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14. ABSTRACT Prostate stem cell antigen (PSCA), a cell surface marker on prostate cancer cells, was used to generated humanized antibody and combined with PET imaging to image prostate tumor. To improve affinity, the humanized anti-PSCA antibody fragments were reformatted and screened for better affinity as well as better tumor imaging ability by PET. During the past year, three minibody variants were affinity-matured, and A11 appears to be the lead agent in terms of specific immunoreactivity and superior imaging contrast. In parallel, specific hPSCA expression was validated in the hPSCA-KI mouse model. Experiment testing A11 in hPSCA-KI mice is in progress, and we expect to obtain exciting results in the next few months. At the same time, crossing to prostate cancer model such as the PTEN conditional knockout is also underway, and we expect to get the compound mice by early next year.					
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INTRODUCTION

Noninvasive molecular imaging is an important tool in monitoring prostate cancer because it can reveal valuable information about the tumor such as location, phenotype, therapy response, as well as detection of metastases. From previous studies, we have utilized a cell surface marker on prostate cancer cells called prostate stem cell antigen (PSCA) [1,2] as a target to generate humanized antibody. Using PET scanning, the radiolabeled humanized antibody was shown to detect tumor in *in vivo* xenograft prostate tumor model, however humanization also caused a 5-fold reduction in antibody affinity compared to the parental antibody [3]. Further loss of affinity is a frequent occurrence when engineered antibody fragments are generated from intact antibodies. To improve affinity of this humanized antibody (designated 2B3), a concerted effort was undertaken with the goal to achieve equivalent affinity to the parental version. The 2B3 variable regions were reformatted into a single-chain Fv (scFv) format, and a yeast library of 2B3 scFvs was screened to obtain clones displaying modified scFvs as well as increased affinity for PSCA. This project was conceived to develop and validate, in preclinical studies, the ability of a humanized anti-PSCA antibody fragment to detect advanced and/or metastatic prostate cancer by PET imaging.

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Specific Aim 1. Development and testing of engineered PSCA antibody fragments in controlled biological model systems.

Aim 1A. Generation of PSCA-specific antibody fragments (year 1).

Aim 1B. Performance characteristics of anti-PSCA engineered antibody in subcutaneous xenograft models (year 1-2).

Minibodies, diabodies and ScFv-Fc fragments that recognize PSCA was produced based on the humanized anti-PSCA antibody 2B3. In the first year of the project, our efforts were concentrated on testing these fragments in prostate cancer model. One minibody called 2B3 demonstrated specific imaging ability in the LAPC9 xenografts, which express high level of PSCA. 2B3 gave great contrast at 21 hr after administration, and cleared rapidly from non-target tissues as well as the bloodstream. Nevertheless, the 2B3 minibody still sustained a 9-fold loss of apparent affinity [4]. In parallel, diabody variants, generated by incorporating different linker lengths and back-mutations to original murine residues were also screened, and found to have better specific tumor retention at 20 hr after administration in the LAPC9 tumor model [5].

During the past year, we continued to screen the scFv library for clones with high affinity for PSCA. Three minibody variants showing higher affinity for PSCA were derived: A2, A11 and C5. All 3 minibodies exhibited better affinity compared to their parental version and were ranked as follows: A2 > A11 > C5. Out of the three variants, when labeled with I-124 and tested in LAPC9 tumor model, A11 showed higher immunoreactivity as well as better PET imaging contrast (fig. 1). This candidate is currently under development for evaluation in clinical imaging study.

