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14. ABSTRACT

Prostate cancer progresses from androgen-dependent to androgen-independent state. The androgen receptor (AR) is expressed throughout progression. We would like to understand the AR role in this progression. Using lox-Cre methodology, we have generated mice in which AR function is abolished in the entire animal (ARKO) or tissue specific manner and generated mice with ARKO in prostate only or in different stages to be used to study prostate cancer (PCa) progresses. We aim to generate mice lacking AR in prostate epithelium., generate inducible ARKO mice to determine potential effects of androgen in AR absence on prostate growth/maintenance, determine AR role in PCA development/progression by crossing ARKO mice with TRAMP mice to examine AR role in TRAMP induced PCa and permit determination of stages in PCa requiring AR function, determine AR role in tumorigenicity of androgen-dependent/androgen-independent ARKO PCa cell lines. The effect of AR loss in these cells will be examined for ability to generate/promote tumors in mice. This year we generated mice with ARKO in the prostate epithelium and will be able to continue the other aims in the proposal in the coming years.

15. SUBJECT TERMS
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Knockout AR in prostate

Introduction:

A summary of this proposal was the following statement:

While androgen receptor (AR) activity is known to be important in the development and maintenance of the normal prostate and in prostate cancer, several aspects of AR function in the prostate have not been able to be addressed until now due to a lack of appropriate animal models. We have developed mice with a floxed AR exon 2, enabling us to inactivate AR in a tissue specific or temporally specific manner. Using these mice, this proposal seeks to address several aspects of the role of AR in the prostate that could not previously be addressed with Tfm mice or by treatment with antiandrogens. The role of epithelial AR function in communication with the stroma of the fully formed prostate will be addressed in Specific Aim 1. This will allow an initial determination of the androgen regulated stromal factors that may contribute to epithelial cell survival. The possible role of recently described rapid nongenomic effects of androgens in the prostate will be addressed using an inducible system to render AR non-functional in the prostate at specific developmental stages in Specific Aim 2. The role of AR at specific stages of prostate cancer progression will be examined in a mouse model system in Specific Aim 3. In Specific Aim 4, suppression of AR expression in prostate cancer cell lines will be examined for the effect of prostate cancer cell tumorigenicity.

The Specific Aims and Sub Aims of the proposal are the following.

Aim 1: To generate male mice specifically lacking a functional AR in the prostate epithelium.

- Aim 1a: To generate mice lacking a functional AR in the prostate.
- Aim 1b: To examine the effect of loss of AR function in the prostate.

Aim 2: To generate an inducible ARKO mouse line.

- Aim 2a: To generate floxAR mice that carry an inducible Cre transgene.
- Aim 2b: To examine the effect of the inducible loss of AR on the development and maintenance of the prostate.

Aim 3: To determine the role of AR in prostate cancer development and progression in mice by crossing the ARKO mice generated in Specific Aims 1 and 2 with TRAMP transgenic mice.

- Aim 3a: To generate mice lacking prostate specific AR function in a mouse model of prostate cancer.
- Aim 3b: To determine the effect of loss of prostatic AR function in the initiation and progression of prostate cancer.
- Aim 3c: To generate mice with an inducible loss of AR function in a mouse model of prostate cancer.
- Aim 3d: To determine the effect of loss of AR function at specific points in the initiation and progression prostate cancer.

Aim 4: To determine the role of AR in the tumorigenicity of androgen dependent and androgen independent AR knockout prostate cancer cells.

- Aim 4a: To generate prostate cancer cell lines lacking AR by somatic homologous recombination
- Aim 4b: To generate prostate cancer cell lines lacking AR by RNA interference (RNAi).
- Aim 4c: To determine the effect of the removal of AR activity on tumor formation and growth in mice.

Our progress and completion of Tasks can be best seen in the following description of the tasks below and in the Figures and legends of the report. These are being written for a manuscript being prepared for publication.

Body:

Our proposed schedule for completion of this proposal included the following tasks during the last 12 months of the grant.

Task 2 (13-24 month)**:** To examine the effect of loss of AR function in the prostate.

