.49) and PSA levels were not detectable. 1 week after 2 mg T pellet implantation, serum PSA was still not detectable, though serum T level increased to an average 5.75 ng ml⁻¹ (range 3.47–8.87 ng ml⁻¹, s.d. 1.63) which is significant higher than that before T pellet implantation (*P* < 0.0001).

In the mice with LNCaP tumor xenografts and serum T level manipulation (*n* = 20), serum PSA values increased significantly along with elevated T levels 1 week after the T pellet implantation. T levels increased from an average of 0.9 to 6.2 ng ml⁻¹ (*P* < 0.001) and PSA levels increased from an average of 12 to 71 ng ml⁻¹ (*P* < 0.001) (Figure 2).

In order to avoid tumor volume interference, besides analyzing the data by standardizing the PSA levels by tumor volumes as above, we also chose 10 pairs of tumor caring mice from groups before and after T pellet implantation. In each pair, tumor volume difference was <5% (*P* = 0.99). The data indicated that both mice serum PSA and T levels increased significantly after T pellet implantation, as T levels increased from an average of 0.98 to 5.85 ng ml⁻¹ (*P* < 0.001) and PSA levels increased from an average of 12 to 50 ng ml⁻¹ (*P* < 0.001) (Figure 3).

DISCUSSION

Prostate specific antigen is produced almost exclusively by prostate epithelial cells, which have AR. Although it is known that the production of PSA is androgen-responsive, the exact role of androgens in the PSA production is not well documented. Cultured LNCaP cells ceased producing PSA after androgen withdrawal and resumed producing PSA after androgen was re-added. It appears that the androgen is a key factor controlling the production of PSA.

Normal serum T levels in adult men are between 2.4 and 9.5 ng ml⁻¹ (Mayo Clinic). On the basis of our results, the average serum T level in nude male mice was roughly 1 ng ml⁻¹, which mimics the serum T level in hypogonadal men. Our results showed that 48% of the mice caring prostate tumor had PSA levels lower than 4 ng ml⁻¹ and that the PSA levels significantly rose after T levels were raised in these tumor caring mice. These results suggest that low T levels could result in low PSA levels in PCa positive mice. On the basis of these data, clinical studies are suggested to confirm whether PCa patients with low serum PSA level is accompanied with low serum T level.

In recent years, numerous studies have reported that men with lower androgen levels not only are more likely to have PCa but also to have more aggressive PCa.^{4–7} It is also reported that the prevalence of hypogonadism is 38.7% in men 45 years or older.⁸ It has also been shown that PCa is present in roughly 15% of the men with a PSA levels of 4.0 ng ml⁻¹ or less.^{1–3} These data suggest that hypogonadal men may make a major part of PCa patients and there may be a higher rate of pseudo-negative result of PCa in hypogonadal men if only serum PSA level is tested. Thus, it is very important to develop more

Table 1: PSA levels in LNCaP tumor xenografts caring mice (25 mice. 9–16 weeks old, weight 16–28 g)

Groups	Percentage	PSA (ng ml ⁻¹) (average, range)	T (ng ml ⁻¹) (average, range)	Tumor volume (mm ³) (average, range)
Low PSA group	48 (12/25)	1.19 (0–3.7)	0.83 (0.32–2.3)	245 (93–693)
High PSA group	52 (13/25)	11.3 (4.6–28.7)	0.86 (0.44–1.04)	511 (277–813)

