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13. SUPPLEMENTARY NOTES

14. ABSTRACT- SPOP mutations occur in 6–15% of cases of prostate cancer (PCa). SPOP mutations are early events that have been found in localized and metastatic stages of PCa but, have shown to be a poor biomarker for risk stratification in patients. Homozygous deletion of CHD1 is the most common recurrent alteration that occurs mostly in the SPOP mutant subclass. CHD1 loss is commonly subclonal, occurs in 5–10% of cases in PCa, 80% of which belong to the mutant SPOP subclass. Tumors carrying combined SPOP mutant; CHD1 loss have distinct gene expression patterns, DNA hypermethylation and highest AR activity compared to other subtypes of PCa. Despite the discovery of this subtype in 2012, molecular mechanisms driving pathogenesis remain to be understood. This stem, in part, from the lack of relevant model systems harboring these specific genetic alterations for use as study tools. Herein, we describe unique genetically engineered mouse models, 3D organoid systems and cell lines that have been developed and deployed in our laboratory to recapitulate clinically observed genotypes in normal prostate cells. Our preliminary data from mouse models supports the tumor suppressor function of SPOP and CHD1. We have also observed a dramatic decrease (by ~50%) in the time taken for the development of HG-PIN when CHD1 deletion co-occurs with SPOP mutation. This reduction in time to develop HG-PIN suggests that loss of CHD1 is required in SPOP mutant subclass to drive it to an aggressive stage. Additionally, it shows that this subclass of prostate cancer is dependent on androgen receptor (AR) signaling for its growth. This project further aims to elucidate genome wide differences in AR signaling in this subclass of PCa testing our hypothesis suggesting that CHD1 deletion modulates transcriptional programs, specifically AR signaling, to confer a highly aggressive phenotype in SPOP mutant prostate cells.

15. SUBJECT TERMS

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Table of Content

Introduction

There is increasing awareness that prostate cancer (PCa) is a heterogeneous disease characterized by specific genomic alterations; this allows stratification for personalizing treatment regimens and improving outcomes. One such subclass is represented by mutations in the SPOP gene, the most recurrent point mutations in PCa. A striking feature of SPOP mutant tumors is that they have increased intrachromosomal rearrangements, specifically in the 5q21 chromosomal locus. This corresponds to loss of the chromodomain helicase DNA binding protein, CHD1. SPOP mutations have a significant tendency to co-occur with CHD1 loss. Based on evidence from clonality studies, it can be gauged that SPOP mutation is an early event in the development of PCa, preceding CHD1 deletion. Interestingly, CHD1 loss is largely restricted to the prostate over other cancer types. Together, this subclass represents ~15% of all primary prostate cancers (~20,000 cases in 2018). Yet, an understanding of the molecular mechanisms driving pathogenesis of this subclass is lacking and the effectiveness of standard therapeutic approaches in this context also remain to be deduced.
Loss of CHD1 occurring as a later event in SPOP mutant subclass suggests it to be a tumor suppressor gene in the prostate. Based on this information we hypothesized that CHD1 loss in SPOP mutant prostate cells is advantageous in accelerating development of cancer and induces an aggressive disease phenotype. Because of its role as a chromatin remodeling protein, we hypothesize that CHD1 loss alters transcriptional programs that contribute to oncogenesis. Since CHD1 loss is largely restricted to the prostate, and androgen receptor (AR) signaling is a central regulator of prostate cell survival, we will specifically investigate changes in AR signaling in this genetic background and its role in driving PCa development.

Keywords

- Prostate Cancer (PCa)
- SPOP
- CHD1
- PTEN
- Androgen Receptor (AR)
- AR activity
- High-grade prostatic intraepithelial neoplasia (HG-PIN)
- Genetically engineered mouse (GEM) model
- Dihydrotestosterone (DHT)
- Enzalutamide
- Bicalutamide
- Nuclear Atypia

Accomplishments

Aim 1: Recapitulate clinically observed phenotypic effects of *SPOP* mutation and *CHD1* deletion in the prostate by developing suitable *in vivo* and *in vitro* models: genetically engineered mice, organoids and cell lines.

Aim 1a: To characterize genetically engineered mouse models of *SPOPmut*/*CHD1null* prostate cancer.

Objective of Research

Despite the discovery of the SPOP mutant (with CHD1 loss) subclass in 2012, a significant impediment to advancing our knowledge of this PCa subclass has existed due to the lack of relevant model systems harboring these genetic alterations. We have developed a genetically engineered mouse (GEM) model with these modifications specifically in the prostate to recapitulate characteristics of the human disease. Use of these GEM (*in vivo*) models provides insight into the functional role of SPOP and CHD1 (as a tumor suppressor) in prostate specific context. Further, it could also be used as a tool to determine the most effective treatment options for this particular subtype of PCa.

