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14. ABSTRACT Approximately half of all prostate cancers are known to harbor the TMPRSS2-ERG translocation resulting in aberrant ERG expression that contributes to prostate tumorigenesis. Despite being an attractive therapeutic target, transcription factors such as ERG have been historically difficult targets for drug development. To address these challenges, we developed a method to measure gene expression patterns in a high throughput format and generated a gene "signature" that differentiates between cells that have active TMPRSS2-ERG activity versus cells in which its activity is suppressed. Using this technique we measured the effect of inhibiting 800 kinases by RNAi on ERG activity in prostate cancer cells to identify kinases that modulate ERG activity. To identify novel small molecules that directly bind to an inhibit ERG activity we tested 100,000 compounds using a recently developed technique of drug screening called small molecule microarrays. The identified compounds were subsequently tested in our gene signature assay to discover novel compounds that inhibit ERG activity in prostate cancer cells. We have also used our gene expression method to test a panel of commercially available and FDA approved drugs and identified multiple drugs that inhibit ERG activity.					
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INTRODUCTION

Approximately half of all prostate cancers harbor a translocation between the transcription factor ERG and the androgen regulated gene TMPRSS2 that results in aberrant ERG expression. Several lines of evidence demonstrate a causal role for TMPRSS2-ERG in tumorigenesis making it an attractive therapeutic target, however, the mechanism remains unclear making it difficult to target its function. Furthermore, despite their clear role in cancer, transcription factors have been historically difficult to target pharmacologically with small molecules. To address these problems, we used a gene expression signature readout for TMPRSS2-ERG that allows us to measure ERG activity even without a detailed mechanistic understanding of the molecular pathways involved in ERG mediated oncogenesis. Using this method we identified novel chemical and genetic modulators of ERG that will provide mechanistic insight into ERG mediated tumorigenesis as well as potential leads for novel therapeutics.

KEYWORDS

Prostate cancer, ERG, gene expression, high throughput screening, small molecule microarray, genetic screen, VCAP

ACCOMPLISHMENTS

What were the major goals of the project?

Task 1. Genetic approach to identify kinases essential for TMPRSS2-ERG activity in prostate cancer cells (months 1-28)

- 1a. Generate and titer lentiviruses expressing shRNAs targeting candidate kinases identified from preliminary results for retesting in secondary assays (months 1-3 – completed December 2013)
- 1b. Measure effect of suppressing candidate kinases on proliferation of cell lines that are positive or negative for TMPRSS2-ERG using viability and BrdU incorporation assays (months 4-9 – completed June 2014)
- 1c. Measure effect of suppressing candidate kinases on transcription of ERG and ERG targets by quantitative PCR (months 10-12 – completed October 2104)
- 1d. For kinases that affect either proliferation or transcription from 1b and 1c, measure effect on invasion using transwell invasion assay, epithelial to mesenchymal transitions (EMT) using immunofluorescence and immunoblotting, and transformation by anchorage independent growth in soft agar (months 13-24 – 75% completed)
- 1e. Bioinformatic analysis of results correlating gene expression changes with different functional outputs critical for ERG function using data mining techniques such as hierarchical, k-means, and consensus clustering (months 25-28 – not started)

Task 2. Test small molecule inhibitors that target candidate kinases identified from genome wide kinase suppression screen (months 1-18)

- 2a. Identify and collect compounds that inhibit candidate kinases identified from genome wide kinome screen (month 1 – completed October 2013)
- 2b. Measure effect of compounds on proliferation of cell lines that are positive or negative for TMPRSS2-ERG using viability and BrdU incorporation assays (months 2-4 – completed January 2014)
- 2c. Measure effect of compounds on transcription of ERG and ERG targets by quantitative PCR (months 5-7 – completed April 2014)

- 2d. For compounds that affect either proliferation or transcription from 2b and 2c, measure effect on invasion using transwell invasion assay, EMT using immunofluorescence and immunoblotting, and transformation by anchorage independent growth in soft agar (months 8-18 – 75% completed)

Task 3. Identify compounds that bind to ERG using small molecule microarrays (SMM) with lysates overexpressing ERG (months 1-12)

- 3a. Request compounds from compound management that scored from preliminary SMM screen using 293T lysates overexpressing ERG (months 1-2 – completed November 2013)
- 3b. Reprint slides with compounds from 3a for counter SMM assays (months 3-4 – completed December 2013)
- 3c. Perform counter SMM assay with 293T lysates expressing unrelated protein (months 5-10 – completed May 2014)
- 3d. Request compounds from compound management that pass counter assays from 3c for secondary assays (months 11-12 – completed June 2014)

