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14. ABSTRACT

We previously generated a PB-Cre4/CAG-SMIL transgenic model allowing Cre-induced expression of c-Myc oncogene and Luc2 (for BLI imaging in vivo) in prostate epithelia. Once turned on, c-Myc and Luc2 expression will not rely on androgen, which allows studying castration response and CRPC. However, most Cre4/CAG-SMIL mice did not develop invasive prostate tumors up to 2-year of age, potentially due to c-Myc induced p53 activation. Hence, we proposed to generate PB-Cre4/CAG-SMIL/p53loxP/loxP mice to conditionally knock out p53 and turn on c-Myc expression in prostate for rapid onset of PCa, and use this model to study CRPC and chemoresistance of CRPC. In the initial year, we have carried out all the proposed studies as described in SOW on schedule, including performing extensive multi-rounds of crossing to generate the target PB-Cre4/CAG-SMIL/p53loxP/loxP mice, and the initial characterization of prostate histopathology to confirm early onset of mPIN in 8-week old PB-Cre4/CAG-SMIL/ p53loxP/loxP mice. We have also identified a potential technical problem and provided alternative approaches to address it.

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Annual Progress Report

W81XWH-13-1-0162

Using a Novel Transgenic Mouse Model to Study c-Myc Oncogenic Pathway in Castration Resistance and Chemoresistance of Prostate Cancer

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W81XWH-13-1-0162 "Using a Novel Transgenic Mouse Model to Study c-Myc Oncogenic Pathway in Castration Resistance and Chemoresistance of Prostate Cancer"

Introduction

c-Myc is the most significantly amplified oncogene in human prostate cancer (PCa) [1, 2], and its gene amplification is associated with advanced disease grade and worse prognosis [3]. In addition, c-Myc overexpression is also very common in PCa as early as PIN [4]. These indicate its critical roles in PCa progression as well as in the development of therapy-resistance, including castration resistance and chemoresistance. Transgenic models are widely used in cancer research. Dr. Sawyer's group has developed the widely used Hi-MYC model using an enhanced probasin promoter to drive c-Myc overexpression in prostate epithelia. These Hi-Myc mice develop invasive prostate carcinomas that share molecular features with human PCa [5]. However, since probasin promoter activity is crucially dependent on androgen, the Hi-Myc tumors lose c-Myc expression after castration [5]. Therefore, the tumor regression in these androgen-depleted Hi-Myc mice represents the mixed effects of an artificial direct effect from loss of oncogene expression and a potential real effect from tumor responses to castration. These greatly complicate the system and make it difficult to concisely study c-Myc oncogenic pathway in androgen signaling, castration-responses, and the development of castration-resistant PCa (CRPC). Accordingly, we have generated a P_{CAG}-*loxP-Stop-loxP-Myc-IRES-Luc2* model (referenced here as *CAG-SMIL* model, Figure 1). P_{CAG} is an enhanced β-actin promoter that can drive universal expression of transgene in mice. The *loxP-Stop-loxP* cassette located between P_{CAG} and the *c-Myc-IRES-Luc2* (an enhanced luciferase from Promega) cassette abolishes the otherwise ubiquitous expression of c-Myc and Luc2. IRES allows bicistronic expression of both genes. After crossing with PB-Cre4 mice overexpressing Cre in prostate epithelium [6], Cre will remove the *loxP-Stop-loxP* cassette to specially turn on c-Myc and Luc2 expression in the prostate of the male *PB-Cre4*/*CAG-SMIL* mice. Importantly, once turned on, the c-Myc and Luc2 expression will be driven by the P_{CAG} promoter independent on androgen. This will allow us to concisely study c-Myc signaling pathway 1) in castration induced prostate tumor regression, 2) in the recurrence of CRPC tumors, and 3) in the development of chemoresistance in CRPC tumors. The Luc2 expression will permanently label the tumors in this model, which allows real-time *in vivo* bioluminescence imaging (BLI) for prostate tumor progression, tumor response to various therapeutic agents, and tumor relapse after the development of therapy (castration and chemotherapy) resistance. Furthermore, by crossing *PB-Cre4*/*CAG-SMIL* mice with mouse lines carrying loxP flanked gene of interest, such as *p53* and *Pten*, we will be able to concisely and efficiently knock out the gene of interest and turn on the expression of c-Myc and Luc2 within the same cell population. We observed delayed prostate tumor progression as well as apparent prostate epithelial cell death in our *PB-Cre4/CAG-SMIL* model. mPIN, but not invasive prostate tumors were formed in most PB-Cre4/CAG-SMIL transgenic mice up to 2 year of age. Since c-Myc overexpression may induce p53 activation and lead to cell senescence or apoptosis [7], the subject of this grant is to cross our *PB-Cre4/CAG-SMIL* mice with $p53^{loxP/loxP}$ mice [8] to conditional knock out *p53* and overexpress c-Myc in prostate for a rapid onset and progression of prostate tumors, and use these mice to study castration resistance and chemoresistance of PCa. Finally, although mutation or loss of *p53* is not a very common event in human PCa at early stage, it is strongly correlated to PCa disease stages, metastasis, and