Progress Summary/Accomplishments

We generated a conditional mouse with the $SPOP^{F133V}$ transgene knocked into the Rosa26 locus using a lox-Stop-lox strategy (Figure 1A). F133V is the most common SPOP mutational variant observed clinically. To express $SPOP^{F133V}$ specifically in the prostate, we crossed $Rosa26^{SPOP\text{-}F133V}$ mice with mice expressing Cre under control of the probasin promoter (Pb-Cre4) (Figure 1A). We also purchased from a commercial vendor, mice with floxed CHD1 alleles (exon 16) and crossed them with Pb-Cre4 transgenic mice to achieve prostate specific deletion of CHD1 (Figure 1A). By breeding these mice, we were able to generate mice harboring both $SPOP^{F133V}$ mutation and CHD1 loss, specifically in the prostate (Figure 1A). All mice are in a pure C57BL/6 background.
Previous results from the Barbieri laboratory have shown that mice expressing $SPOP^{F133V}$ in the prostate display- 1.) Minimal histological changes by one year in $Pten^{+/+}$ background, 2.) Focal high-grade prostatic intraepithelial neoplasia (HG-PIN) with striking nuclear atypia at 6 months of age in heterozygous Pten loss ($Pten^{f/+}$) backgorund, and 3.) At 3 months of age in homozygous Pten loss ($Pten^{f/f}$) background. Heterozygous and homozygous loss of Pten has a minimal phenotype by itself at 6 months and 3 months respectively, and rarely transition into diffusive HG-PIN or invasive cancer.

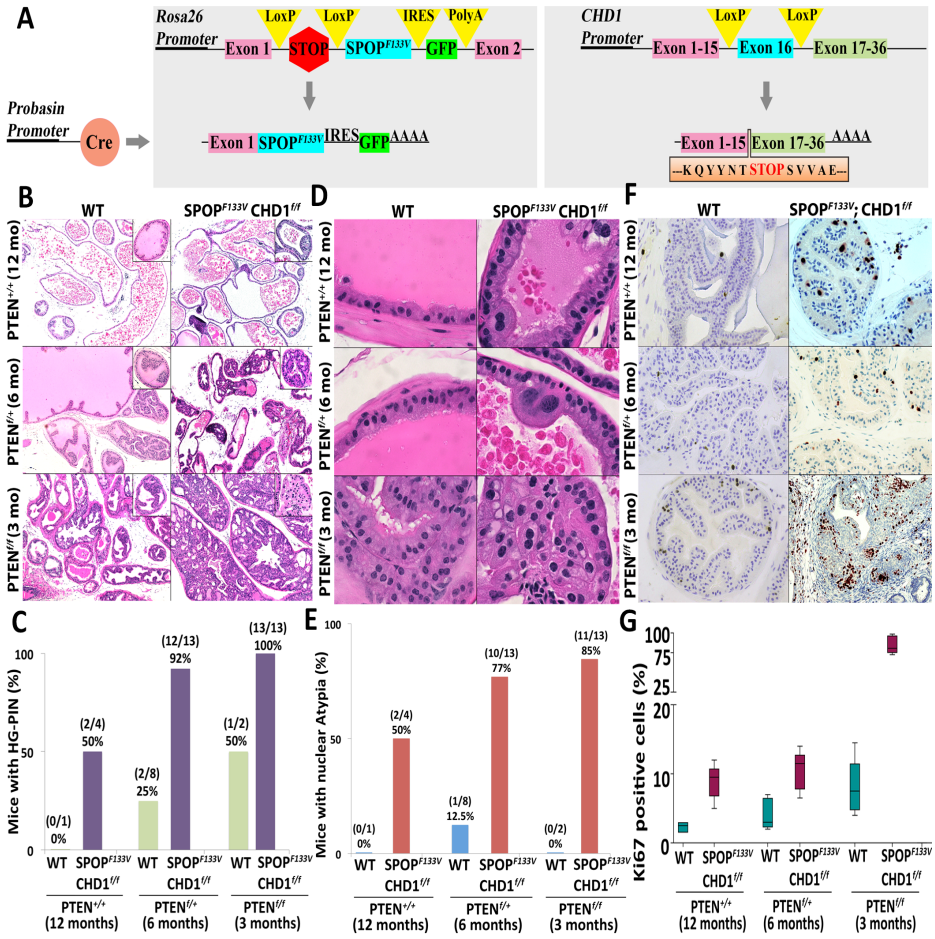

Figure 1: Co-occurrence of SPOP mutation and CHD1 deletion is associated with early onset of high-grade prostate intraepithelial neoplasia (HG PIN). (A.) Shows schematics of conditional SPOP^{F133V} insert in Rosa 26 locus, and CHD1gene floxed between exon 15 and 17, with their respective transcripts generated after Cre expression driven by probasin (Pb) promoter. (B-E) show Hematoxylin and Eosin (H&E) staining and (F-G) shows Ki67 staining via immunohistochemistry (IHC), of mouse prostates- with or without SPOP^{F133V}; CHD1$^{f/f}$ expression in PTEN background.

