Establishment of a Serum Tumor Marker for Preclinical Trials of Mouse Prostate Cancer Models

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Abstract

Current prostate cancer research in both basic and preclinical trial studies employ genetically engineered mouse models. However, unlike in human prostate cancer patients, rodents have no counterpart of prostatic-specific antigen (PSA) for monitoring prostate cancer initiation and progression. In this study, we established a mouse serum tumor marker from a mouse homologue of human prostate secretory protein of 94 amino acids (PSP94). Immunohistochemistry studies on different histologic grades from both transgenic and knock-in mouse prostate cancer models showed the down-regulation of tissue PSP94 expression (P < 0.001), the same as for PSA and PSP94 in humans. The presence of mouse serum PSP94 was shown by affinity column and immunoprecipitation purification using a polyclonal mouse PSP94 antibody. A competitive ELISA protocol was established to quantify serum PSP94 levels with a sensitivity of 1 ng/mL. Quantified serum levels of mouse PSP94 ranged from 49.84 ng/mL in wild-type mice to 113.86, 400.45, and 930.90 ng/mL in mouse prostatic intraepithelial neoplasia with microinvasion, well differentiated, moderately differentiated, and poorly differentiated prostate cancer genetically engineered prostate cancer mice, respectively (P < 0.01, n = 68). This increase in serum PSP94 is also well correlated with age and tumor weight. Through longitudinal monitoring of serum PSP94 levels of castrated mice (androgen ablation therapy), we found a correlation between responsiveness/refractory prostate tissues and serum PSP94 levels. The utility of mouse serum PSP94 as a marker in hormone therapy was further confirmed by three-dimensional ultrasound imaging. The establishment of the first rodent prostate cancer serum biomarker will greatly facilitate both basic and preclinical research on human prostate cancer.

Prostate cancer remains the most frequently diagnosed malignancy among North American males and is second only to lung cancer in mortality (1). Genetics, diet, and lifestyle are just some of the factors that contribute to the development of prostate cancer (2). PSA is the only prostate cancer marker widely used for population screening, diagnosis, and monitoring of prostate cancer. The use of PSA as a serum marker has increased our ability to detect prostate cancer, select therapy, and to monitor outcomes (3–5).

Prostate cancer has proven to be a complicated disease due to its heterogeneous and multifocal nature (6, 7). Consequently, much research has been devoted to elucidating the mechanisms of the disease. This has led to the construction of genetically engineered mouse models of prostate cancer using transgenic and knock-out techniques that attempt to model the human clinical situation in all aspects (for reviews, see refs. 6, 8). Rat probasin gene-based transgenic adenocarcinoma prostate (TRAMP; ref. 9) and LPB-SV40 tag (LADY; ref. 10) models are currently the most prevalent murine prostate cancer models.

Unlike in humans, murine models of prostate cancer currently lack established biomarkers for the disease, specifically serum biomarkers (review refs. 6, 7). A serum marker is an affordable noninvasive screening test that can monitor treatment efficacy and disease recurrence disease-free state. The ability to noninvasively quantify tumor burden in living conditional tumor model mice will ultimately lead to the development of more accurate models of human cancer that are better suited to evaluating and optimizing preclinical cancer therapy.

Because mice do not produce and express a human PSA analogous, the search begins for other equals in mice. Prostatic secretory protein of 94 amino acids (PSP94), also known as β-microseminoprotein (11–15), is one of the three most abundantly secreted proteins (0.5-1 mg/mL in semen) from the prostate gland (the others being PSA and prostatic acid phosphatase). Both PSA and PSP94 can leak out from the
prostate glandular ducts into the blood circulation at a detectable concentration (3, 4, 14, 15). The abnormal protein levels in serum in prostate cancer patients indicate irregular or erratic control of the secretion of prostate cancer cells and possibly defective or less efficient tissue barrier (3–5, 16, 17). The similar tissue distribution of PSA and PSP94 in secretory tissues and their similar means of escape from prostate secretory ducts into the general circulation suggest a similar control mechanism of secretion of these two proteins (3, 4, 14, 15). PSP94 expression in prostate tissue has been shown as having a statistical correlation with histologic grade (13, 18–21). This association is inversely correlated (that is, as tumor grade advances PSP94 expression decreases), the same as in PSA (3, 4, 16, 17). As with PSA, much research has investigated the use of PSP94 as a prostate cancer marker in humans in terms of serum bound free forms, urine levels, and tissue expression (14, 15).