castration-resistance [9, 10]. Therefore, conditional ablation of *p53* in the *PB-Cre4/CAG-SMIL* mice is a valid approach to model a large fraction of advanced PCa, which is the exact PCa type that should be targeted for our proposed studies on castration-resistance and chemoresistance of PCa.

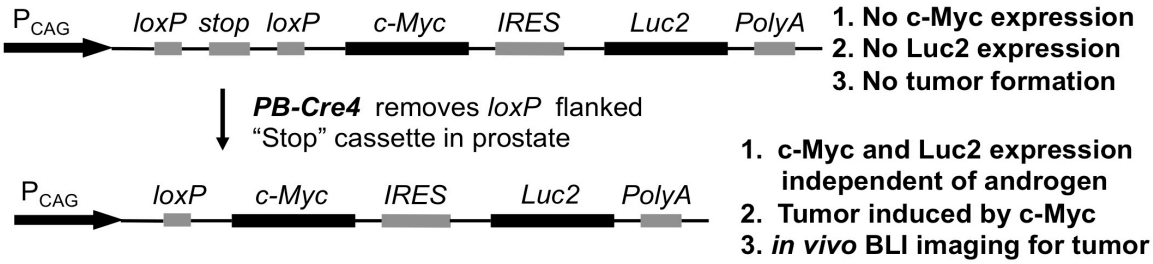

Figure 1. Diagram of the CAG-SMIL transgenic model (CAG promoter driving LoxP flanked "Stop" cassette, followed by c-Myc, IRES, and Luc2).

Keywords:

c-Myc, prostate cancer, castration resistance, chemoresistance, prostate tumor model

Overall Project Summary

We have made significant progress in this first year of funding. Our research progress is in line with what we have proposed in the SOW, which includes the following three major tasks.

Major Task1: Characterize the tumor development in the *PB-Cre4/CAG-SMIL/ p53$^{loxP/loxP}$* mice and the control *PB-Cre4/CAG-SMIL* mice. These include Subtasks (1) Generate and expand the population of *PB-Cre4/CAG-SMIL/ p53$^{loxP/loxP}$* mice and *PB-Cre4/CAG-SMIL* mice (1-18 months) and (2) Perform full necropsy on mice from each group every two months after the BLI imaging. Collect prostate tissues / tumors for histopathology / immunohistochemistry (IHC), Western blot and/or qRT-PCR analysis (8-24 months).