However, the prostates of mice harboring loss of CHD1 in concurrence with the expression of mutant SPOP^{F133V} demonstrated- 1.) Focal HG-PIN with nuclear atypia, which was observed in 50% of the mice compared to control (Figure 1B-E), by one year in Pten$^{+/+}$ background; 2.) Accelerated onset of diffuse HG-PIN with nuclear atypia by 6 months in a conditional heterozygous Pten loss (Pten$^{f/+}$) background (Figure 1B-E) and; 3.) Even more severely diffused HG-PIN with nuclear atypia by 3 months in a conditional homozygous Pten loss (Pten$^{f/f}$) background (Figure 1B-E). In addition, HG-PIN glands showed higher Ki67 staining, a proliferation marker, compared to their respective Pten controls (Figure 1F-G).

These results suggest that the prostatic loss of CHD1 in a SPOP mutant background causes the disease to progress faster by almost 50%. This is in strong support of our hypothesis that CHD1 loss cooperates with SPOP mutation to accelerate pathogenesis of the disease. Furthermore, loss of CHD1 itself has a minimal phenotype in a Pten$^{f/+}$ background, with only focal HG-PIN even in mice aged 2 years.

Future Activities

We aim to increase the sample size of each cohort, particularly in control groups. We also plan to study the mice, with same genetic makeup, over extended period to determine the time point of

cancer development. In addition, we aim to test clinically relevant treatment approaches: castration, drug treatment (enzalutamide). If alternate druggable pathways emerge from molecular analysis, we would use these mouse models for *in vivo* target validation.

Aim 1b: Characterize cell lines/organoids generated from mouse prostates and study the effect of *SPOP* mutation and/or *CHD1* loss on cell growth and viability.

Objective of Research

SPOP mutation occurs early in the natural history of PCa and defines a distinct genetic subtype not represented by cell lines; therefore, it may be necessary to study its effects in normal prostate cells. Previous reports studying the biology and downstream effects of SPOP mutations have relied on ectopic overexpression of SPOP proteins in cell lines with multiple preexisting genetic alterations, complicating interpretation. Moreover, there are no cell lines available to study the combined effect of SPOP mutation and CHD1 deletion. Organoid platforms represent an opportunity to study the effect of cancer-associated alterations in genetically normal cells that can recapitulate the cell biology and epithelial architecture of the prostate. Generation of these oragnoids model provide a valuable tool to study the molecular basis, in terms of changes in regulatory pathways, of the cancer progression in subclass. Furthermore, these organoid models can be used for target identification as well as drug development for this specific subclass of PCa.

Progress Summary/Accomplishments

The Barbieri laboratory has already established and characterized organoids expressing SPOP^{F133V} mutation, which has been observed to enhance AR activity and PI3K/mTOR signaling. Herein, we also established organoid lines from the prostates of CHD1$^{f/f}$ (Pten wt) and SPOP^{F133V} mice, and mice with only CHD1$^{f/f}$ (Pten wt).

To express the respective deletion (CHD1$^{f/f}$) and/or mutation (SPOP^{F133V}), the cells obtained from these tissues were introduced with Cre through adenovirus (AdenoCre-RFP) that lacks its replication machinery. This strategy helped in generation of cell lines that had their internal control i.e., cells that were not introduced with Cre. Rather, these control cells were in infected with adenovirus lacking Cre (Adeno-RFP). Use of adenovirus provided us almost 100% efficiency in introducing Cre and therefore, respective mutations and/or deletions in these cell lines. Further, cells from control and Cre induced lines were- 1.) Confirmed to carry their respective deletion/mutation (Figure 2) and, 2.) Used to generate and maintain 2D as well as 3D organoids lines for these studies.

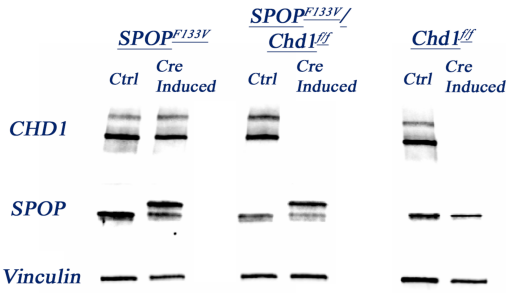

Figure 2: Generation of murine prostate cell line and organoid models via transduction with Adeno-RFP (Ctrl) or AdenoCre-RFP (Cre Induced).

