Published in final edited form as:

Prostate. 2012 October 1; 72(14): 1532–1541. doi:10.1002/pros.22508.

Chemoaffinity Capture of Pre-Targeted Prostate Cancer Cells with Magnetic Beads

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Abstract

BACKGROUND—Prostate circulating tumor cells (PCTCs) in circulation are shed from either a primary tumor or metastases, which are directly responsible for most prostate cancer deaths. Quantifying exfoliated PCTCs may serve as an indicator for the clinical management of prostate cancer, isolating and removing of PCTCs could potentially reduce prostate cancer metastasis, and culturing and characterizing captured PCTCs could facilitate the development of personalized treatment options. Prostate-specific membrane antigen (PSMA) is an established biomarker for prostate cancer being strongly expressed on prostate tumor cells associated with high-grade primary, androgen independent, and metastatic tumors.

METHODS—Suspensions of PSMA+ (LNCaP) cells were pre-targeted with the irreversible PSMA inhibitor biotin-PEG₁₂-CTT-54 to serve as a bait to capture PSMA+ cells using streptavidin-coated magnetic beads. Decreasing numbers of LNCaP cells were spiked into blood to determine the cell captured efficiency, recovery and viability.

RESULTS—High selectivity, recovery, and viability were achieved for the capture of PSMA+ cells in both model experiments with mixtures of LNCaP cells and WBCs as well as blood samples spiked with LNCaP cells. As low as 10 cells were captured from 1 mL of blood with nearly 90% viability. More importantly, captured cells could be subsequently propagated *in vitro*.

CONCLUSIONS—This methodology for the detection, isolation, and culture of PCTCs from peripheral blood can serve as an effective tool for the detection of metastatic prostate cancer, treatment monitoring, and the development of personalized therapy based on the responsiveness of PCTCs to chemotherapeutic strategies.

Keywords

prostate cancer; circulating tumor cells; PSMA; prostate-specific membrane antigen; flow cytometry; magnetic bead

1. INTRODUCTION

Prostate cancer is the most common diagnosed cancer for men in the US.(1) In 2010, over 217,730 new cases of prostate cancer were recorded and the mortalities reached approximately 32,000.(1) The leading cause of death in prostate cancer patients is a

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consequence of overt metastasis in distal sites.(2) The fundamental biology of these prostate metastases differ from the primary tumor and change over time, which may result in unresponsiveness to certain therapies.(3) Therefore, isolating, propagating, and characterizing metastatic cells from patients opens opportunities for studying the biology of disseminated tumor cells and for developing successful and personalized treatment.

Currently, detection methods for circulating tumor cells (CTCs) of prostate cancer include real-time polymerase chain reactions (RT-PCR) assays for PSA mRNA and immunocytochemical techniques, such as CellSearch.(4–11) Although the PSA RT-PCR assay provides high sensitivity, it does not allow for quantification or recovery of CTCs.(12) The CellSearch system, which utilizes an immunomagnetic enrichment strategy based on the epithelial cell adhesion molecule (EpCAM) antigen, can detect CTCs in patients with metastatic disease from a plethora of extensive carcinomas, such as breast, colon, prostate, etc.(4–11) However, the subpopulation of CTCs that downregulate EpCAM expression during the epithelial to mesenchymal transition (EMT) and are thought to exhibit stem-cell properties conducive to invasive micrometastasis, cannot be detected.(13,14) Therefore, methods for detecting circulating and invasive prostate tumor cells currently remain limited.

The cell-surface enzyme prostate-specific membrane antigen (PSMA) is an important and unique biomarker in prostate cancer research. It is strongly expressed on prostate tumor cells associated with high-grade primary, androgen independent, and metastatic tumors.(15) Recent studies have demonstrated that PSMA expression is heterogeneous in primary prostate tumors and metastases, but completely PSMA-negative prostate carcinomas are infrequent.(16) This has inspired considerable effort toward the development of PSMA-targeted diagnostic and therapeutic technologies. However, its usage in cell-capture applications has not been widely studied. In the past decade, various chemical scaffolds of PSMA inhibitors, such as phosphinate and urea structures, have been developed and conjugated to fluorescent dyes for cell detection.(17–20) However, these chemical scaffolds have not been utilized in cell-capture applications. In our previous studies, we demonstrated that a fluorescent phosphoramidate PSMA inhibitor could effectively detect prostate tumor cells in blood by flow cytometry.(21) The focus of this study was to confirm that based on PSMA expression prostate tumor cells could be captured selectively by magnetic isolation, quantified by flow cytometry, and subsequently propagated *in vitro*.

