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TITLE: Mechanism of Action of Prostate Stem Cell Antigen Targeted Antibody Therapy and Its Relevance to Clinical Application in Prostate Cancer

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14. ABSTRACT

We have recently demonstrated that a monoclonal antibody against prostate stem cell antigen (PSCA) can exert anti-tumor activity in a xenograft animal model, suggesting oncogenic activity of PSCA in prostate cancer. Therefore the our goal is to elucidate the role of PSCA in the development of prostate cancer. A better understanding of PSCA function and its antibody activity will enable rational patient selection and trial design, all of which are particularly relevant to subsequent clinical trials of PSCA antibody. There were difficulties in using the LAPC9 xenograft cells to study the effect of suppressing PSCA, but we have since established conditions for infecting LAPC9 xenograft tumor cells in vitro, and confirmed knockdown ability of siPSCA lentivirus. We have examined PSCA$^{-/-}$/Nkx3.1$^{-/-}$ double knockout mice at early time point and find no difference in the lagged time to PIN formation compared to control group. We have also recognized the difficulty in generating the PSCA$^{-/-}$ /conditional PTEN$^{-/-}$ compound mice, and have engaged in an alternative approach by using the tissue recombination assay. Overall, we have overcome technical difficulties and expected to make significant progress in the next year.

15. SUBJECT TERMS
Prostate cancer, prostate stem cell antigen, preclinical mouse model, antibody therapy, antitumor activity

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INTRODUCTION

Previous studies from our laboratory have shown that PSCA is overexpressed in human primary and metastatic prostate cancers. Furthermore, we have recently demonstrated that a monoclonal antibody against PSCA can exert anti-tumor activity in a xenograft animal model, and that this activity was a direct effect mediated by cross-linking. These data suggest that PSCA may have growth-promoting and/or oncogenic activity in prostate cancer. Therefore the our goal is to elucidate the role of PSCA in the development of prostate cancer in order to better understand the mechanism of action of PSCA antibody in tumor inhibition. A better understanding of PSCA function and antibody activity will enable rational patient selection and trial design, all of which are particularly relevant to subsequent clinical trials of PSCA antibody. To accomplish this, we propose to evaluate the requirement of PSCA expression in both xenografts and transgenic models of prostate cancer. We will also generate transgenic models of prostate cancer that express the human PSCA gene to determine the efficacy and toxicity of PSCA antibody therapy.

PROGRESS REPORT

Specific Aim 1. *Investigate the permissive role(s) of PSCA in cancer cell proliferation.*
This aim is being carrying out in parallel to specific aim 2.

Task 1. *Effect of gene silencing of PSCA in cell lines with endogenous PSCA expression*.
We selected two shRNA constructs (CSCG-GFP-siPSCA) with the most PSCA-knockdown efficiency and subcloned them to generate lentivirus for better infectivity into LAPC9 cells. The backbone of this lentiviral vector also contains GFP to monitor the infectivity and allow isolation of infected cells. Freshly isolated LAPC9 xenograft tumor cells were infected with shRNA constructs or CSCG-GFP alone and the effect on PSCA expression was examined by flow cytometry [figure 1]. After the initial infection, PSCA expression in the siPSCA group was reduced by ~ 43% (green line) compared to the CSCG-GFP infected cells (blue line). While PSCA was not suppressed completely, however with repeated rounds of lentiviral infection we expect to obtain high percentage of reduction in PSCA expression. Once this is achieved, the cells will be sorted for GFP expression to obtain purer infected population for in vivo experiment.