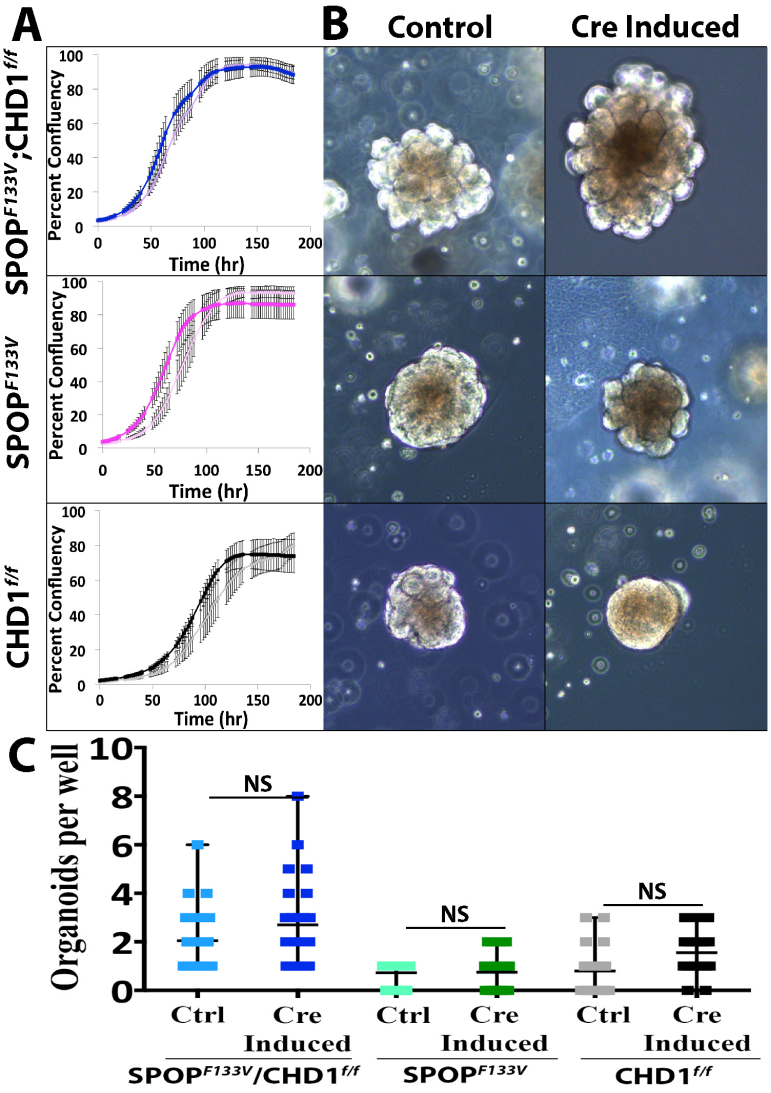

Figure 3: Growth rate of mouse prostate cell lines and organoids. (A.) Shows growth rate- comparing control and cre induced cells from each cell line in 2D/monolayer form. (B and C) Show organoids, and number of organoids, respectively, formed by each cell line (comparing control and cre induced cells).

The TCGA data suggests that both SPOP and CHD1 play a role of a tumor suppressor in prostate cancer, since mutation in former and deletion of latter occur in the cancer patients. Based on these findings, we hypothesized that mutation in SPOP or, loss of CHD1 or, a combination of both would promote cell growth. Therefore, we determined changes in growth rate of the abovementioned Cre induced cell lines with respect to their respective controls. The growth rate was studied in both 2D/monolayer form as well as 3D/organoid form.

In the monolayer form, we plated 1000 cells per well in a 96 well plate, for each cell line (in 12 technical replicates) and observed percent confluency in a well over a time period (200 hr) that represents the rate of growth of the respective cell line. We compared the control cells with Cre induced cells for each cell line. Based on these studies, we found no significant differences in the growth rate between the control and Cre induced cells (Figure 3A).

Further, to study the growth rate in 3D/organoid form, 2D cells (from each cell type) were plated in matrigel to have 10 cells in 40ul of matrigel aliquot per well in a 96 well plate. These cells were then incubated for 7 days, to promote organoid growth, prior to obtaining readout. The readout for these experiments was the number of organoids grown per well (in 6 technical replicates), for each (control and Cre induced) cell/organoid line. For this study also, we compared the control cells with Cre induced cells for each cell line. We found no significant differences in the number of organoids grown between the control and Cre induced cells (Figure 3B-C).

Although, these studies do not support the tumor suppressor role of SPOP and CHD1, we speculate that the observed indifference could be due to media conditions that promote cell growth in primary cell cultures. This could perhaps obscure the ability of SPOP mutant and/or CHD1 deleted cells to survive better than their controls.