For Subtask 1, we have crossed male *PB-Cre4/CAG-SMIL* mice with *p53$^{loxP/loxP}$* mice to generate the *PB-Cre4/CAG-SMIL/p53$^{wt/loxP}$* mice. We then crossed the obtained male *PB-Cre4/CAG-SMIL/p53$^{wt/loxP}$* with *p53$^{loxP/loxP}$* mice to generate the targeted *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice for conditional knockout of p53 and overexpression of c-Myc in prostate epithelial cells. We are now expanding the population of *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice and *PB-Cre4/CAG-SMIL* mice for the proposed studies in all tasks.

For Subtask 2, we have performed BLI imaging and the initial full necropsy on the *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice of 8-week old along with the age-matched control *PB-Cre4/p53$^{loxP/loxP}$* mice. BLI imaging confirmed high Luc2 activities in the prostate tissues of the *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice. In addition, lower but significant Luc2 activities were also observed in the epididymis of these mice, indicating off-target efforts of the PB-Cre4 transgene (data now shown).

After full necropsy, we performed histopathological analysis including H&E staining and IHC staining for AR, c-Myc, Ki67 on the prostate and the epididymis tissues of these 8-week old *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice and *PB-Cre4/p53$^{loxP/loxP}$* mice. As shown in Figure 2, c-Myc is highly expressed in the prostate tissue of PB-Cre4/CAG-SMIL/*p53$^{loxP/loxP}$* mice, but not in the control PB-Cre4/*p53$^{loxP/loxP}$* mice. In addition, while the prostate tissue from *PB-Cre4/p53$^{loxP/loxP}$* mouse appears normal, the PB-Cre4/CAG-SMIL/*p53$^{loxP/loxP}$* mouse developed mPIN, supporting that overexpression of c-Myc and loss of p53 together promotes early onset of mPIN, which exhibits enhanced proliferation (Ki67 staining). This disease progression is significantly more rapid than that of PB-Cre4/CAG-SMIL alone. This rapid onset of mPIN provides essential data supporting the feasibility of using this mouse model to study castration resistance (Major Task 2) and chemoresistance (Major Task 3) of PCa in years 2 and 3.