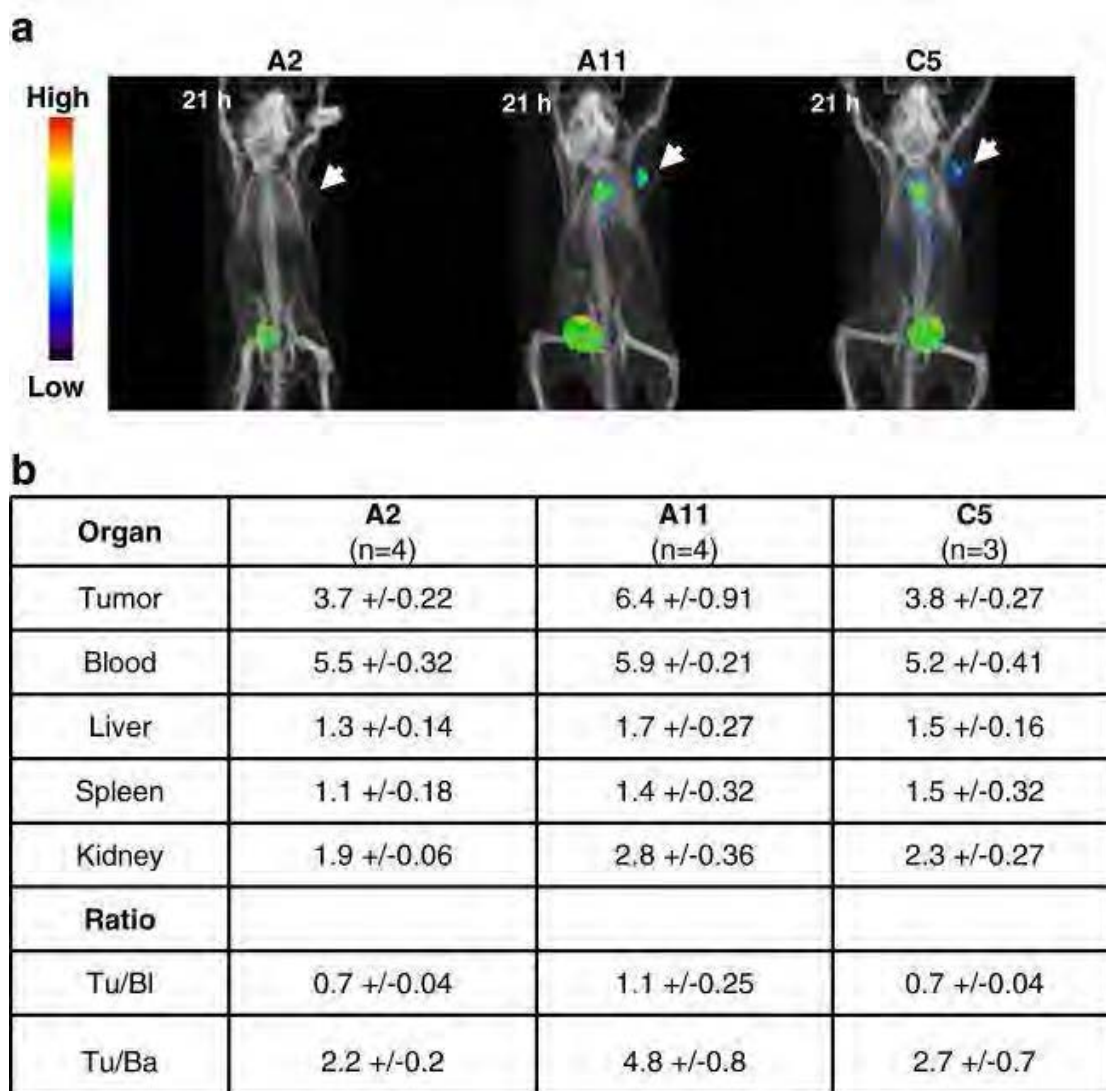


Fig. 1. In vivo analysis of affinity-matured, I-124-labeled minibodies A2, A11 and C5. **a/** PET/CT scan of SCID mice bearing LAPC9 prostate cancer xenografts. Mice were injected with radiolabeled minibody variants and scanned at 21 hr later. Arrow indicates tumor site. **b/** Biodistribution, Tu/Bl (tumor/blood), and Tu/Ba (tumor/background) imaging data at 21 hr after injection. Tumor and normal organ uptakes are expressed as % injected dose per gram (%ID/g +/- SE).

Specific Aim 2. Preclinical assessment of PSCA antibody fragments to image transgenic models of prostate cancer.

Aim 2A. Generation of human PSCA knock-in model for evaluation of PSCA antibody fragments (year 1).

In the first year of this project, a knock-in (KI) mouse model was successfully generated, in which the human (h) PSCA cDNA substituted for the murine PSCA gene. Germline transmission were confirmed using a PCR genotyping assay, and the mice were bred to C57Bl/6 background. During the past year, we were able to screen the 3 different hPSCA-KI lines (hP4, hP5, hP10) for hPSCA expression in the organs previously known to express PSCA (fig. 2). At mRNA expression level, hP4 and hP10 both showed good expression of hPSCA in the prostate, bladder and higher level in the stomach. In contrast, hP5 showed lower hPSCA

expression in both prostate and bladder, yet higher level in the stomach. Based on this preliminary screen, hP4 and hP10 were chosen for further expansion. We concentrated on the hP4 line because its breeding colony was more productive.