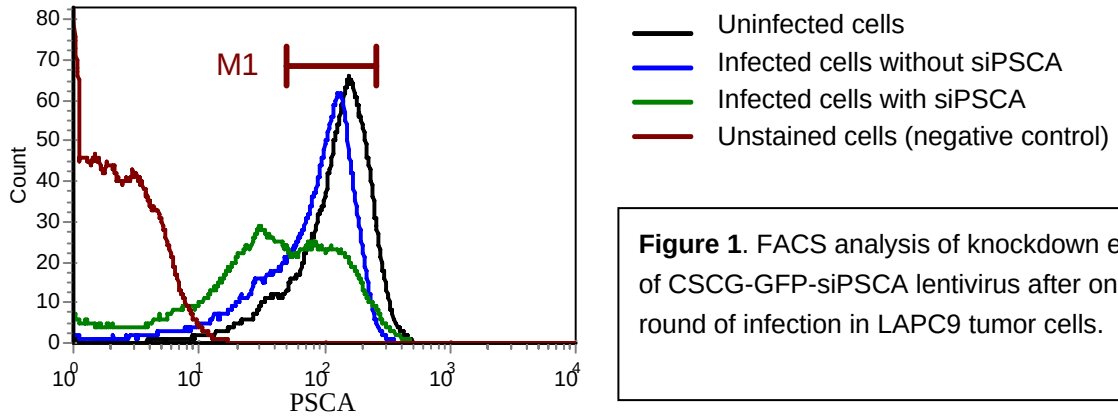

Figure 1. FACS analysis of knockdown efficacy of CSCG-GFP-siPSCA lentivirus after one round of infection in LAPC9 tumor cells.

One technical difficulty we have encountered is that LAPC9 cells are xenografts which cannot be stably maintained in vitro as other cell lines, thus it was a challenge to perform repeated infections on the cells while keeping them alive. To overcome this problem, we have tested and established conditions for primary culturing of LAPC9 for 5 to 10 days ex vivo (figure 2). This time frame allows us to infect the cells, check and select for population with reduced PSCA expression, follow by re-implanting in mice. We expect to gain positive result from this experiment in the next few months.

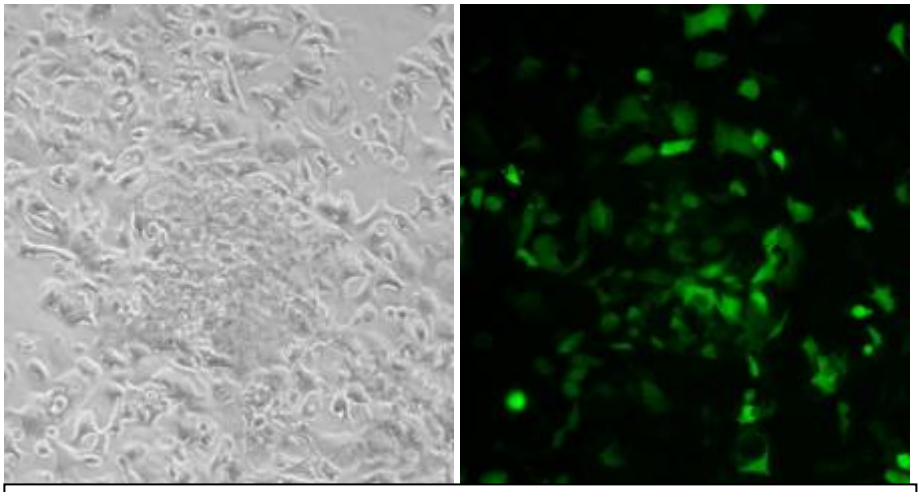

Figure 2. Ex vivo primary culture of LAPC9 cells infected with CSCG-GFP-siPSCA lentivirus. Left panel: light field; right panel: dark field.

Initially we were concentrating on using a prostate tumor cells with endogenous PSCA expression, but we are now testing our knockdown construct in the bladder cell line SW780 which also express PSCA and can be propagated in vitro. This will allow us to investigate the effects of PSCA suppression on cell growth and possibly invasion by in vitro assay.

Task 2. *Effect of overexpressing PSCA in normal prostate epithelial cells (PrECs) and other heterologous cell lines*.
New lines of PrECs are being generated and maintained for PSCA overexpression. We will also use the PZ-HPV7 cell line, which is a commercially available immortalized PrEC, that does not express PSCA for comparison. Currently we are yet to obtain result from these experiments.