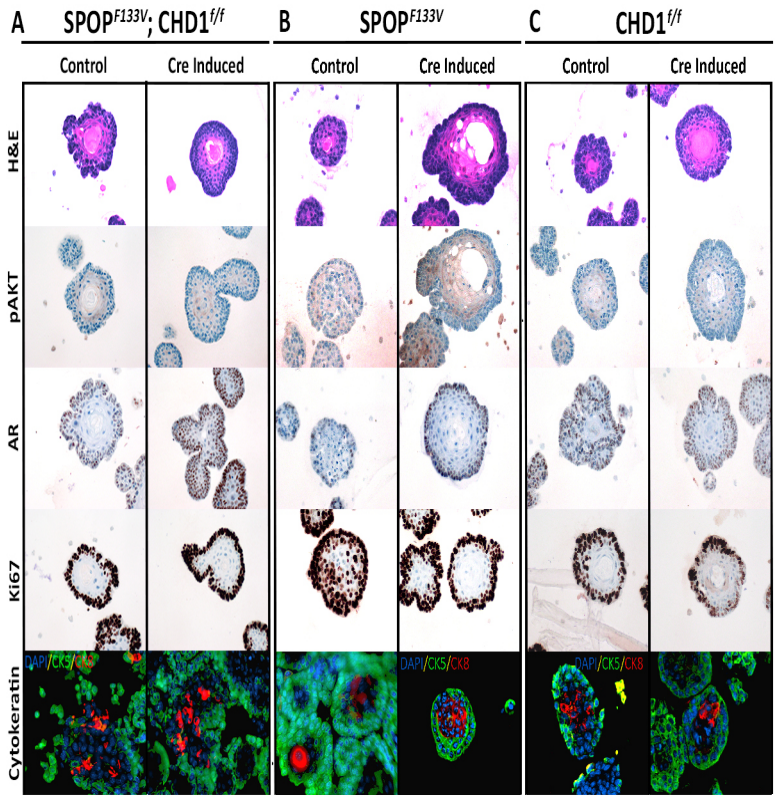

Figure 4: CHD1 cooperates with SPOP mutation in modulating AR expression. (A-C) show comparison between control and Cre Induced organoids for- H&E staining (first row), IHC for phosphor-Akt (pAkt), AR, and Ki67 (2nd, 3rd, and 4th row) and Immunofluorescence (IF) for CK5 and CK8 (last row), in 3 different oragnoid lines (SPOP^{F133V}; CHD1$^{f/f}$, SPOP^{F133V}, and CHD1$^{f/}$ respectively.

However, organoids recapitulated features of prostate histology, retained AR and demonstrated expression of CK5 and CK8 in basal and luminal cells respectively (Figure 4). It has been previously observed that the prostates from mouse containing SPOP^{F133V} mutations show high AR and pAKT expression. Although, increase expression of pAKT was not observed, higher AR expression was observed in Cre induced organoids containing SPOP^{F133V} mutation (Figure 4B). The higher expression of AR was also observed in organoids (cre induced) containing CHD1 loss along with SPOP^{F133V} mutation (Figure 4A). This suggests that the cells with loss of CHD1 and SPOP mutation maintain high AR expression as well as its activity.

Future Activities

We plan to study the changes in growth rate of each cell line by modulating the media conditions. We also plan to study that migration and/or invasion abilities of these cell lines to delineate the tumor suppressor role of SPOP and CHD1.

Aim 2: Define the importance of androgen receptor mediated signaling in *SPOPmut/CHD1null* prostate cells

Objective of Research

CHD1 plays a crucial role in activating transcriptional pathways by co-localizing with RNA Pol II. Therefore, its loss can induce transcriptional reprogramming in cells. Interestingly, deletion of CHD1 is largely restricted to prostate cancer. It is well known that the androgen receptor (AR) plays a dominant role in the pathogenesis of human PCa. The TCGA data reveals a higher AR score in SPOP mutant PCa. Additionally, CHD1, a chromatin remodeler protein, has been shown to localize with AR and influence AR-dependent transcription. It has also been observed that the SPOP mutant mouse model maintains high AR activity and increased expression of AR co-

activators, such as Ep300. Based on this information we hypothesize that- increased expression of AR and its co-activators in a SPOP mutant cell alters the cistrome of AR. This alteration of cistrome is further facilitated by CHD1 deletion because of its role as a chromatin modeler. This induces more aggressive disease phenotype in a cell by broadening the realm of AR target genes and diluting its dependency on conventional AR signaling.

Progress Summary/Accomplishments

To test this hypothesis, we first elucidated the dependency of CHD1 deleted prostate cells on androgens such as dihydrotestosterone (DHT), in the background of SPOP mutation using the organoid model systems developed in Aim 1. After determination of suitable conditions in which AR modulation can be studied, we plan to perform RNA-seq and ChIP-seq using the defined conditions to examine the alterations in the expression of AR targets and differences in the AR cistrome in the context of contributing to pathogenesis of this subclass. We elucidated the effect of androgen (DHT) by conducting experiments in organoid lines to determine changes in AR expression and activity under variable DHT conditions. The readouts were determined using western blot and qPCR for FKBP5 (one of the AR target genes). For these experiments, we initially plated 5000 cells per 50µl of matrigel aliquot in 1nM DHT. After incubation for 72 hrs, which was time for organoid formation from these cells, we changed the media under the following conditions for each organoid line: 1.) 1nM DHT and 2.) 0nM DHT in media for the duration of the experiment (6 hr, 12 hr, 24 hr, 48 hr, and 96 hr); 3.) DHT withdrawal for 72 hours followed by the re-addition of DHT for 24 hours at concentration of: 0.1nM, 1nM, 10nM and 100nM.