To investigate the use of mouse PSP94 as a serum biomarker for mouse prostate cancer model studies, we did a study to show, detect, and quantify PSP94 in the mouse serum.

**Materials and Methods**

**Expression and purification of polyhistidine containing recombinant mouse PSP94.** A cDNA fragment coding for the mature form of mouse PSP94 (22) was amplified and inserted into the polyhistidine containing vector (pTrcHis A, Invitrogen, Carlsbad, CA) as reported previously (23). An *Escherichia coli* strain DH15a (Invitrogen) was used to purify large amounts of recombinant protein (23), following the manufacturer’s recommendations. Matrix-assisted laser desorption ionization and electrospray ionization tandem mass spectrometry protein sequencing was used for the identification of pTrcHis-Mouse PSP94. For sequencing, 10 μg of recombinant protein were run on 15% SDS-PAGE, the band was excised from the gel, and in-gel trypsin digestion was done.

**Generation of rabbit antiserum against recombinant fusion proteins of pTrcHis-mPSP94.** Rabbit polyclonal antibodies against pTrcHis mouse PSP94 proteins were obtained using a standard procedure (SOP #370-01, University of Western Ontario Animal Care Committee). In brief, ~2.5 μg of recombinant mouse PSP94 (1.0 mL) was emulsified in 1.0 mL of Freund’s adjuvant (incomplete, from Sigma, St. Louis, MO) and injected i.m. into rabbits 1 kg in size. A second booster (1.5 mg) injection was done 2 weeks later.

**Genetically engineered mouse prostate cancer models.** In previous studies, we used a 3.84-kb promoter enhancer region of the *PSP94* gene, which PSP94 expression in prostate tissue has been shown as having a statistical correlation with histologic grade (13, 18–21). This association is inversely correlated (that is, as tumor grade advances PSP94 expression decreases), the same as in PSA (3, 4, 16, 17). As with PSA, much research has investigated the use of PSP94 as a prostate cancer marker in humans in terms of serum bound free forms, urine levels, and tissue expression (14, 15).

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antiserum was diluted 1:40,000 in 1.5% bovine serum albumin/PBS with E. coli (isomerase block) lysate and preincubated for 1 hour. Blocked antiserum was then added to standards/samples and incubated for 1.5 hours. Competitor mixtures were then added to immunoplates and incubated for 30 minutes. Horseradish peroxidase–conjugated goat antiserum anti-rabbit IgG was diluted (1:1,000) and incubated for 1 hour. The plate was then washed and incubated in 0.4 mg/ml o-phenylene diamine dihydrochloride (Sigma) and 0.05% H2O2 in developing buffer [35 mmol/L citric acid, 67 mmol/L Na2HPO4 (pH 5.0)] for 20 minutes. Standard curves were plotted by relative absorbency (B/B0) against competitor standard protein (ng/mL). Relative absorbency (B/B0) was calculated as follows: B = A595 nm of the sample – nonspecific binding (NSB). NSB was determined by testing A595 nm with excess standard (1 µg/ml) in the competition reaction to entirely block the antibody (i.e., under maximum competition and minimum antibody binding to the plate). B0 = A595 nm of the maximum antibody binding (no competition) – NSB.