2. MATERIALS AND METHODS

2.1 Cell Lines, Reagents, and General Procedures

LNCaP and PC-3 cells were obtained from the American Type Culture Collection (Manassa, VA). All other chemicals and cell-culture reagents were purchased from Fisher Scientific (Sommerville, NJ), Pierce (Rockford, IL), or Sigma-Aldrich (St. Louis, MO). The 15 μ m nonfluorescent polystyrene microsphere beads (Flow Cytometry size calibration Kit) were obtained from Invitrogen (Carlsbad, CA). The 1 μ m Streptavidin coated Magnetic Beads were obtained from Pierce (Rockford, IL). Porcine blood was obtained from the Swine Center (Pullman, WA). The inhibitor CTT-54 was available from a prior study.(21)

2.2 Preparation of biotin-PEG₁₂-CTT-54

Biotin-PEG₁₂-CTT-54 was synthesized by a method similar to one previously reported for the preparation of a fluorescent conjugate of CTT-54.(21) A solution of NHS-PEG₁₂-Biotin (25 mg, 27 μ mol) in 100 μ L DMSO was added to a stirred solution of the CTT-54 (8.5 mg, 13 μ mol, 100 μ L of 20 mM in H₂O), 160 μ L H₂O, and 40 μ L of 1M NaHCO₃, and stirred for 6 hr. The pH of the solution was then adjusted to 9.3 by an addition of 8 μ L of 1M Na₂CO₃. 25 mg of Si-Isocyanate resin (SiliCycle, Inc., Quebec, Canada) was added to the solution to

scavenge the unreacted inhibitor CTT-54 by stirring overnight at room temperature. The solution was subsequently centrifuged (9,000 rpm, 10 min) and the supernatant was lyophilized in a 2 mL microcentrifuge tube. Unreacted or hydrolyzed NHS-PEG₁₂-Biotin was removed by successively triturating the lyophilized solid 10 times with 1 mL portions of DMSO and centrifuging the mixture (1 min at 13,000 rpm) after each wash. The biotin-conjugated inhibitor was resuspended in 50 mM Tris buffer (pH 7.5) to give a final concentration of 4 mM. MALDI-HRMS (M+K)⁺: C₅₀H₈₈N₆O₂₇PSK, expected 1307.487, found 1307.468.

2.3 IC₅₀ Determination and Reversibility of Enzyme Inhibition

Inhibition studies were performed as described previously with only minor modifications. (22,23) Working solutions of the substrate (*N*-[4-(phenylazo)- benzoyl]-glutamyl- γ -glutamic acid, PABG γ G) and biotin-PEG₁₂-CTT-54 were made in TRIS buffer (50 mM, pH 7.4). Working solutions of purified PSMA were diluted in TRIS buffer (50 mM, pH 7.4 containing 1% Triton X-100) to provide 15% to 20% conversion of substrate to product in the absence of inhibitor. A typical incubation mixture (final volume 250 mL) was prepared by the addition of either 25 mL of an inhibitor solution or 25 mL TRIS buffer (50 mM, pH 7.4) to 175 mL TRIS buffer (50 mM, pH 7.4) in a test tube. PABG γ G (25 mL, 10 mM) was added to the above solution. The enzymatic reaction was initiated by the addition of 25 mL of the PSMA working solution. In all cases, the final concentration of PABG γ G was 1 μ M while the enzyme was incubated with five serially diluted inhibitor concentrations providing a range of inhibition from 10% to 90%. The reaction was allowed to proceed for 15 min with constant shaking at 37 °C then terminated by the addition of 25 mL methanolic TFA (2% trifluoroacetic acid by volume in methanol) followed by vortexing. The quenched incubation mixture was quickly buffered by the addition of 25 mL K₂HPO₄ (0.1 M), vortexed, and centrifuged (10 min at 7,000 g). An 85 mL aliquot of the resulting supernatant was subsequently quantified by HPLC as previously described. (24,25) IC₅₀ values were calculated using KaleidaGraph 3.6 (Synergy Software).