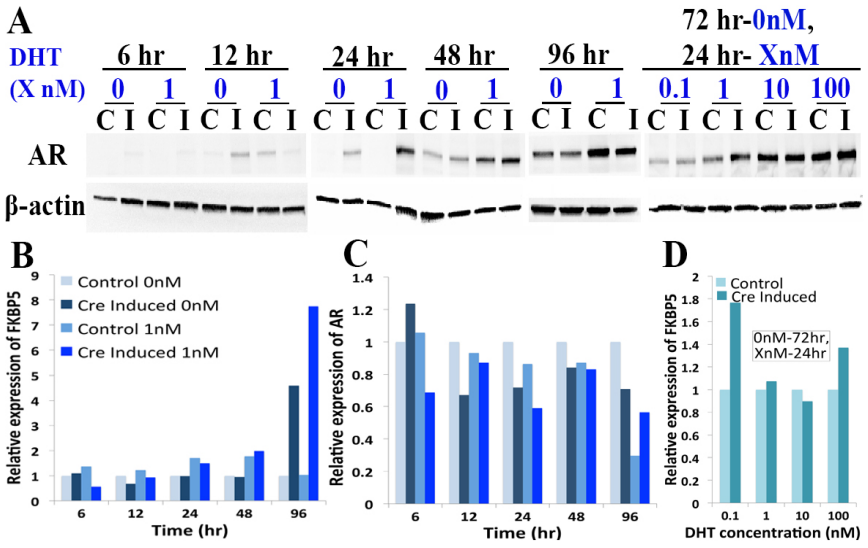

Figure 5: Dependence of SPOP^{F133V}; CHD1$^{f/f}$ organoid model systems on AR signaling. Panel (A.) shows changes in AR expression, with respect to time and DHT concentration (nM). Panel (B-D) show changes mRNA levels of FKBP5 (B and D) interpreted as AR activity and AR (C), with respect to time and DHT concentration (nM). C: control cells; I: Cre Induced cells.

We observed that in presence of 1nM DHT expression of androgen receptor (AR) increased with time, with the highest expression observed at 96 hr (Figure 5A). This increase in AR protein expression was not due to changes in mRNA levels (Figure 5C). Consequently, higher AR activity was observed in these organoids at the same time point as well (Figure 5B). AR expression was also observed to increase with time in 0nM DHT condition; however, the expression remained low compared to 1nM DHT case (Figure 5A). It was also observed that DHT withdrawal for 72 hr

followed by re-addition of DHT for 24 hr at different concentrations increased the AR expression and activity only starting at 10nM DHT (Figure 5A, 5D).
These results provide us with the information that by modulating concentration of DHT in media, induction or sensitivity to the AR changes at decent amounts under different conditions. Further, the pattern of induction changes between the variable DHT conditions implying that AR signaling plays an important role in each of these different contexts in these mouse prostate cells. In addition, it also helps us identify the parameters, in terms of DHT dose and time period of incubation, that can be used for RNA-seq.

Future Activities

We have experimentally validated (from above results) that 96 hours of DHT depletion to be optimal in displacing AR from the nucleus and reduce its activity. Therefore, we plan to perform RNA-seq on these organoids under following conditions- 1.) 1nM DHT for 96 hr, 2.) 0nM DHT for 96 hr, and 3.) DHT (withdrawal- 0nM) for 72 hr followed by re-addition at 10nM concentration for 24 hr. As CHD1 loss is hypothesized to affect global transcription, including many housekeeping genes and ribosomal RNAs that are commonly used for normalization in traditional gene expression analysis protocols, we will include spiked-in RNA standards to allow normalization to cell numbers and to adjust for experimental variations. Differentially expressed targets of importance will be validated by PCR in cell lines, organoids and mouse prostate tissue.
To complement RNA-seq studies and test our hypothesis underscoring reprogramming of AR targets, we will perform AR ChIP-seq in each of the cell/organoid lines. We will design the experiment to match the conditions mentioned above, i.e, 1nM DHT and 0nM DHT in the media for 96 hours. We expect these two conditions to provide necessary information about true AR targets in each genetic background while conserving resources. However, we will validate AR targets of interest by ChIP-PCR under all conditions tested for RNA-seq. Additionally, genomic loci with differential AR binding across cell lines will be validated in organoids.

Specific Aim 3: Investigate therapeutic potential of androgen deprivation and AR antagonism in *SPOPmut/CHD1null* prostate cancer and nominate targetable pathways.