Results

Characterization of a polyclonal antibody against recombinant pTrcHis mPSP94. We have previously reported the production of several kinds of antibodies for mouse PSP94 studies, all of which lacked high titer and specificity (23). As shown in SDS-PAGE (Fig. 1A), according to the standard protocol using the heavy metal affinity column provided by the manufacturer, two dominant protein bands were always copurified at an apparent molecular weight of 20.8 and 15 kDa separately. Matrix-assisted laser desorption ionization and electrospray ionization tandem mass spectrometry sequencing was done on these two purified proteins, which were identified as isomerase and pTrcHis mPSP94, respectively, and both contained several histidine rich areas (data not shown). Considering that mouse PSP94 is a small-sized peptide (93 amino acids, 10.6 kDa) of weaker antigenicity, we used the larger bacteria band (peptidyl-prolyl cis/trans-isomerase from E. coli, 20.8 kDa) as a carrier protein, which should act the same as other glutathione S-transferase fusion proteins we used (23) to elicit a stronger immune response. A higher dose of antigen (2.5 mg pTrcHis mPSP94/ rabbit) was used to obtain polyclonal rabbit antiserum. Western blotting analysis showed the antibody to be both sensitive and specific (Fig. 1A and B) for isomerase and pTrcHis mPSP94.

Western blot analysis was also conducted on natural mouse PSP94 from the ventral prostate lysate and found to be very specific (Figs. 1E and 3B). Because the antibody detected a wide band with smearing, a kit was used to detect glycosylation of PSP94 (Fig. 1C). A broad range of glycosylated mouse PSP94 proteins, shown as positive magenta bands, were observed (Fig. 1C), indicating the protein is heavily glycosylated (pTrcHis mPSP94 was used as a negative control). As a control, human PSP94 protein (10 µg) purified from human semen (30) was loaded for a comparison. Figure 1D shows
a Coomassie blue SDS-PAGE staining of the purified human PSP94 protein. Glycosylation detection for human seminal PSP94 (Fig. 1D) was negative. Deglycosylation reaction was done to confirm the glycosylation of mouse PSP94 from prostate tissue lysates. Western blots (Fig. 1E) showed a stronger band with lower molecular weight (14.3 kb) after deglycosylation, probably due to focusing of the deglycosylated PSP94 protein.

**Down-regulation of the prostate tissue PSP94 expression is associated with different tumor grades as shown by immunohistochemistry.** The antibody for pTrcHis mPSP94 was further characterized via immunohistochemistry staining and found to be specific for the cytoplasm of the epithelial cells of the prostate gland (Fig. 2). No stromal staining was detected. PSP94 expression was tested in 100 different tissue fixed prostate gland (Fig. 2). No stromal staining was detected. Be specific for the cytoplasm of the epithelial cells of the characterised via immunohistochemistry staining and found to be specific for the cytoplasm of the epithelial cells of the prostate gland (Fig. 2). No stromal staining was detected.

**Fig. 2.** Histologic analysis of prostate tissue PSP94 expression from genetically engineered prostate cancer mouse models. A, strong PSP94 expression (≥2) in wild-type prostate tissue with a 76% to 100% extent of staining (20×). A, highlighting the stais free stroma (inset, 40×). B, strong PSP94 expression (≥2) in low-grade mPIN prostate tissue with a 76% to 100% extent of staining (20×). Insert, stained cytoplasm of the secretory cells where PSP94 is located (40×). C, strong PSP94 expression (≥2) in high-grade mPIN area with an extent of 76% to 100% (20×). Arrow points to the stained cytoplasm of the secretory cells (40×). D, moderate intensity of PSP94 staining (1+) in well-differentiated prostate cancer with an extent of 51% to 75% (20×). At higher magnification (40×), nuclear atypia is clear as well as staining localization of PSP94 to the cytoplasm of the secretory cells. E, moderate intensity of PSP94 staining (1+) in moderately differentiated prostate cancer with an extent of 51% to 75% (20×). Inset, boxed area of nuclear atypia and proliferating cells with weaker staining localized in the cytoplasm of the secretory cells (arrow). F, negative PSP94 expression (0) in poorly differentiated prostate cancer with higher magnification revealing nuclear atypia (arrow B) and cell proliferation (arrow A). G, plot of the correlation of immunoreactivity with prostate cancer progression. Abbreviations: WT, normal wild-type mice; LGmPIN, low-grade mouse PIN; HGMmPIN, high-grade mouse PIN; WDCaP, well-differentiated prostate cancer; MDCaP, moderately differentiated prostate cancer; PDCaP, poorly differentiated prostate cancer. Difference between grade and immunoreactivity is significant (P < 0.01).