Reversibility of inhibition was determined as follows. A solution of purified PSMA, (26) at a concentration 100-fold greater than that required to achieve approximately 20% conversion of 1 μ M substrate to product in 15 min, was incubated in Tris buffer (50mM, pH 7.4, containing 1% Triton X-100) with biotin-PEG₁₂-CTT-54 at a concentration 10-fold greater than its IC₅₀ value at 37 °C for 10 minutes. This mixture was then diluted 100-fold into a reaction buffer (50 mM Tris buffer, pH 7.4) containing a saturating concentration of substrate (10 μ M PAB-Glu- γ -Glu) and kept at 37 °C. At increasing time points (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min) a 250 μ L aliquot was removed and quenched with a 25 μ L methanolic solution of TFA (2% by volume) followed by vortexing and centrifugation (10 min at 7000 g). An 85 μ L aliquot of the resulting supernatant was subsequently analyzed by HPLC and enzyme substrate and product were quantified as described previously. (26) Progress curves of product formation were generated to monitor the recovery of enzymatic activity for inhibited PSMA and compared to a control sample in which no inhibitor was added.

2.4 In Vitro Cell Capture Experiments

LNCaP (PSMA positive; PSMA+) cells and PC-3 (PSMA negative; PSMA-) cells were cultured in T-75 flasks with complete growth medium [RPMI 1640 containing 10% heat-inactivated fetal bovine serum (FBS), 100 U of penicillin and 100 μ g/mL streptomycin] in a humidified incubator at 37 °C and 5% CO₂. LNCaP cells were cultured 5 days and PC-3 were cultured 4 days before conducting the following experiments.

Cell preparation—Both LNCaP and PC-3 were grown in T-75 flasks to approximately 70% confluency. Cells were then washed twice in 37 °C pre-warmed *medium A* (phosphate-free RPMI 1640 containing 1% FBS), and then detached with a 0.25% trypsin 0.53 mM EDTA solution (5 mL) for 6 mins at 37 °C. 5 mL of *medium A* was added to each flask. The cells were distributed into five 2 mL tubes (~ 100 cells/tube). The cells were then centrifuged at 900 g at 4 °C for 5 mins. Following removal of the medium, the cells were resuspended in *medium A*. Flow cytometry was used to determine the concentration of cells for the start of each experiment.

In vitro cell capture—A specific number of cells in *medium A* were put into each tube and were incubated with or without 1 μ M of Biotin-PEG₁₂-CTT-54 in a shaking water bath (40 rpm) at 37 °C for 30 mins. The sample was then washed twice with *medium A* and centrifuged at 900 g at 4 °C for 5 mins. The cell pellets were resuspended in 450 μ L of *medium A* with 20 μ L of 1 μ M Streptavidin coated Magnetic Beads (bead concentration 10 mg/mL). The sample was incubated in a tube shaker rotator at 4 °C for one hour. Cells were captured on the magnetic beads by placing the sample tube against an external magnetic stand. The sample was washed twice with *medium A*. Cell that were captured on the magnetic beads were resuspended in 100 μ L *medium B* (phosphate-free RPMI 1640 containing 1% FBS, 0.2% propidium iodide, PI). The cells in supernatants and in wash solutions (not captured) were centrifuged for 900 g at 4 °C for 5 mins, then finally resuspended in 100 μ L of *medium B*. All cell capture experiments were performed in triplicate and were analyzed by flow cytometry on the same day.