Objective of Research

Androgen deprivation therapy and AR antagonism are standard approaches to the treatment of prostate cancer. Based on data generated in Specific Aim 2, we examined the dependence of SPOPmut/CHD1null tumors on the AR axis by inhibiting this pathway and examining resulting phenotypes. Further, we aim to explore alternate targetable pathways that are viable for drug testing.

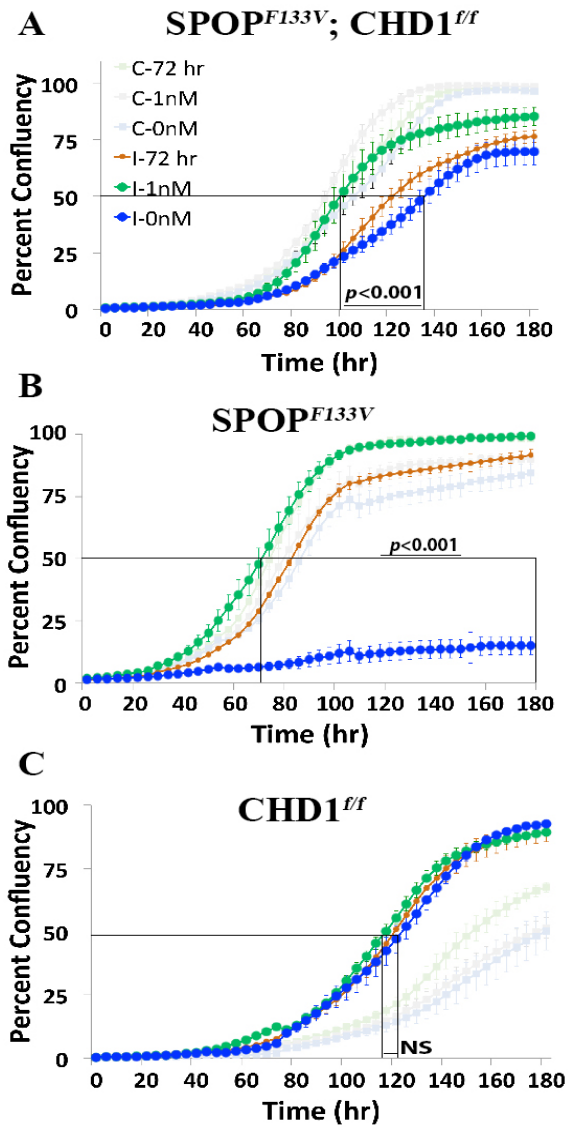

Figure 6: Growth response of SPOP mutant and/or CHD1 deleted cell lines to DHT. C: control cells; I: Cre Induced cells

Progress Summary/Accomplishments

From our abovementioned results (in Aim 2a), we observed that SPOP^{F133V}/CHD1null cells are dependent on the AR expression and signaling. Therefore, to determine its consequence in terms of a phenotypic affect, we determined whether androgen deprivation would effectively decrease cell viability. Using the aforementioned cell lines, we first modulated DHT concentrations in the media.

We plated 1000 cells/well in a 96 well plates and grew each cell line (control and Cre induced) under- 1.) 0nM DHT, 2.) 1nM DHT, and 3.) 0nM DHT for 72 hrs followed by 1nM DHT till total of 180 hrs. As readout, we measured percent confluency representing growth or proliferation of cells in a well.

We observed that the cell lines that carried SPOP mutation alone or in combination with CHD1 loss were highly sensitive to DHT (Figure 6A, 6B). These cell lines showed reduced growth or proliferation in absence of DHT (Figure 6A, 6B). In cells with SPOP^{F133V} only, under 0nM DHT conditions, the growth rate was extremely low with an inability to reach even 50% confluency, compared to cells under 1nM DHT that reached 50% confluency within ~70hrs (Figure 6B). Similarly, in cells carrying SPOP^{F133V} in combination with CHD1 loss, cells had slower growth rate under 0nM DHT compared to cells growth under 1nM DHT conditions (Figure 6A). However, the growth rate in this case under 0nM DHT conditions was slightly higher than in cells containing SPOP^{F133V} only (Figure 6A, 6B). In cells carrying SPOP^{F133V} in combination with CHD1 loss, 50% confluency was reached at ~100 hrs and ~138 hrs with 1nM and 0nM DHT respectively (Figure 6A).

In addition, the DHT dependence was observed in the (Cre induced) cells carrying SPOP mutation with or without CHD1 deletion but not in the control cells from the same cell lines (Figure 6A, 6B). This suggests that the sensitivity observed in these cases is exclusively due to presence of respective mutations and/or deletions. On the other hand, the cells containing only CHD1 loss were insensitive to DHT (Figure 6C). Together, these results suggest that cells containing SPOP mutation are highly depended on AR mediated signaling for their growth. Further, these results

also suggest that loss of CHD1 in SPOP mutant cells makes them slightly insensitive to DHT or perhaps dilutes its dependency on conventional AR signaling.