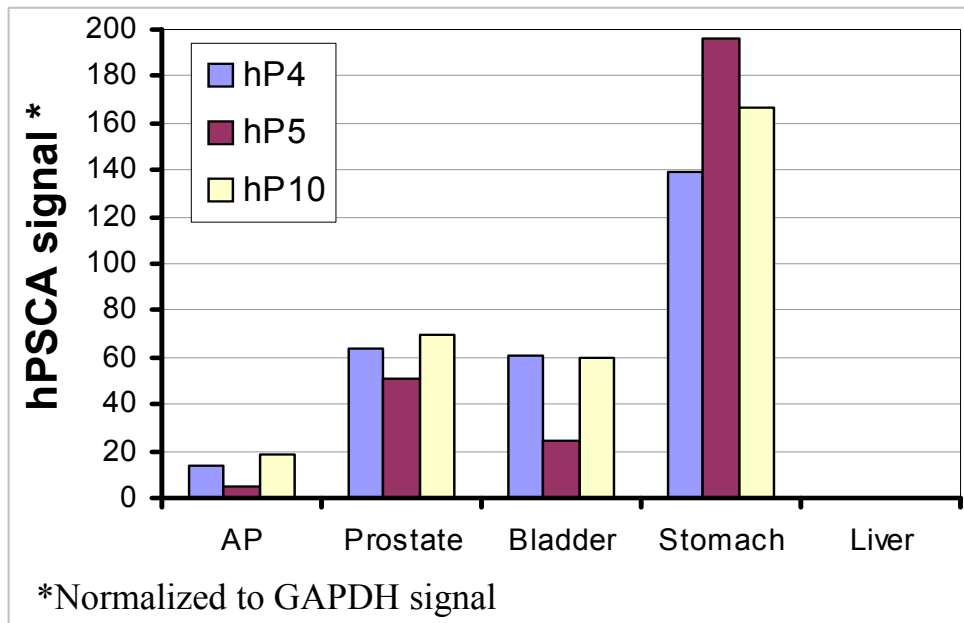


Fig. 2. Human PSCA mRNA expression level in 3 hPSCA-KI lines. PSCA was quantified by RT-PCR and normalized to GAPDH signals. hPSCA expression was comparable in prostate and bladder, while much higher in the stomach. Liver was included as negative control. AP: anterior prostate.

Other major organs were also screened for hPSCA to evaluate its distribution in the hP4 line (table 1). As expected, hPSCA expression was either much lower or negative in other organs compared to bladder, thus demonstrating the specific expression pattern of hPSCA in this knockin line.

hPSCA knockin mouse tissues	hPSCA Gene expression	Previous Reports on mPSCA expression
Kidney	+/-	low
Lung	+/-	?
Thymus	+	?
Testis	++	low
Colon	++	low
Bladder	++++	medium
Liver, spleen, heart, intestine, esophagus	negative	negative

To evaluate protein expression of hPSCA in hP4 KI line, the mouse anti-human PSCA antibody 1G8 was used to detect hPSCA in mouse tissues (fig. 3). Specific staining was observed in the epithelia of prostate, bladder and stomach, while the underlining stromal layer remain

unstained. Currently, experiment using the lead minibody A11 established in aim 1 is underway to image hP4 mice to evaluate normal uptake of the minibody in these mice.

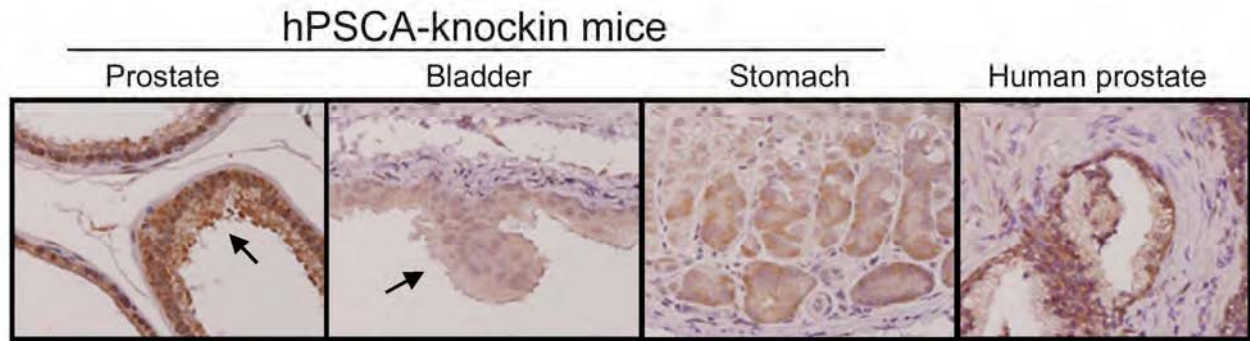


Fig. 3. Immunohistochemistry of hPSCA in the prostate, bladder and stomach of hPSCA-KI hP4 line. hPSCA protein was detected by mouse monoclonal antibody 1G8. Human prostate was included as positive control. Arrow indicates specific epithelial staining.