Erythrocyte lysis—10 mL of fresh pig blood was mixed with 40 mL of 0.87% Tris-NH₄Cl pH 7.3 at 37 °C until the lysis of the red blood cells was complete, which was indicated by a color change from red to glassy red-black. The sample was then centrifuged at 1000 g for 8 mins at 4 °C, the supernatant was removed, and the cell pellet was washed twice with PBS-20% ACD [phosphate buffered saline (PBS) containing 20% acid citrate dextrose (ACD)]. The cell pellet was then resuspended into 1 mL of *medium A*.

Non-specific capture of leukocytes—After erythrocytes lysis, 100 μ L of the leukocyte suspension in *medium A* (from 1 mL of pig blood) was used to determine the non-specific binding of the leukocytes by subjecting them to the protocol described above for the *In vitro cell capture* experiments.

Cell capture in the presence of leukocytes—After erythrocytes lysis, 100 μ L of the leukocyte suspension in *medium A* (from 1 mL of pig blood) was combined with various numbers of LNCaP cells (22,000; 7,000; 1,760; 440; 110; 60; 15). The cell mixtures were then subjected to the protocol described above for *In vitro cell capture*.

2.5 Cell Capture from Blood

Decreasing numbers of LNCaP cells (258,200; 50,100; 11,090; 2,100; 630 and 200 as determined by flow cytometry) were added to 10 mL of porcine blood. This mixture was then subjected to the *Erythrocyte lysis* protocol described above. The cell pellet was then suspended into 2 mL of *medium A* and 200 μ L of the suspension was subjected to protocol above for *In vitro cell capture*. This 200 μ L cell suspension represented 25,820; 5,010; 1,109; 210; 63 and 20 cells in 1 mL of blood processed. Triplicate determinations of these experiments were performed and the total cell numbers for each sample were enumerated by flow cytometry.

2.6 Quantification of LNCaP cells by flow cytometry

To 100 μ L of each sample prepared for flow cytometry was added 20 μ L of nonfluorescent polystyrene microsphere counting beads (approximately 1,500,000 beads/mL, Flow cytometry Size Calibration Kit, Invitrogen). The samples were then subjected to the flow cytometry. Data acquisition for each sample was completed after 10,000 these gated counting beads were detected; acquisition time and the flow rate were recorded.

2.7 Flow Cytometry

A Beckton-Dickinson FACSCalibur flow cytometer equipped with argon and red lasers, a Macintosh computer, and Cell Quest software (Becton-Dickinson Immunocytometry Systems, San Jose, CA) was used to collect data. FCS Express software (DeNovo software, Thornton, Ontario, Canada) was used to analyze the data. FL-4 channel was used to detect cell labeling by anti-human CD326 (EpCAM) antibody eFluor 660. FL-2 channel was used to detect fluorescent intensity from propidium iodide (PI) to monitor cell viability.

2.8 Fluorescent Microscopy & Imaging

Cells were visualized under a 60X water immersion objective using a Zeiss LSM 510 META Laser Scanning Microscope.

3. RESULTS

The selectivity of the magnetic bead capture strategy using biotin-PEG₁₂-CTT-54 as well as the viability of captured cells were first determined. For selectivity determinations, LNCaP (PSMA+) and PC-3 (PSMA-) cells were used and they were treated both with and without biotin-PEG₁₂-CTT-54. Treated cells were subsequently incubated with streptavidin-coated magnetic beads to bind to capture those cells presenting extracellular biotin through the PSMA enzyme-inhibitor complex with biotin-PEG₁₂-CTT-54 using an external magnet. The results of the selectivity experiments are illustrated in Figure 2 and Figure S3 (supplemental material). The cell suspension samples for each experiment were doped with 15 μ m nonfluorescent microsphere counting beads to serve as internal standard for precise cellular quantification by flow cytometry.(9,27,28) Data acquisition for each sample was completed after 10,000 gated beads were detected, which allowed for a total count of detected cells. As a reference, the 2D dot-plot of the individual counting beads and magnetic beads were shown in Figure S1 of the supplemental material in which gates for these two bead populations were defined (counting beads were defined as Gate 1). Cells were also treated with anti-human CD326 (EpCAM) antibody eFluor 660 to label and further confirm the identity of LNCaP cells in the flow cytometry histograms using the FL4-H fluorescence channel.(29,30) In the histograms A-C of Figure 2, LNCaP cells were defined by Gate 2, which was established in the gating strategy described in Figure S2 for EpCAM+ cells. As seen in histograms B, C, E, and F of Figure 2, LNCaP cells were efficiently captured on the streptavidin coated magnetic beads after being pre-targeted with biotin-PEG₁₂-CTT-54 (histograms B and E). However, it was noted that capture efficiency decreased with lower concentrations of cells (Table 1). Minimum non-specific cell capture was observed when LNCaP cells were not pre-targeted with biotin-PEG₁₂-CTT-54 (Figure S3). Specificity of cell capture was further confirmed as PSMA-negative PC-3 cells were not captured with streptavidin coated magnetic beads when they were incubated either with or without biotin-PEG₁₂-CTT-54 (Figure S4).