Pes-ARKO mice contain a prostate epithelial specific promoter (1) driving cre-recombinase. Expression of the probasin promoter transgene has been reported to be increasingly expressed from 2-7 weeks and sustained expression is observed throughout life (2). To verify AR gene deletion within prostate epithelium of pes-ARKO mice, candidate mice were genotyped for probasin-cre transgene and conditional flox-AR allele (Fig. a). We also evaluated the specificity of recombination in several key organs by RT-PCR using primers directed towards exons 1 and 3 of the AR gene. Deletion of AR exon 2 was confirmed by the detection of truncated transcripts via RT-PCR present within the ventral prostate and dorsal-lateral prostate of pes-ARKO mice only (Fig. b). The lobe specific expression is consistent with the probasin promoter transgene driven expression in other models (3). No other tissues in wild-type (WT) or pes-ARKO mice contained truncated forms of AR DNA. There were no differences in external characteristics, including genital-anal distances between WT and pes-ARKO mice (Fig. c). The internal urogenital organs also showed no differences between WT and pes-ARKO mice (Fig. d, upper panels). In contrast, pes-ARKO mice had significantly larger ventral prostates at week 24 (Fig. d, lower right). Neither the dorso-lateral prostate nor anterior prostate within pes-ARKO mice significantly changed in size.

Task 4 (13-24 month)**:** To examine the effect of the inducible loss of AR on the development and maintenance of the prostate. We are working on this task, but due to the low breeding ratios we have not been able to complete the experiments yet.

Task 6 (13-24 month)**:** To determine the effect of loss of prostatic AR function in the initiation and progression of prostate cancer.

We confirmed the progressive loss of AR by immunohistochemistry. A labeling index for epithelial AR was determined and tabulated (Fig. 1e, left). AR protein localized to epithelial nuclei slowly decreased with age in pes-ARKO mice compared to WT littermates. By week 24 epithelial AR detection was rare. To indirectly evaluate epithelial AR signaling, we quantified staining intensity of probasin, an androgen regulated protein in mature animals. Probasin intensity was similar in pes-ARKO and WT littermates until 12 weeks of age, when the reduced level in pes-ARKO samples compared to WT approached significance ($P=0.057$). By week 24 and thereafter, this difference was significant ($P=0.027$) (Fig. 1e, right). These data suggest that probasin expression is normal before significant loss of AR and epithelial AR decline precedes the loss of an AR-dependent secreted protein.

To determine if pes-ARKO mice contain abnormalities other than enlarged ventral prostates, we evaluated fertility. We found that there were no significant differences in litter-size when either WT or pes-ARKO males were mated to WT females (Fig. f). To rule out the possibility of altered ventral prostate size might be due to circulating androgen levels, we measured serum testosterone levels by ELISA. We observed no difference between the WT and pes-ARKO males at 12 or 24 weeks of age (Fig. f, right). Together, results shown in Figures a-f demonstrate an effective deletion of the AR that is confined to the prostatic epithelium and that consequences of the AR gene deletion appears to be restricted to the prostate and are without the influence of serum testosterone.

Task 7 (1-13 month)**:** To generate mice with an inducible loss of AR function in a mouse model of prostate cancer. We are working on this task, but due to the low breeding ratios we have not been able to complete the experiments yet.

Task 8 (13-36 month)**:** To determine the effect of loss of AR function at specific points in the initiation and progression of prostate cancer. These experiments are ongoing and long term so final results will be reported in next progress report. The experiments reported for Task 6 are part of the experiments planned to complete this task.

Task 9 (1-18 month)**:** To generate prostate cancer cell lines lacking AR by somatic homologous recombination. These cell lines have been more difficult to develop than expected, but are in the final stages of being tested and experiment results will be reported in next progress report.

Task 10 (1-12 month)**:** To generate prostate cancer cell lines lacking AR by RNA interference (RNAi). These cell lines have been difficult to develop, but are in the final stages of being tested and experiment results will be reported in next progress report.

Key Research Accomplishments and Reportable Outcomes:
Key Accomplishment List
•Task 1, 3, and 5 have been completed and we continue to generate more mice for experiments in other Aims and tasks.

•Task 2 (13-24): To examine the effect of loss of AR function in the prostate. Several experiments have been completed or are in the process of being completed. Results are in the Figures presented.

•Task 6 (13-24 month)**:** To determine the effect of loss of prostatic AR function in the initiation and progression of prostate cancer. Several experiments have been completed or are in the process of being completed. Results are in the Figure presented.