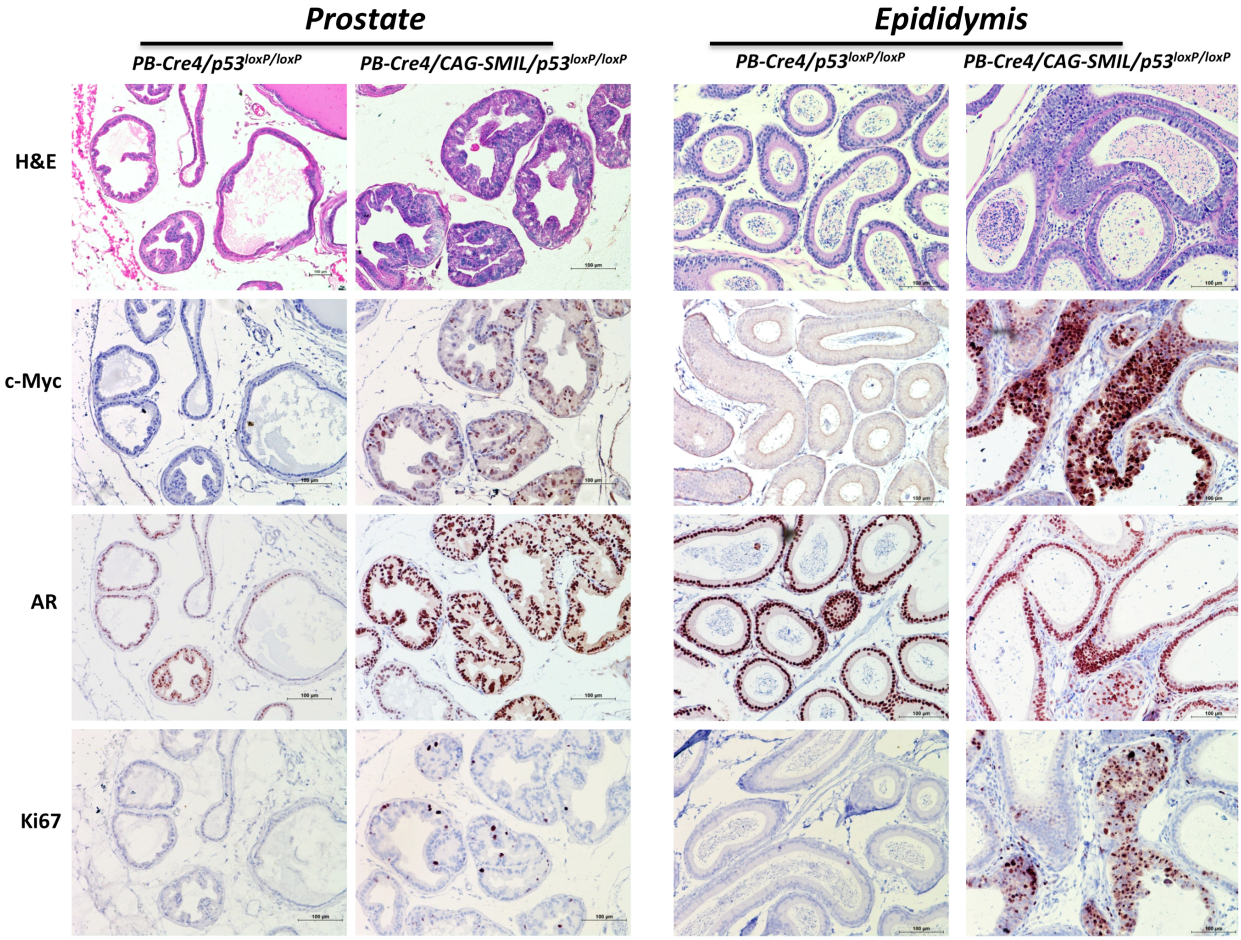

Figure 1. Histopathological characterization of the prostate and epididymis from representative 8-week old *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mouse and *PB-Cre4/p53$^{loxP/loxP}$* mouse.

Although PB-Cre4 transgenic mice have been extensively used in prostate tumor modeling and research, significant off-target Cre activity was detected in the epididymis in our study as evidenced by both BLI imagining and IHC of c-Myc expression in epididymis (Figure 2

and data not shown). This off-target Cre activity led to overexpression of c-Myc, knockout of p53, extensive cell proliferation, and rapid development of hyperplasia / low grade dysplasia in the epididymis of the 8-week old *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice. The epididymis from control *PB-Cre4/p53$^{loxP/loxP}$* mice showed normal histology without appreciable amount of c-Myc expression or cell proliferation. Therefore, due to the off-target effects of PB-Cre4 transgene, our model may develop aggressive tumors of epididymis, therefore, may provide a transgenic model for tumor of epididymis together with prostate tumor. Tumor of epididymis is a rare type of cancer in human, and most of them are benign. The aggressive types of epididymis tumors are extremely rare. Therefore, this may significantly limit the clinical application of our model as a tumor model for epididymis tumors. However, we will continue monitor and characterize the nature and disease progression of these epididymis tumors and investigate whether it is a model for this rare cancer.

The potential rapid growth of tumors from epididymis (as evidenced by extensive cell proliferation) may bring technical problems in using our *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* model to study prostate tumors, especially if metastatic tumors are involved. However, we predict that we may solve such problems by performing castration in young *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice (such as those of 6-week old, to be optimized). Castration procedure will remove both testis and epididymis; therefore, erase the concerns on tumors of epididymis. In this case, subcutaneous implant of testosterone pellets may be used to continue support prostate tumor growth and our proposed studies on CRPC in Major Task 2 and chemoresistance of CRPC in Major Task 3 can be done by simply removing the testosterone pellets from these mice as an alternative "castration" approach.