In order to determine the viability of the captured cells, suspensions of captured cells were stained with propidium iodide (PI) and analyzed by flow cytometry.(31) As shown in histogram G of Figure 2, the population of captured LNCaP cells (Gate 4, histogram E Figure 2) was examined for cell viability by uptake of PI. Of those cells captured, 86% were

found to be viable. As shown in Table 1, the cell viability of captured cells was approximately 90% regardless of the capture efficiency. This data confirms that the method of cell capture is gentle and amenable to the subsequent culturing of captured cells. In addition to cellular quantification by flow cytometry, phase contrast images of captured LNCaP cells were obtained (Figure 3). Captured cells were subsequently sub-cultured in the normal growth medium and were successfully propagated without removal of the magnetic beads from the cells (Figure 3). It was noted that the morphology of the cultured captured cells was similar to that of LNCaP cells normally cultured.

After the selectivity and cell viability of the magnetic bead capture strategy using biotin-PEG₁₂-CTT-54 were determined, the specificity of capture, efficiency of cell capture, viability of captured PSMA+ cells, and detection limit were determined in the presence excess white blood cells (WBCs) from porcine blood. Porcine blood was used in the experiments because its ready availability and known similarity to human blood (32–35). It was first confirmed that WBCs could not be captured non-specifically by magnetic beads either with or without pre-targeting with biotin-PEG₁₂-CTT-54. Data to support these findings is found in Figures S5 and S6. It should be noted that because WBCs lack both EpCAM and PSMA, they can be distinguished from LNCaP cells by flow cytometry and were not expected to be susceptible to capture by pre-targeting with biotin-PEG₁₂-CTT-54.

In contrast to WBCs, LNCaP cells pre-targeted with biotin-PEG₁₂-CTT-54 were selectively captured on the streptavidin coated magnetic beads (histograms B, C, E, and F of Figure 4). Blood sample preparation prior to flow cytometric analysis required treatment with ammonium chloride to lyse erythrocytes and remove the platelets from the blood.(21) These conditions did not affect either the capture efficiency and viability of captured LNCaP cells (Figure S8 and Table S3).

The capture efficiency and viability of decreasing numbers of LNCaP cells in the presence of a large and constant amount of WBCs were then determined (Table 2). LNCaP cells were efficiently captured on the streptavidin coated magnetic beads after being pre-targeted with biotin-PEG₁₂-CTT-54 (histograms B and E, Figure 4). However, it was noted that capture efficiency decreased with lower concentrations of cells (Table 2). In order to determine the viability of the captured cells, suspensions of captured cells were stained with propidium iodide (PI) and analyzed by flow cytometry.(31) As illustrated in histogram G of Figure 4, the population of captured LNCaP cells (Gate 4, histogram E Figure 4) was examined for cell viability by uptake of PI. As shown in Table 1, the cell viability of captured cells was approximately 90% regardless of the capture efficiency. This data further confirms that the method of cell capture is gentle and amenable to the subsequent culturing of captured cells. Minimum non-specific cell capture was observed when LNCaP cells were not pre-targeted with biotin-PEG₁₂-CTT-54 (Figure S7).