Down-regulation of the prostate tissue PSP94 expression is associated with different tumor grades as shown by immunohistochemistry. The antibody for pTrcHis mPSP94 was further characterized via immunohistochemistry staining and found to be specific for the cytoplasm of the epithelial cells of the prostate gland (Fig. 2). Next, we intend to test, as for PSA and PSP94 in humans, whether mPSP94 is able to “leak” into the mouse vasculature. To show the presence of mouse PSP94 in the serum of wild-type mice by affinity column separation and purification. Next, PSP94 from mouse ventral prostate lysates was passed through the column to determine if the column could purify
natural mouse PSP94 (Fig. 3B). The column successfully purified mouse PSP94 with a yield of roughly 40%. Little to no mouse PSP94 was observed in the column pass; however, a substantial amount was noted in the first column wash. Finally, 2 and 4 mL of wild-type mouse serum were passed through the column, and mouse PSP94 was eluted (Fig. 3C). Column passes and washes yielded no mouse PSP94 but did have some nonspecific protein, most likely due to the high protein content of serum. Ventral prostate lysate mouse PSP94 band was used as a positive control and was found to match up with serum elutions. Furthermore, increasing amounts of mouse PSP94 were noted for larger sample sizes of serum.

Further demonstration of the existence of PSP94 in mouse serum was by immunoprecipitation experiments. The same logical steps were taken as in affinity column, producing the same results (Fig. 3). First, recombinant pTrcHis mPSP94 was precipitated to show the technique was working properly (Fig. 3D). Second, natural mouse PSP94 was precipitated from ventral prostate lysate (Fig. 3E). Like in affinity column, substantial amounts of mouse PSP94 washed off in the pass and first wash; however, a significant amount was visible in the precipitate. In the same blot, 2 mL of serum were precipitated. Due to the high protein content of serum, the pass had significant nonspecific background. No mouse PSP94 was detected in the subsequent washes and the precipitate revealed a multiband mouse PSP94. The multiple bands observed for immunoprecipitation and not Affinity column purification may be due to the fact that in immunoprecipitation the antibody is free in solution and may bind to PSP94 differently. Glycosylation of mPSP94 may also play a role in this phenomenon. Both affinity column and immunoprecipitation produced comparable amounts of mouse PSP94 precipitate for 2 mL of serum.

**Establishment of competitive ELISA standard curves using recombinant pTrcHis mouse PSP94 as coating antigen.** To show that mouse serum PSP94 is quantifiable, two sets of immunoassays were done for obtaining standard curves that will quantify serum mouse PSP94 levels and show the assay is functional for natural mouse PSP94. The first standard graph used recombinant pTrcHis mPSP94 as both the coating antigen and the competitor (Fig. 4A). This assay consistently produced a linear logarithmic graph with a negative slope, indicating the assay was working. The next step was to confirm the sensitivity of the assay applied to natural mouse PSP94. Figure 4B is an example of the resultant standard curve when using recombinant pTrcHis mPSP94 as the coating antigen, and mouse PSP94 from the ventral prostate lysate as the competitor. This standard graph produced no slope and showed the assay could not distinguish between different levels of mouse PSP94. It was thought that because the carrier protein isomerase most likely produced the most antibodies in our polyclonal antibody, it was overpowering our immunopassay. This was not noticed in our standard graph using recombinant pTrcHis mPSP94 as the competitor because, in that case, isomerase coated to the plate had competition. Consequently, *E. coli* lysate containing isomerase was used to block our polyclonal antibody and remove any signal produced by isomerase competition. Two new standard curves (Fig. 4C and D) were generated using this technique. Again, using pTrcHis as the competitor, the assay produced a linear graph with a negative slope (Fig. 4C). The same results were obtained when using the ventral prostate lysate (natural mouse PSP94) as the competitor, indicating the assay was functional for detecting mouse PSP94 (Fig. 4D). This new assay using *E. coli* lysate to block out isomerase activity was consequently used to quantify mouse PSP94 in our unknown serum samples.