Major Task 2: Study how the *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* prostate tumors respond to castration and the molecular signatures of castration resistance of these tumors. These include Subtask (1) At 6-8 month of age (or time to be optimized), *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice will be performed castration or sham operated. Prostate tumors will be collected for histopathology / IHC, Western blot and/or qRT-PCR analysis for their acute response to castration and the development of castration-resistant prostate tumors (12-30). Subtask (2) cDNA Microarray will be performed on the above tumors for the molecular signature of castration-resistant prostate tumors (18-36).

Since the proposed studies in Major Task 2 begin at Month 12, limited studies have been carried out in the first year. However, we did begin performing castration on the *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice, *PB-Cre4/CAG-SMIL* mice and *PB-Cre4/p53$^{loxP/loxP}$* mice, along with various controls. The results from these studies will be updated in the next Annual Report.

Major Task 3: Study how the castration-resistant PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$ prostate tumors respond to chemotherapy (docetaxel) and the molecular signatures of chemo-resistance of these tumors. These include Subtask (1) *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice with castration-resistant prostate tumors will receive weekly intravenous administration of docetaxel or solvent control. Prostate tumors will be collected for histopathology / IHC, Western blot and/or qRT-PCR analysis for their acute response to chemotherapy and the development of chemo-resistant prostate tumors (20-36 months). Subtask (2) cDNA microarray will be

performed on the above tumors for the molecular signature of chemo-resistant CRPC tumors (24-36 months).

Due to the nature of the proposed studies in Major Task 3, these studies will not begin until Month 20. Therefore, no study has been carried out in this Task in this initial year.

Key Research Accomplishments

- Received local IACUC approval and ACURO approval for the proposed animal studies.
- Generation of the *PB-Cre4/CAG-SMIL/p53$^{loxP/wt}$* and *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice for Cre-mediated knockout of p53 and overexpression of c-Myc in prostate epithelial cells.
- Verification of the Cre-induced Luc2 expression in the prostate tissues of male *PB-Cre4/CAG-SMIL/p53$^{loxP/wt}$* and *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice *in vivo* (BLI imaging).
- Full necropsy on the *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice of 8-week of age. Initial characterization of histopathology of the prostate tissues, including IHC staining for c-Myc, AR, and Ki67 etc.
- Initial characterization of the c-Myc overexpression and its induced histopathology changes in epididymis and / or testis.

Conclusion

In this first year, we have carried out the proposed Year 1 studies as described in the SOW on schedule. Initial histopathological characterization on the prostate tissues from 8-week old *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice confirms overexpression of c-Myc transgene and cell proliferation in the prostate epithelial cells, together with rapid on-set of mPIN. This provides essential data supporting the feasibility of using this mouse model to study castration resistance and chemoresistance of PCa in years 2 and 3.

Although PB-Cre4 transgenic mice have been extensively used in prostate tumor modeling and research, significant off-target Cre activity was detected in the epididymis in our study. This led to overexpression of c-Myc, knockout of p53, extensive cell proliferation, and rapid development of hyperplasia / low-grade dysplasia in the epididymis of the 8-week old *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice. The potential rapid growth of tumors from epididymis may bring technical problems in using our *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* model to study prostate tumors. However, such problems may be solved by performing castration in young *PB-Cre4/CAG-SMIL/p53$^{loxP/loxP}$* mice (such as those of 6-week old, to be optimized). Castration procedure will remove both testis and epididymis; therefore, erase the concerns on tumors of

epididymis. In this case, subcutaneous implant of testosterone pellets may be used to continue support prostate tumor growth in these castrated mice.

In summary, we have successfully carried out the proposed studies in the first year on schedule, which provided strong data supporting studies in the years 2 and 3. Although tumors from epididymis might be a concern, we expect to be able to solve this problem by performing castration in young mice and expect to be able to finish the proposed studies at the conclusion of the award.

Publications, Abstracts, and Presentations: Nothing to report

Inventions, patents and licenses: Nothing to report

Reportable Outcomes: Nothing to report

Other Achievements: Nothing to report

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Appendices: None