•Task 7 (1-13 month)**:** To generate mice with an inducible loss of AR function in a mouse model of prostate cancer (ind-pes-ARKO). This task has been initiated, the proper genotypes have been produced in small numbers.

•Task 8 (13-36 month)**:** To determine the effect of loss of AR function at specific points in the initiation and progression of prostate cancer. Several experiments have been completed or are in the process of being completed. Results are in the Figures presented.

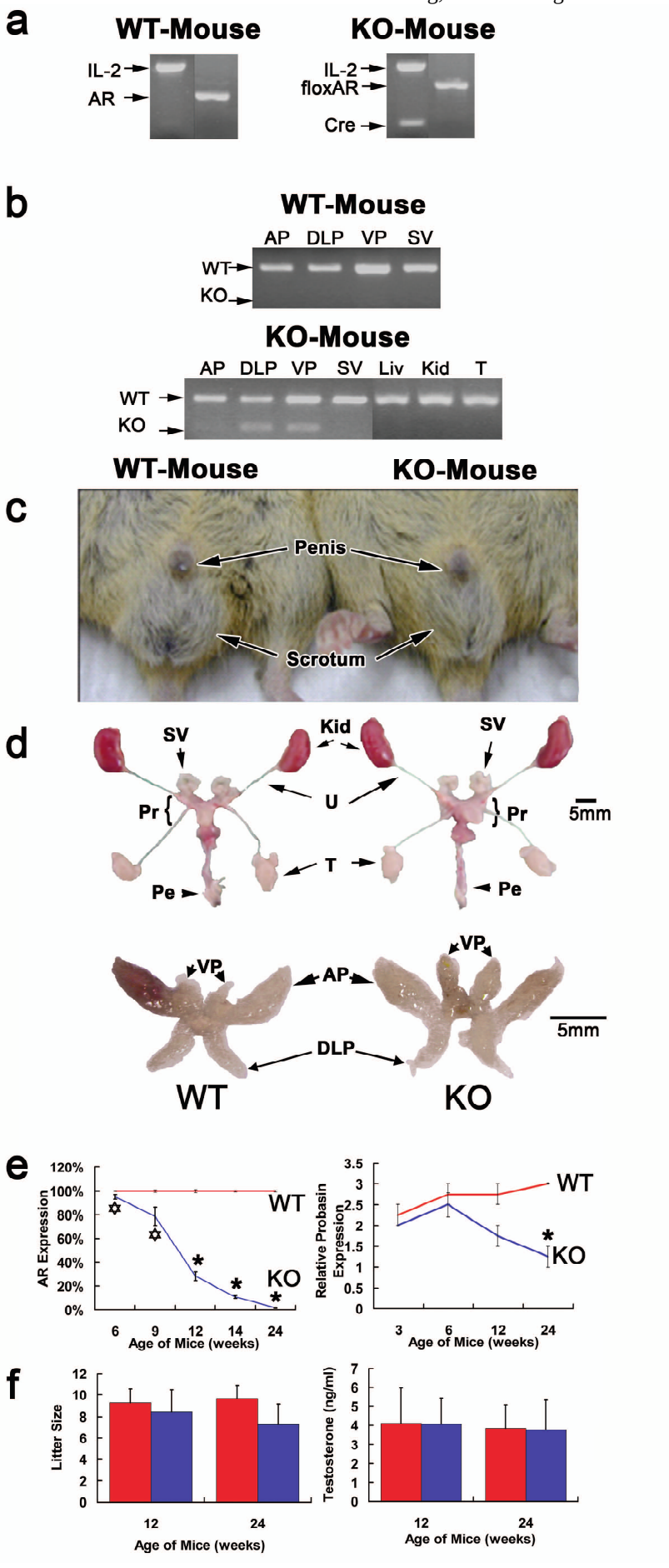
•Task 9 (1-18 month)**:** To generate prostate cancer cell lines lacking AR by somatic homologous recombination.

•Task 10 (1-12 month)**:** To generate prostate cancer cell lines lacking AR by RNA interference (RNAi). This task was initiated, but no statistically significant results during the reporting period.

Task 11(19-36 month)**:** To determine the effect of the removal of AR activity on tumor formation and growth in mice. Several experiments have been completed or are in the process of being completed. Results are in the Figure presented at the end of the report following References and Appendices.