**Quantification of mouse PSP94 in serum from mice of different age groups via established competitive ELISA techniques.** From the established standard graph technique (Fig. 4C), serum
mouse PSP94 levels were quantified (Fig. 4E). In our system, 50 µL of serum separated from mouse tail blood were assayed by ELISA for each well. Average serum levels for wild-type mice were 49.84 ng/mL. Average serum levels for KIMAP mice with mPIN with microinvasion, well-differentiated prostate cancer, and moderately/poorly differentiated prostate cancer were 113.86, 400.45, and 930.90 ng/mL, respectively (Fig. 4E). Compared with wild-type mice, serum PSP94 levels increase significantly (P < 0.001, ANOVA) with both tumor grade and age in KIMAP mice. This increase is synchronous to increases of grades from well- to moderately differentiated prostate cancer in our KIMAP model (19, 20). No significant difference was noted between wild type and mPIN with microinvasion (P = 0.941).

Next, to correlate serum PSP94 levels with tumor weight, a correlation of tumor weight and age in KIMAP was established (Fig. 4F). No detectable differences were observed before the age of 33 weeks (Fig. 4F). Larger error bar for 72- to 91-week-old mice was found reflecting nonsynchronous tumor growth before castration and 2 and 5 weeks after castration. As shown in Fig. 5A, there was a significant decrease in serum PSP94 levels (P < 0.001) after castration. Histologic analysis of prostate tissue showed significant involution as a result of the androgen deprivation (Fig. 5B and C).

To show use of serum PSP94 marker to monitor hormone therapy (castration) in genetically engineered prostate cancer mouse models. To show use of serum PSP94 in preclinical trial studies, PSP94 levels were tested for their responsiveness and refractory to androgen deprivation therapy in our transgenic (PSP-TGAMP) and knock-in (PSP-KIMAP) mouse prostate cancer models. Castration was used for mimicking hormone therapy in two groups of mice with prostate cancer. The first group was used to test for tumors in well- and moderately differentiated prostate cancer development (not at the late stage of prostate cancer development with massive metastasis) in our recently established knock-in prostate cancer (PSP-KIMAP) model (19, 20). Five PSP-KIMAP mice at the age of 10 months were subjected to orchidectomy operation. Tail blood samples were collected before castration and 2 and 5 weeks after castration. As shown in Fig. 5A, there was a significant decrease in serum PSP94 levels (P < 0.001) after castration.
AR and PSP94 showed a reduction in expression compared with noncastrated mice (Fig. 2B, D, and E). Because AR staining was still present (Fig. 5D), the observed PSP94 expression may be attributed to testosterone secreted from the adrenal gland.

To test for refractory of hormone therapy in preclinical trials, genetically engineered prostate cancer mice at later stages of prostate cancer with large tumor mass were selected for hormone therapy (castration). Only PSP-TGMAP mice were selected. A new method developed in our laboratory for acquisition and analysis of three-dimensional ultrasound images of mouse prostate cancer models (24) was employed to assist the diagnosis by PSP94 serum marker. This is because of the following considerations: (a) PSP-TGMAP mice revealed exuberant tumor growth with neuroendocrine features (as did the TRAMP and Lady models), which the genetically engineered prostate cancer tumor mass could reach extraordinary sizes of up to 15 g/35 body weight in just 2 weeks time (19, 20, 24). (b) Our University Council on Animal Care protocols mandate a 2-week time interval limit for collecting tail blood and more frequent collection is not possible. (3) It is important to assess the effectiveness of the serum marker. A total of three mice were castrated and two were imaged.