PSA: prostate specific antigen; T: testosterone



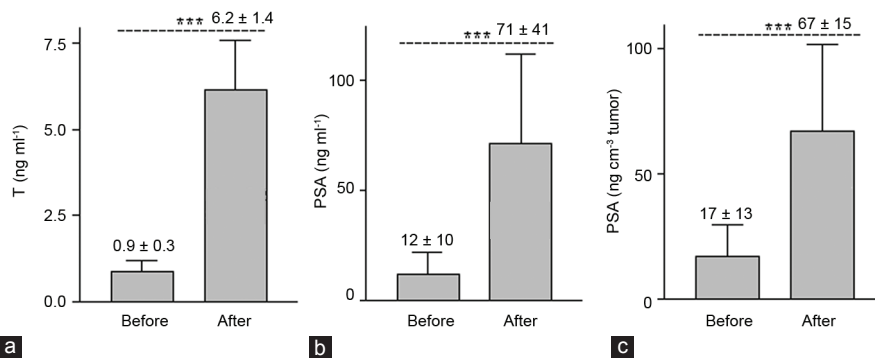


Figure 2: Serum T (a), PSA (b), and tumor volume standardized PSA levels (c) of LNCaP tumor caring mice before and 1 week after 2 mg T pellet implantation. The data showed mice serum PSA levels significantly increased after T pellet implanted along with raised serum T levels. ****P* < 0.001. T: testosterone; PSA: prostate specific antigen.

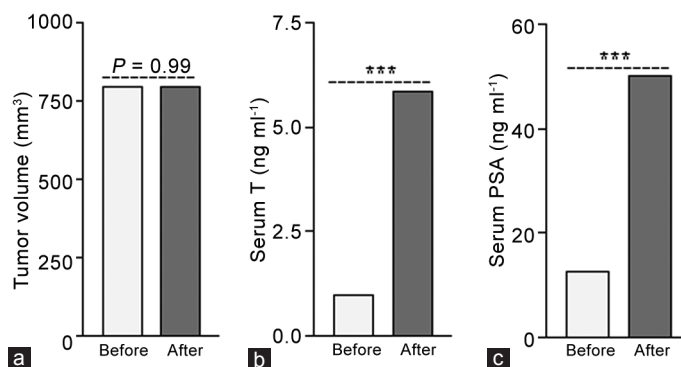


Figure 3: Tumor volume (a), serum T (b) and PSA levels (c) in 10 pairs of LNCaP tumor caring mice of groups before and 1 week after 2 mg T pellet implantation. In each pair, the difference of tumor volume is <5%. The data showed serum PSA levels significantly increased after T pellet implanted along with raised serum T levels in mice with same tumor volumes. ****P* < 0.001. T: testosterone; PSA: prostate specific antigen.

accurate applications for diagnosis of PCa in hypogonadal men. The result of our study demonstrated that raising serum T levels of normal nude mice without PCa did not have any effect on serum PSA levels. This result is similar to clinical studies which reported raising serum T level by testosterone replacement therapy (TRT) does not appear to significantly influence serum PSA level in men without PCa.^{9,10} Our data also indicated raising serum T levels in mice caring PCa xenografts from hypogonadal to eugonadal levels will also raise serum PSA levels significantly. This data is also supported clinically that both serum T and PSA levels increased significantly in hypogonadal men with PCa after initiating TRT.¹⁰ On the basis of our study, we suggest applying both T and PSA test to avoid pseudo-negative results of PCa and for men with both low T and low PSA values, it is necessary to recheck PSA levels after normalizing his T level.

A concern for initiating TRT to normalize T levels is that raising serum T levels will increase the risk of PCa incidence and enhance PCa growth. This concern is based on Huggins’ theory¹¹ which prevailed for more than 70 years. It has been believed that a severe reduction of serum androgen levels caused regression of PCa and that increasing androgen levels enhanced PCa growth. However, recent studies reported that men with lower androgen levels not only are more likely to have PCa but also to have more aggressive PCa.⁴⁻⁷ There is also data suggesting that TRT in hypogonadal men with a history of PCa have lower PCa recurrence rates.¹²⁻¹⁴ We have previously studied the relationship of androgen levels and proliferation of PCa cells *in vitro* and found that physiologically normal levels of androgen inhibit the proliferation of

PCa cells *in vitro*, though very low levels androgens are essential for PCa cells initial growth.¹⁵ These studies challenged Huggins’ theory and suggested applying TRT in hypogonadal men to normalizing their androgen level will be safe while decreasing pseudo-negative results of PCa diagnosis.