Reportable Outcomes:
• flox AR mice
• pes-ARKO mouse model

Figure Characterization and studyof pes-ARKO mice. a, Wild-type (WT, left) and pes-ARKO mice (KO, right) genotyping. IL-2 RNA occur in both mice and serve as internal positive controls. Transgenes: cre (110bps) and floxAR (540bps) are only in pes-ARKO mice. Only WT mice have wild-type AR gene. **b**, Q-PCR from priming in exon 1 and exon 3 of the AR gene shows one band for WT AR (305 bps) in WT mice. In pes-ARKO mice (KO), both WT band (WT AR occurs in stroma) and the KO band (153 bps; deletion of exon 2) appears in dorso-lateral prostates (DLP) and ventral prostate (VP), but only the WT band appears in other tissues. **c**, Results from external (c) and internal (d) organs looked the same in both strains, except for VP. Note larger size of VP from pes-ARKO mice. **e**, Expression of AR (left) and probasin (right) in VPs of WT vs. pes-ARKO mice with age. Probasin levels decline along with epithelial AR. **f**, Pups/litter from WT female x WT males (red bar) or x pes-ARKO (blue bar) males were similar, left. Serum testosterone levels (**f**, right) were similar in WT (red bar) and pes-ARKO (blue bar) male mice at week12 and 24. Key: seminal vesicle (SV), kidney (Kid), ureter (U), anterior prostate (AP), dorsolateral prostate (DLP), ventral prostate (VP) all lobes of prostate (Pr), testes (T), glans penis (Pe); *P<0.05, ***P<0.001.


a
WT-Mouse
KO-Mouse
IL-2
AR
IL-2
floxAR
Cre
b
WT-Mouse
AP DLP VP SV
WT
KO
KO-Mouse
AP DLP VP SV Liv Kid T
WT
KO
c
WT-Mouse
KO-Mouse
Penis
Scrotum
d
SV
Kid
SV
Pr
U
Pr
5mm
T
Pe
Pe
VP
AP
VP
5mm
DLP
WT
KO
e
AR Expression
120%
100%
80%
60%
40%
20%
0%
WT
KO
6 9 12 14 24
Age of Mice (weeks)
Relative Probasin Expression
3.5
3
2.5
2
1.5
1
0.5
0
WT
KO
3 6 12 24
Age of Mice (weeks)
f
Litter Size
12
10
8
6
4
2
0
12 24
Age of Mice (weeks)
Testosterone (ng/ml)
7
6
5
4
3
2
1
0
12 24
Age of Mice (weeks)

Conclusions:

A key signature of the adult normal prostate gland is the lack of proliferation even in the presence of growth stimulating androgens. This is in contrast to benign prostate hyperplasia and prostate cancer, in which epithelial cells acquire the ability to proliferate. Here we show 2 seminal findings in the areas of cell biology and cancer research. First we report that in mature prostatic epithelium, AR is critical for maintaining differentiated phenotype and overall homeostasis of the gland. Moreover, selective removal of epithelial AR signaling stimulates mitogenesis of the otherwise growth quiescent prostate. Normal prostate growth may require delicate temporal and spatial balance between the proliferative role of stromal AR and the growth-suppressive role of epithelial AR. Our findings recast the role of androgen/AR signaling within the prostate, and consequently call into question the current therapeutic strategy for prostate disease, which relies solely and indiscriminately on antagonizing stromal ARs to prevent proliferation without considering epithelial AR's suppressive roles.

The other tasks for the second and third year are well on the way to completion and will be reported with study results in the final report. The inducible mouse model, and other cell lines that are in production are very time consuming and difficult to produce, but are being produced in enough numbers for further experiments to be completed within the third year of the grant. All of the initiated tasks will allow us to continue with the other tasks towards completion of the entire project within the 3 years.

References:

1 Greenberg, N. M. et al. The rat probasin gene promoter directs hormonally and developmentally regulated expression of a heterologous gene specifically to the prostate in transgenic mice. Mol Endocrinol 8, 230-9 (1994).

2. Wu, X. et al. Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech Dev 101, 61-9 (2001).

3. Greenberg, N. M. et al. Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A 92, 3439-43 (1995).

Appendices: None