Figure 6A illustrates that serum levels of PSP94 in castrated mice decreased 2 weeks after castration and then stabilized at a higher level than normal wild-type mice. This may indicate the incomplete responsiveness of prostate cancer after castration. Furthermore, longitudinal three-dimensional ultrasound imaging of a castrated mouse in Fig. 6B revealed that the tumor decreased slightly in size from 4.37 to 3.97 mm in diameter (Fig. 6B, row 1) 1 week after castration. Continual tumor expansion was observed in three-dimensional imaging (rows 2–3) from 1 to 7 weeks after castration. Based on the longitudinal imaging data and measurements of tumor diameters, a tumor growth curve was established (Fig. 6C), along with noncastrated mice (n = 3) as a control. The growth curve shows that castration (hormone therapy) in mice with late stages of prostate cancer slowed and delayed the rapid tumor growth rate and increased survivability. Responsiveness to castration (hormone therapy) was brief (first week after castration), which is consistent with the results from the serum PSP94 tests (Fig. 6A). Histologic analysis (Fig. 6D) also showed that in castrated mice, even in poorly differentiated prostate cancer tissues, atrophy is evident, which is the reason for increased survivability (Fig. 6C). Immunohistochemistry experiments with several prostate cancer marker genes (AR and PSP94) were done to further show that the androgen therapy was, in fact, working. The expression levels for each of the two markers used were decreased when compared with the levels of genetically engineered prostate cancer mice with the same cancer grade (Fig. 6D).

**Discussion**

PSA as the "gold standard" for the early detection and tracking of tumor progression has revolutionized the management of prostate cancer (3, 4). Rising PSA levels after prostatectomy or radiotherapy generally predicts clinical recurrence. In contrast to the number of markers developed for human prostate cancer, rodents have few tumor markers and no identified PSA counterpart (gene or protein). A prostate-specific serum tumor marker as effective as or more effective than PSA would greatly assist in longitudinal observations of prostate cancer development in genetically engineered prostate cancer models in both basic and preclinical trial studies. By exploring PSP94 as a serum marker for mouse prostate cancer models, we will contribute uniquely to the establishment of a preclinical trial model for prostate cancer.

In contrast to PSA showing no counterpart in rodent species, PSP94 has analogous proteins in humans, primates, pigs, and rodents. These studies have shown that PSP94 is a conserved but also a rapidly evolving, protein (minireview; refs. 22, 26). We have previously shown that, at the transcriptional and protein expression levels, rodent PSP94 expression is strictly prostate tissue specific (26, 34). We have also found serum bound forms of PSP94 (14, 15), similar to serum-bound forms of PSA (35, 36). PSA and PSP94 may thus have a similar application as serum markers for prostate cancer.

Before establishing mPSP94 as a serum biomarker in mice, we failed in constructing an antibody against mouse natural PSP94 with high specificity and immunoreactivity (23). In this study, we have discovered that, unlike human PSP94, mouse PSP94 is glycosylated. This may suggest that glycosylation of mouse PSP94 protein contributes an important role as an immunogen. Glycosylation of mPSP94 may play a role in the reduced affinity of our recombinant antibody to natural mouse PSP94 by blocking epitope structures of mouse PSP94.
observation is further supported by the fact that our antibody has a significantly higher affinity for our recombinant pTrcHis protein, which is not glycosylated (Fig. 1). This reduction in affinity caused problems when establishing standard curves for quantifying mouse serum PSP94 (Fig. 4) and must be addressed to refine the immunoassay.

We have shown that PSP94 is present in mouse serum by both affinity column and immunoprecipitation methods. Through the established competitive ELISA technique in this study, we have also shown that the concentration of mouse serum PSP94 levels is higher than human PSA levels, although the range (ng/mL) for human PSA and PSP94 is similar.