Specific Aim 2. *Evaluate the role of PSCA in the causation and progression of prostate cancer*.
This aim is being carrying out in parallel to specific aim 1.

Task 1. *Effect of PSCA deficiency in the PTEN$^{-/-}$ transgenic model*.
Due to the difficulty in obtaining the PSCA$^{-/-}$/conditional PTEN$^{-/-}$ compound mice, we are trying an alternative approach. This involves obtaining prostate cells from the PSCA knockout mice and infecting the cells with a PTEN knockdown construct, following by combination with the mouse embryonic urogenital sinus mesenchymal cells and engraftment under the kidney capsule of immunodeficient host

mice. Such assay has been utilized previously [1] to study murine prostate tissue regeneration, and more recently [2] as a tool to evaluate the result of genetic perturbation in murine prostate cells within 8 weeks. We are expanding the PSCA knockout line to move ahead with this experiment, and are expecting to complete this task over the next year.

Task 2. *Effect of PSCA deficiency in the Nkx3.1$^{-/-}$ transgenic model*.
We have examined the PSCA$^{-/-}$/Nkx3.1$^{-/-}$ double homozygous compound mice and their Nkx3.1$^{-/-}$ control cohort for difference in the duration to PIN formation in the prostate. The first problem we encountered was that all the mice took much longer time to develop prostatic intra-epithelial neoplasia (PIN), even in the Nkx3.1$^{-/-}$ control group. The most obvious abnormalities was observed in the anterior prostates at 16 weeks, and here there was no difference between the double knockout group and the control group. Currently we are still acquiring tissues from the two mice groups at later time points for histology assessment.

Specific Aim 3. *Assess the efficacy and physiological effects of the antibody in a preclinical model expressing human PSCA.*

Task 1. *Development of transgenic model of prostate cancer expressing human PSCA.*
We have since revised our strategies for developing this preclinical model. Initially we proposed to generate a transgenic mouse overexpressing human (h)PSCA using the human PSCA promoter while its mouse counterpart is not disturbed, and subsequently cross it with PTEN null mice. However, we did acknowledge the concern that targeting human PSCA alone may not be sufficient to inhibit tumor growth since the expression of mouse PSCA is not targeted. Therefore, our current approach is to specifically place the human PSCA cDNA under the mouse promoter by “knock-in” gene targeting, thus effectively silencing the mouse PSCA by expressing its human counterpart. We expect the construct for gene targeting to be engineered in the next 6 months.

Task 2. *Studies of PSCA antibodies in transgenic models.*
This task will be performed once the preclinical model is established.

KEY RESEARCH ACCOMPLISHMENTS

- Establish conditions for infecting LAPC9 xenograft tumor cells in vitro.
- Confirm knockdown ability of siPSCA lentivirus.
- Examine PSCA$^{-/-}$/Nkx3.1$^{-/-}$ double knockout mice at early time point and find no difference in the extent of time taken to PIN formation compared to control group.
- Revise approach to breeding PSCA$^{-/-}$/conditional PTEN$^{-/-}$ compound mice by using the tissue recombination assay.
- Revise approach to making the preclinical model by using gene targeting “knock-in” technology.

REPORTABLE OUTCOMES

None

CONCLUSION

We have encountered difficulties in using the LAPC9 xenograft cells to study the effect of suppressing PSCA, but we have worked out conditions to overcome this problem and expect to make significant progress over the next year. Although the $PSCA^{-/-}$/$Nkx3.1^{-/-}$ double knockout mice that we generated did not show any difference in the time duration to PIN formation in comparison to control group, this does not rule out the role of PSCA in prostate cancer. It is possible that PSCA is more important in the progress to metastatic cancer than in the primary tumor stage, since we have shown that PSCA protein and mRNA are highly elevated in clinical specimen of prostate cancer metastases [3]. We have also recognized the difficulty in generating the $PSCA^{-/-}$/conditional $PTEN^{-/-}$ compound mice, and have engaged in an alternative approach, which we hope will deliver the results in a more definite time frame.

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