CONCLUSION

This study indicates that androgen is a key factor controlling the production of PSA and low serum PSA levels in mice with PCa xenografts is related to low serum T levels. Raising serum T levels in tumor caring mice will also significantly increase serum PSA level. This may have clinical implications when screening PSA in men who have occult PCa, though further human clinical trials are needed.

AUTHOR CONTRIBUTIONS

Both WS and MK were responsible for conception, experimental design, and drafting the manuscript and intellectual content. WS was in charge of performing experiments, data acquisition and analysis. VS was in charge of performing animal surgery. All authors read and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

Dr. Weitao Song and Dr. Vikram Soni - no conflicts of interest. Dr. Mohit Khera - consultant for Merck, Lilly, and Auxilium.

REFERENCES

- Morgentaler A, Rhoden EL. Prevalence of prostate cancer among hypogonadal



- men with prostate-specific antigen levels of 4.0 ng/mL or less. *Urology* 2006; 68: 1263–7.
- 2 Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, *et al*. Prevalence of prostate cancer among men with a prostate-specific antigen level \leq 4.0 ng per milliliter. *N Engl J Med* 2004; 350: 2239–46.
 - 3 Babaian RJ, Johnston DA, Naccarato W, Ayala A, Bhadkamkar VA, *et al*. The incidence of prostate cancer in a screening population with a serum prostate specific antigen between 2.5 and 4.0 ng/ml: relation to biopsy strategy. *J Urol* 2001; 165: 757–60.
 - 4 Hoffman MA, DeWolf WC, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? *J Urol* 2000; 163: 824–7.
 - 5 Lane BR, Stephenson AJ, Magi-Galluzzi C, Lakin MM, Klein EA. Low testosterone and risk of biochemical recurrence and poorly differentiated prostate cancer at radical prostatectomy. *Urology* 2008; 72: 1240–5.
 - 6 Ribeiro M, Ruff P, Falkson G. Low serum testosterone and a younger age predict for a poor outcome in metastatic prostate cancer. *Am J Clin Oncol* 1997; 20: 605–8.
 - 7 Teloken C, Da Ros CT, Caraver F, Weber FA, Cavalheiro AP, *et al*. Low serum testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy: hypogonadism represents bad prognosis in prostate cancer. *J Urol* 2005; 174: 2178–80.
 - 8 Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 years: the HIM study. *Int J Clin Pract* 2006; 60: 762–9.
 - 9 Grober ED, Lamb DJ, Khera M, Murthy L, Lipshultz LI. Correlation between simultaneous PSA and serum testosterone concentrations among eugonadal, untreated hypogonadal and hypogonadal men receiving testosterone replacement therapy. *Int J Impot Res* 2008; 20: 561–5.
 - 10 Coward RM, Simhan J, Carson CC 3rd. Prostate-specific antigen changes and prostate cancer in hypogonadal men treated with testosterone replacement therapy. *BJU Int* 2009; 103: 1179–83.
 - 11 Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941; 1: 293–7.
 - 12 Khera M, Grober ED, Najari B, Colen JS, Mohamed O, *et al*. Testosterone replacement therapy following radical prostatectomy. *J Sex Med* 2009; 6: 1165–70.
 - 13 Morgentaler A. Testosterone therapy for men at risk for or with history of prostate cancer. *Curr Treat Options Oncol* 2006; 7: 363–9.
 - 14 Rhoden EL, Averbek MA. Testosterone therapy and prostate carcinoma. *Curr Urol Rep* 2009; 10: 453–9.
 - 15 Song W, Khera M. Physiological normal levels of androgen inhibit proliferation of prostate cancer cells *in vitro*. *Asian J Androl* 2014; 16: 864–8.