Aim 2B. Generation of hPSCA knockin X conditional-PTEN knockout (year 2-3).

We have expanded the hPSCA-KI line and already started crossing with the conditional PTEN knockout (KO) model. To speed up the process, PTEN loxp/lox female and PTEN loxp/lox; probasin-cre male mice were obtained from Hong Wu lab and used to cross to hPSCA-KI homozygotes according the breeding scheme below (fig. 4). Currently we are trying at stage 1.

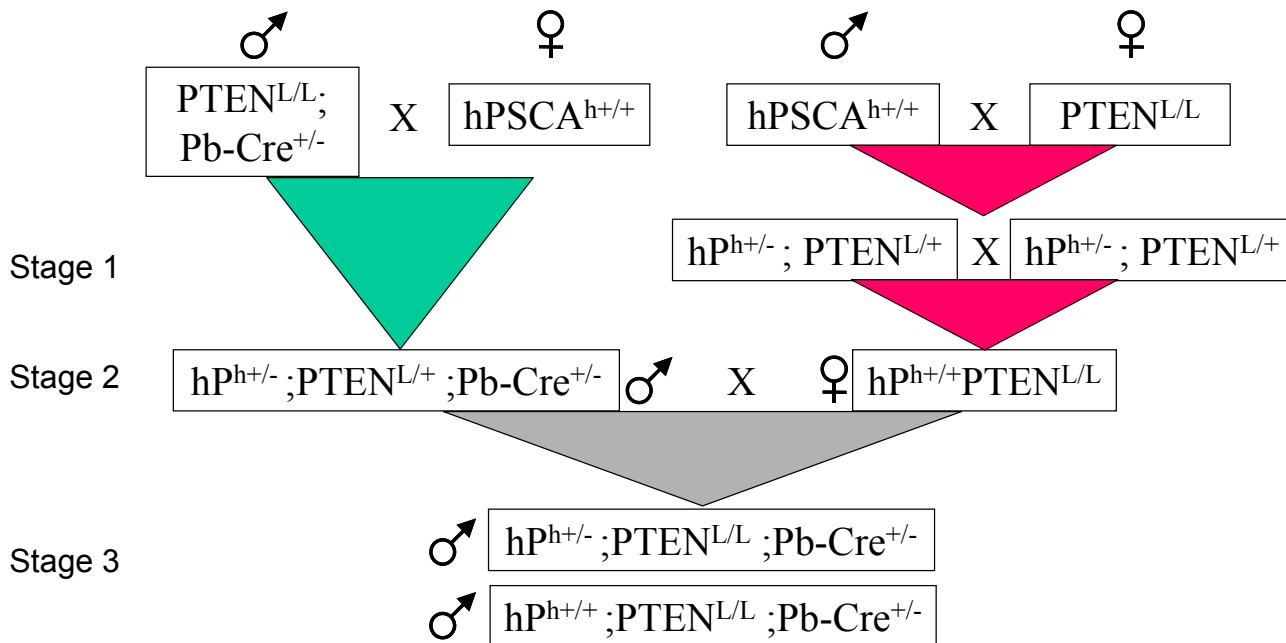


Fig. 4. Breeding scheme to obtain the compound mice: hPSCA knockin; PTEN conditional knockout.

KEY RESEARCH ACCOMPLISHMENTS

- Three minibody variants (A2, A11, C5) were shown to have improved affinity over parental minibody.

- A11 appeared to be the best candidate with superior immunoreactivity to PSCA positive tumor, and better imaging contrast.
- Expansion hPSCA-KI mouse models and validate specific hPSCA expression in the prostate, bladder and stomach.
- Organize breeding cross of hPSCA-KI to PTEN conditional KO.

REPORTABLE OUTCOMES

- Manuscript published this past year that were supported in part by this grants:
Lepin EJ, Leyton JV, Zhou Y, Olafsen T, Salazar FB, McCabe KE, Hahm S, Marks JD, Reiter RE, Wu AM. An affinity matured minibody for PET imaging of prostate stem cell antigen (PSCA)-expressing tumors. *Eur J Nucl Med Mol Imaging*. 2010 Aug;37(8):1529-38.

CONCLUSION

In the past year, we were able to narrow down a microPET lead agent, minibody A11, which demonstrated excellent imaging contrast and immunoreactivity in the LAPC9 prostate cancer xenograft model. In parallel, specific hPSCA expression was validated in the hPSCA-KI mouse model. Experiment testing A11 in hPSCA-KI mice is in progress, and we expect to obtain exciting results in the next few months. At the same time, crossing to prostate cancer model such as the PTEN conditional knockout is also underway, and we expect to get the compound mice by early next year.

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