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14. ABSTRACT Defining the origin of prostate cancer cells is fundamentally important and will guide future research to focus on cells from which prostate cancer cells are derived. Prostate cancer is thought to be derived from luminal epithelial cells in the prostate, because a hallmark of prostate cancer is the loss of basal epithelial cells and prostate cancer cells exhibit a luminal epithelial cell phenotype including the expression of AR and PSA. However, the luminal origin of prostate cancer has been challenged by a number of recent publications. This project will determine whether prostate cancer cells are derived from luminal or basal epithelial cells in an EAF2-/-; PTEN+/- mouse model, and determine whether luminal-derived prostate cancer cells behave differently from basal-derived prostate cancer cells. In the last year of the funding period, we have generated 1 breeding pair (consisting of 1 male and 1 female) of PSA-CreERT2; R26RmT/mG;EAF2+/-;PTEN+/- mice for Specific Aim 1. Also, we have obtained CK14-CreERT2 mice and began breeding to generate CK14-CreERT2; R26RmT/mG;EAF2-/-;PTEN+/- mice for Specific Aim 2.					
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Introduction

Defining the origin of prostate cancer cells is fundamentally important and will guide future research to focus on cells from which prostate cancer cells are derived. Prostate cancer is thought to be derived from luminal epithelial cells in the prostate, because a hallmark of prostate cancer is the loss of basal epithelial cells and prostate cancer cells exhibit a luminal epithelial cell phenotype including the expression of AR and PSA [1]. The capability of luminal epithelial cells as origin of prostate cancer is also supported by over expression of oncogenes such as cMYC and T-antigen or knockout of important tumor suppressor such as PTEN, specifically in prostate luminal epithelial cells. However, the luminal origin of prostate cancer has been challenged by a number of recent publications [2, 3]. This project will determine whether prostate cancer cells are derived from luminal or basal epithelial cells in an EAF2^{-/-}; PTEN^{+/-} mouse model, and determine whether luminal-derived prostate cancer cells behave differently from basal-derived prostate cancer cells.

Body

This project will use genetic lineage tracing [4, 5] [6] and the EAF2^{-/-};PTEN^{+/-} mouse prostate cancer model [7] to determine whether prostate cancer cells are derived from basal and/or luminal epithelial cells in the prostate *in vivo*. All the mice in this study will be on C57BL/6J background.

Task 1: To determine whether prostate cancer can be derived from luminal epithelial cells in the EAF2^{-/-};PTEN^{+/-} mouse prostate cancer model using the PSA-CreER^{T2}-based genetic lineage tracing (months 1-30)

- A. Obtain IACUC approval and generate PSA-CreER^{T2}; R26RmT/mG; EAF2^{-/-}; PTEN^{+/-} mice (month 1-16)

Status: We have written and received approval for IACUC protocol 12020202. Generation of mice is ongoing, currently we have generated 7 breeding pairs (consisting of 1 male and 1 female) with the following genotypes: PSA-CreER^{T2}; EAF2^{-/-};PTEN^{+/-} mated to R26RmT/mG. These breeding pairs have been established to generate 80 male PSA-CreER^{T2}; R26RmT/mG;EAF2^{-/-};PTEN^{+/-} mice required to complete Specific Aim 1. The first litters of pups from these mice consisted of 28 pups of which 1 male and 1 female had the following phenotype: PSA-CreER^{T2}; R26RmT/mG;EAF2^{+/-};PTEN^{+/-} (Figure 1). These mice will be utilized as a breeding pair to enhance the efficiency of breeding. Additionally, a cohort of 10 male R26RmT/mG mice generated through the breeding strategy are being utilized to determine the rate of spontaneous labeling in the prostate following multiple cycles of regression and regrowth. A subgroup of 4 animals was castrated at 10 weeks of age and will be subjected to 5 rounds of regression and regrowth. These animals will be examined to determine what percentage, if any, of epithelial cells can be labeled through spontaneous recombination. These animals will serve as additional experimental controls.

- B. Genotyping to verify genotype of genetically modified animals (month 1-18).

Status: Genotyping of pups is ongoing, tail clippings are taken for genotyping analyses at 21 days of age at weaning. See Figure 1.