Lastly, decreasing number of PSMA+ prostate tumor cells were spiked directly into the porcine blood, and the cell recovery, captured efficiency, and viability of these cells were determined. The gating strategies outlined in Figures 4 were used and the results were summarized in Table 3. From 1 mL of blood in which LNCaP cells were added, an average of 10 cells were detected by flow cytometry in the most dilute sample; 20 cells expected. Approximately 92% of these detected cells were captured with 84% being viable.

4. DISCUSSION

The objective of this study was to develop a clinically relevant protocol for the selective capture, quantification, and *in vitro* propagation of circulating tumor prostate tumor cells from blood. The strategy was designed to first pre-target the target cells with a biotinylated

inhibitor (biotin-PEG₁₂-CTT-54) of the enzyme-biomarker PSMA followed by selective capture using streptavidin-coated magnetic beads. Biotin-PEG₁₂-CTT-54 was found to be irreversible in its binding mode to PSMA with an IC₅₀ of 10 nM, confirming that conjugation of biotin-PEG₁₂ to the inhibitor core CTT-54 (IC₅₀ = 14 nM)(36,37) did not affect the overall binding affinity.

We found that biotin-PEG₁₂-CTT-54 can effectively serve as a pre-targeting bait to efficiently capture of PSMA-positive LNCaP cells with high selectivity, recovery, and viability. This was true for both model experiments with mixtures of LNCaP cells and WBCs (Figure 4, Table 2) as well as blood samples spiked with LNCaP cells (Table 3). Despite the observed internalization of PSMA that occurs when it is bound to either mAb and low-molecular weight inhibitors, (17–20,36,38) there remains a sufficient number of pre-targeted biotin-PEG₁₂-CTT-54 molecules on the cell surface, to allow for effective cell capture. In our attempts to maximize the amount of available cell-surface pre-targeted biotin by blocking internalization of the PSMA-inhibitor complex (preliminary experiments, data not shown), we noticed that pre-targeting LNCaP cells with biotin-PEG₁₂-CTT-54 for 1 h at 4 °C or 30 min at 37 °C with 0.1% sodium azide resulted in decreased capture efficiency and viability, respectively. Furthermore, we found that the capture of LNCaP cells with a pre-formed complex of biotin-PEG₁₂-CTT-54 with streptavidin-coated magnetic beads was considerably less effective in capturing LNCaP cells than pre-targeting cells with biotin-PEG₁₂-CTT-54.

A comparison of histograms A and B in Figure 2 revealed that captured LNCaP cells exhibit a greater degree of side scattering compared to control LNCaP cells. This observation is consistent with the microscopic image of captured LNCaP cells in Figure 3 in which the cell surface of LNCaP cells is decorated with streptavidin-coated magnetic beads resulting in altered cell granularity.

The capture efficiency of LNCaP cells pre-targeted with biotin-PEG₁₂-CTT-54 and then incubated with streptavidin-coated magnetic beads was determined by flow cytometry. In model experiments (Figures 2 and 4), the capture efficiency was reduced for samples with small numbers of LNCaP cells but for the studies in which LNCaP were cells spiked into blood samples, the capture efficiency remained relatively constant across the cell dilution range examined (Table 3). Of those cells detected by flow cytometry, either captured or those that escaped capture, approximately 90% were consistently captured on the streptavidin-coated magnetic beads in both the model conditions (Figures 2 and 4) and in blood samples spiked with LNCaP cells. Although minor, a small amount of non-specific binding of both PSMA+ and PSMA– cells to streptavidin coated magnetic beads was observed (Figure S3 and S4). We attributed this to the known interaction of the RYD sequence of streptavidin to extracellular fibronectin.(39)

In addition to the encouraging cell capture data described above, we found that captured cells could be subsequently propagated *in vitro* as (Figure 3). These results demonstrate that release from the magnetic beads does not negatively impact the viability or proliferation of capture cells.(40) This finding suggests that in clinical practice, a simplified manual operation to capture and culture CTCs could be developed in which an additional step to release cells from magnetic beads is omitted. To date, there are no reports on the successful propagation of captured PCTCs from the peripheral blood of a patient. In contrast, the results presented herein are encouraging, assuming that cell culture conditions can be identified for the propagation of captured PCTCs, and could enable the exploration of the tumor biology of captured PCTCs.