Future Activities

From the above results, we can conclude that SPOP^{F133V}/CHD1null cells are dependent on the AR expression and signaling. Therefore, similar to androgen deprivation, use of AR antagonists such as bicalutamide, and enzalutamide, could effectively decrease cell viability as well. We aim to study the effect of these drugs using the same cell lines, under various dose concentrations of bicalutamide and enzalutamide. Readouts will include cell proliferation and survival assays, as well AR target gene expression by PCR. These results can be further validated in xenograft mouse models. The Barbieri laboratory has successfully developed protocols for grafting SPOP^{F133V}; Pten$^{f/f}$ organoids subcutaneously into immunocompromised mice, providing an *in vivo* platform for mechanistic and preclinical therapeutic studies. Additionally, castration of host mice results in regression of SPOP^{F133V}; Pten$^{f/f}$ grafts, it provides a critical rationale for exploration of AR signaling inhibition and sensitivity in these models. As readout, we aim to track tumor volumes using SPOP^{F133V}; CHD1$^{f/f}$ organoid grafts in response to AR antagonism. This will ensure increased clinical success of these therapies within this subclass.

Professional Development

Scientific Presentation:

May 2018 Belfer Basic Research Working Group (BBRWG) **'Characterization of combined SPOP mutant and CHD1 deleted subtype of prostate cancer'**, New York, NY.

Dissemination of Results: Nothing to report

Impact

Impact on Principle Discipline: Prostate cancer (PCa) is a clinically heterogeneous disease with distinct molecular features. SPOP mutation and CHD1 loss characterize 10-15% of primary prostate cancers. Of the estimated 164,690 cases of PCa that will be detected in 2018, roughly 20,000 men will carry these genetic alterations. There is a significant tendency for CHD1 deletions to co-occur with SPOP mutations in primary prostate cancer. While SPOP mutations are clonal, CHD1 loss represents a subclonal event; therefore, SPOP mutations occur prior to CHD1 loss during PCa development. The overarching goal of this project is to improve our understanding of the collaboration between CHD1 loss and SPOP mutation in driving pathogenesis of this PCa subclass, test changes in androgen receptor (AR) signaling and define the efficiency of androgen deprivation or AR antagonistic therapies in this PCa subtype.

These studies will enable us to understand the biology driving tumorigenesis within this subclass as well as elucidate response/resistance of these cells to conventional pharmacological therapies. This project will also allow us to identify deregulated pathways with potential for employing targeted therapies. In addition, information about specific deregulated pathways could be used to detect and quantify aberrant changes in the expression of key participating proteins and therefore, biomarker development. These strategies can be translated to the clinic to deliver efficient therapies with the highest likelihood of success in reducing lethality associated with this PCa subclass. Taken together, this project presents a significant discovery opportunity to uncover seminal data that would contribute greatly to the advancement of precision medicine in PCa, with the ultimate goal of improving patient outcomes in this subclass.

Impact on other Disciplines: Nothing to report

Impact on Technology Transfer: Nothing to report

Impact Beyond Science and Technology: Nothing to report

Changes/Challenges

Changes in approach: Nothing to report

Actual or Anticipated problems:

Aim 2: Define the importance of androgen receptor mediated signaling in *SPOPmut/CHD1null* prostate cells

From the CHIP-seq data, we plan to bioinformatically deduce differentially expressed AR target genes in each genetic background and correlate this with differences in AR binding. In the background of SPOP mutation, we expect CHD1 loss to alter AR binding across the prostate, cause hyper-activation of certain AR targets and contribute to enhanced cell survival and growth. The underlying hypothesis is that SPOP mutation enhances prostate cell dependence on AR signaling, which is further reinforced by CHD1 loss. It is also possible that CHD1 loss reprograms other transcription factors, and not AR, to promote tumorigenesis. Therefore, we will also elucidate other differentially expressed pathways in the genetic backgrounds tested during RNA-seq, as well as perform network analysis and GSEA to identify signaling cascades and transcriptional programs contributing to tumor development. Additional pathways will be validated using suitable assay readouts (PCR, activation of key proteins by western blotting or immunohistochemistry) in organoids and mouse prostates. We will also supplement each of these results with CHD1 ChIP-seq data being generated in our laboratory as part of a related project. Together, these experiments will pinpoint molecular pathways that drive PCa development when CHD1 loss coordinates with SPOP

mutation. Completion of these studies will also shed light on why CHD1 loss by itself is unfavorable for prostate cell survival, and therefore, co-exists with SPOP mutation.

Changes in expenditure, use of animals, or biohazards: Nothing to report

Products: Nothing to report

Participants and other Collaborating Organizations:

Name:	Kaveri Arora
Project Role:	PI
Nearest person months worked	12
Contribution to Project	**Designed, performed, and analyze all experiments for this project**
Funding Support:	**Department of Defense Post-Doctoral Fellowship Award PC160937**

Changes in personnel: Nothing to report

Other Organization Involved: Nothing to report