- C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells (month 4-20)

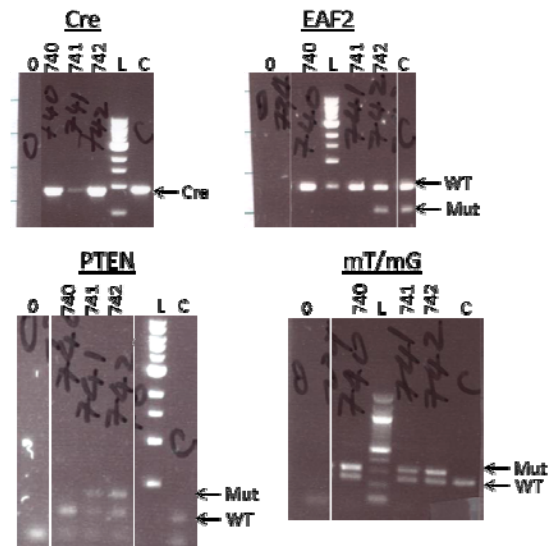


Figure 1. Genotypic analysis of offspring from PSA-CreER^{T2}; EAF2^{-/-};PTEN^{+/-} mated to R26RmT/mG mice. Tail DNA was isolated from offspring at weaning and examined by PCR. Animal #742 is PSA-CreER^{T2}; R26RmT/mG;EAF2^{+/-};PTEN^{+/-}. Molecular weight ladders are shown (L) as well as negative (0) and positive controls (C).

Status: We are expecting the first experimental animals to be born within the next 30 days. These animals will be ready for intraperitoneal injection of tamoxifen at 8 weeks of age. Expected revised timeline months 14-20.

D. Histological and genetic lineage analysis of prostate cancer in mice (month 15-30)

Task 2: To determine whether prostate cancer cells can be derived from basal epithelial cells in the EAF2^{-/-};PTEN^{+/-} mouse prostate cancer model (months 10-32).

A. Generate CK5-CreER^{T2}; R26RmT/mG; EAF2^{-/-}; PTEN^{+/-} mice (month 10-20)

Status: We encountered some difficulties with importing the CK5- or CK14-CreER^{T2} mice at Dr. Brigid Hogan's lab in Duke were positive with virus, and our animal facility was unable to accept the mice. Dr. Xin Li from Baylor has kindly offered us CK14-CreER^{T2} mice. Unfortunately, these animals were also infected with virus. Luckily, Jackson Lab has CK14-CreER^{T2} available. We placed order immediately and it took about 6 months for them to revive the colony and ship the animals to us. Crosses of between CK14-CreER^{T2} and EAF2^{-/-} mice have been initiated and the first generation of CK14-CreER^{T2}; EAF2^{+/-} consisted of 6 offspring (Figure 2). Mice will also be crossed with R26RmT/mG;EAF2^{-/-};PTEN^{+/-} generated in Task 1A to enhance the efficiency of breeding.

B. Genotype genetically modified animals (month 12-22).

Status: Genotyping of pups is ongoing, tail clippings are taken for genotyping analyses at 21 days of age at weaning. See Figure 2.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells (month 14-20).

Status: We are expecting the first experimental animals to be born within the next 3 months. These animals will be ready for intraperitoneal injection of tamoxifen at 8 weeks of age. Expected revised timeline months 17-23.

D. Histological and genetic lineage analysis of prostate cancer in mice (month 16-32)

Task 3: To determine whether prostate cancer cells derived from luminal epithelial cells are different from those from basal cells in the EAF2^{-/-};PTEN^{+/-} mouse prostate cancer model (months 16-36).

A. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers, and neuroendocrine markers in luminal-derived prostate cancer (month 20-32).

B. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers, and neuroendocrine markers in basal-derived prostate cancer (month 20-32).

C. Determine the effect of castration on luminal-derived and basal-derived prostate cancer (month 16-36).

D. Data analysis and manuscript writing (month 30-36).

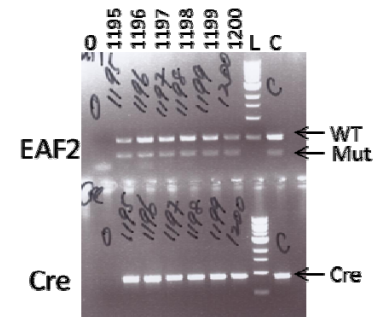


Figure 2. Genotypic analysis of offspring from CK14-CreER^{T2} mated to EAF2^{-/-} mice . Tail DNA was isolated from offspring at weaning and examined by PCR. Animals are CK14-CreER^{T2}; EAF2^{+/-}. Molecular weight ladders are shown (L) as well as negative (0) and positive controls (C).

Key Research Accomplishments

1. Obtained approval of the IACUC protocol for this project.
2. Generated 1 breeding pair (consisting of 1 male and 1 female) of PSA-CreER^{T2}; R26RmT/mG;EAF2+/-;PTEN+/-.
3. Obtained CK14-CreER^{T2} mice.
4. Generated CK14-CreER^{T2}; EAF2+/- mice (4 males and 2 females).

Reportable Outcomes

None.

Conclusion

We are in the process of generating transgenic mice to accomplish proposed specific aims.

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