The viable capture of PSMA+ cells from blood demonstrates the necessary proof-of-concept data to validate the use of an irreversible PSMA inhibitor for targeted prostate tumor cell isolation. We expect that the protocol established here can be translated for the isolation and culture of PCTCs from human peripheral blood and also freshly collected blood samples from prostate cancer patients in the future. At the lower limit, an average of 10 cells from 1 mL of blood samples were captured, nearly all of which were viable. In summary, we have successfully demonstrated that PSMA+ prostate tumor cells from blood can be selectively isolated by magnetic selection by first pre-targeting cells with the irreversible PSMA inhibitor biotin-PEG₁₂-CTT-54. Quantification of captured cells can be achieved by routine flow cytometry and most importantly, captured cells are viable and can be propagated in culture. We expect that this methodology for the detection, isolation, and culture of PCTCs from peripheral blood can serve as an effective tool for the detection of metastatic prostate cancer, treatment monitoring, and the development of personalized therapy based on the responsiveness of PCTCs to chemotherapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health (R21CA135463, R01CA140617) and the Department of Defense Prostate Cancer Research Program (W81XWH-10-PCRP-PCTA, PC102144, predoctoral fellowship for L.Y. Wu: views and opinions of, and endorsements by the authors do not reflect those of the US Army or the Department of Defense). The authors would also like to extend their gratitude to the WSU center for NMR Spectroscopy, the WSU Flow Cytometry and Cell Analysis Core, D. Peters at the WSU Swine Center, G. Muske and the WSU Lab for Biotechnology and Bioanalysis I (LBB1), and to both C. Davitt and V. Lynch-Holm at the WSU Franceschi Microscopy and Imaging Center.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1

Flow cytometric analysis of capture efficiency and viability of different LNCaP population.

Total cell count ^a	%Captured ^b	% viability ^b
75,749	97 (2.3)	92 (0.7)
37,874	97 (2.6)	88 (3.5)
9,469	97 (0.1)	87 (1.6)
2,367	92 (0.3)	93 (2.9)
592	73 (8.8)	91 (3.3)
148	49 (6.8)	94 (2.4)

^aBased on a stock suspension estimated to be 200,000 cells/mL.

^bAverage of triplicate determinations, standard deviation in parentheses.

Table 2

Flow Cytometric Analysis of capture efficiency and viability LNCaP cells in the presence of WBCs

Total cell Count ^a	% Captured ^b	% Viability ^b
21.446	97 (0.5)	93 (1.0)
7.060	94 (4.3)	91 (15)
1.765	95 (1.5)	90 (2.2)
441	85 (11.3)	89 (4.5)
110	86 (4.3)	85 (3.1)
60	70 (6.6)	91 (2.5)
15	67 (4.1)	90 (1.1)

^aBased on a stock suspension estimated to be 200,000 cells/mL^bAverage of triplicate determinations, standard deviation in parentheses.

Table 3

Flow Cytometric analysis of capture efficiency and viability of LNCaP cells isolated from blood

Expected ^a	Detected ^b	% Recovery ^b	% Captured ^b	% Viability ^b
25.820	10.593 (494)	41 (1.9)	84 (4.2)	89 (0.9)
5.010	1.989 (118)	40 (2.4)	78 (4.3)	90 (2.4)
1.109	545 (29)	49 (2.6)	84 (1.6)	82 (1.8)
210	93 (11)	44 (5.4)	92 (4.0)	90 (3.4)
63	25 (6)	40 (8.7)	84 (4.1)	91 (1.6)
20	10 (4)	50 (21.8)	92 (14.4)	84 (4.7)

^aBased on a stock suspension estimated to be 200,000 cells/mL.^bAverage of triplicate determinations: standard deviation in parentheses.