The use of mouse PSP94 as a serum marker lies in its ability to distinguish between mice with or without prostate cancer even before the tumor is palpable. In this study, mice with genetically engineered prostate cancer from both transgenic and knock-in mouse models were investigated. Both models represent two types of tumor developmental kinetics, fast and protract (18–20, 24). At a tissue level, we showed that mouse PSP94 protein expression decreases as prostate cancer progresses in a statistically significant way (Fig. 2G). This decrease correlated with tumor intensity, rather than the extent of the tumor staining (Fig. 2), indicating that intensity is the leading factor for determining the rate at which PSP94 may leak into the surrounding vasculature. Through ELISA techniques, we showed that PSP94 increased with tumor grade and age in our KIMAP model of prostate cancer indicating that PSP94 serum levels were affected by cancer grade (P < 0.01, ANOVA). Furthermore, significant elevation of serum PSP94 was found to correlate with significant increases in average tumor weight in a majority of our KIMAP mice (19, 20).

An important issue for preclinical trials is to test the responsiveness and refractory of prostate cancer to hormone therapy. We have shown the use of a serum marker in monitoring preclinical trials for hormone therapy of genetically engineered prostate cancer mice in our newly established PSP-KIMAP model, with the majority of well- and moderately differentiated prostate cancer.

By doing castration experiments, we have shown that serum PSP94 could be used as an indicator for the responsiveness of hormone therapy. We have also tested refractory to hormone therapy in PSP-TGMAP mice with a later stage of prostate cancer development. In the PSP-TGMAP model, as with in the TRAMP model, transgenic mice reveal rapid and exuberant

**Fig. 6.** Combined tests of mouse serum PSP94 levels and three-dimensional (3-D) ultrasound imaging before and after androgen deprivation therapy (castration) for TGMAP mice at late stage of genetically engineered prostate cancer (GE-CaP) development. A, serum mPSP94 levels (ng) before and after castration showing a significant reduction in response to therapy followed by refractory (n = 3, P < 0.05). B, longitudinal detection by three-dimensional ultrasound imaging in living castrated mice with rapid ventral prostate tumor development. 1-6, representative two-dimensional slices from six different three-dimensional ultrasound images before castration and at different time points after castration. 1, mouse was 21 weeks old when the tumor was first detected (measuring 4.37 mm in diameter) and was then castrated (day 1). 2-5, the same tumor on days 8, 15, 22, 29, and 43 as the tumor diameter went from 3.97 to 5.54, 6.37, 8.25, and 11.81 mm, respectively. Ventral surface of the abdominal wall (top) and deeper abdominal and retroperitoneal structures (bottom). Bar, 1 mm. Prostate (CaP) and bladder (B1) positions. C, determination of growth curve in PSP-TGMAP mice based on three-dimensional ultrasound imaging data of (B). Rapid tumor growth rate is shown in this graph for transgenic mice with genetically engineered prostate cancers. The control group was PSP-TGMAP mice (n = 3) without castration. The maximum sagittal diameter of the tumor was plotted against the day after initial observation/castration. D, histologic analysis of the castrated prostate tissues demonstrating involution of gland structure and altered PSP94 and AR expression (10×). Before castration was from the age matched, late stage of prostate cancer in TGMAP mice. Involution in the prostate tissue under castration was evident. Immunohistochemistry analysis of the prostate tissue showed weak PSP94 and AR expression in poorly differentiated prostate cancer.
prostate cancer growth with logarithmic volume expanding in a very short time (18–20, 24). Because of the speed of tumor growth and time constraints for mouse blood sampling, daily monitoring of serum levels will not be possible and thus other techniques must be used. We showed that three-dimensional ultrasound imaging could supplement the tumor biochemical marker for longitudinal observations in living conditions in the preclinical trial. The recently developed three-dimensional ultrasound imaging technology applied to genetically engineered prostate cancer mice in our laboratories has shown many advantages (24). By combining serum marker analysis and three-dimensional ultrasound imaging, we have developed a solid technique for monitoring prostate cancer longitudinally with consistent accuracy. Biochemical serum markers and biomedical imaging in living animals have great value for both basic and preclinical trial studies of prostate cancer.

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Establishment of a Serum Tumor Marker for Preclinical Trials of Mouse Prostate Cancer Models

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