Task 4. Identify compounds that bind to ERG using small molecule microarrays with purified ERG protein (months 1-24)

- 4a. Express and purify functional ERG protein from mammalian cells for SMM assay using immunoaffinity tag (months 1-6 – completed March 2014)
- 4b. Perform SMM screen of 84,000 compounds using purified ERG protein (months 7-12 – completed June 2014)
- 4c. Request compounds from compound management that scored from 4b (months 13-14 – completed July 2014)
- 4d. Reprint slides from 4c for counter SMM assays (months 15-16 – completed September 2014)
- 4e. Perform counter SMM assays with unrelated protein and antibody control (months 17-22 – completed December 2014)
- 4f. Request compounds from compound management that pass counter assays from 4e for secondary assays (months 23-24 – February 2015)

Task 5. Measure gene expression signature of candidate SMM compounds (months 25-30)

- 5a. Perform L1000 gene signature assay on compounds identified from Task 3 and Task 4 at concentrations of 10 uM and 30 uM (months 25-27 – completed June 2015)
- 5b. Perform dose curves with L1000 on compounds that modulate the gene signature from 5a (months 28-30 – completed August 2015)

Task 6. Functional assays with compounds that bind to ERG and modulate gene signature (months 31-48)

- 6a. Perform cytotoxicity assay to eliminate nonspecific toxic drugs (months 31- 32 – completed August 2015)
- 6b. Perform proliferation assays in cell lines that are positive or negative for TMPRSS2-ERG (months 33-35 – completed August 2015)
- 6c. Perform ERG dependent differentiation assays using high throughput flow cytometry (months 36-38, not started)
- 6d. Measure transcription of ERG targets by quantitative PCR (months 39-42, 75% completed)
- 6e. For compounds that affect either proliferation, differentiation, or transcription from 6a, 6b, or 6c, measure invasion using transwell invasion assay, EMT using immunofluorescence and immunoblotting, and transformation by anchorage independent growth in soft agar (months 43-48, 10% completed)

Task 7. Biophysical assays with compounds that bind to ERG and modulate gene signature (months 25-42)

- 7a. Scale up protein production and purification for biophysical assays (months 25-30, completed June 2015)
- 7b. Assay development for thermal shift assay with purified ERG protein (months 31-36, 75% completed)

- 7b. Assay development for surface Plasmon resonance with purified ERG protein (months 31-36, 75% completed)
- 7c. Perform thermal shift and surface Plasmon resonance on compounds and determine binding constants (months 37-42, not started)

Task 8. Identify FDA approved drugs that modulate TMPRSS2-ERG gene signature (months 1-24)

- 8a. Perform L1000 gene expression assay on panel of 1800 FDA approved drugs in ERG positive or ERG negative cell lines (months 1-6, completed December 2013)
- 8b. Rearray drugs from 8a and perform dose curve with L1000 gene expression assay in cell lines that are positive or negative for ERG to identify drugs that inhibit ERG activity (months 7-12, completed June 2014)
- 8c. Measure effect of drugs from 8b on proliferation of cell lines that are positive or negative for TMPRSS2-ERG using viability assay and BrdU incorporation (months 13-15, completed June 2014)
- 8d. Measure effect of drugs from 8b on transcription of ERG targets by quantitative PCR (months 16-18, completed December 2014)
- 8e. For compounds that affect either proliferation or transcription from 8c or 8d, measure effect on invasion using transwell invasion assay, EMT using immunofluorescence and immunoblotting, and transformation by anchorage independent growth in soft agar (months 19-24, 75% completed)

What was accomplished under these goals?

Specific Aim 1 - Genetic approach to identify kinases essential for TMPRSS2-ERG activity in prostate cancer cells.

1) Major activities:

We validated the leads that were identified from the gene expression high throughput screen of shRNAs targeting 808 kinases using quantitative PCR. We also assessed their activity in cell based assays mediating ERG dependent viability.

2) Specific objectives

- Collect and express shRNAs using lentivirus for validation studies
- Perform QPCR after suppression of kinases by shRNA to confirm screen results
- Measure cell viability after suppression of candidate kinases

3) Results and conclusions/other achievements

As discussed in the previous progress report, we defined an ERG gene expression signature using a translocation positive VCAP prostate cell line in which ERG expression was suppressed by RNAi. The ERG gene signature served as our readout for ERG activity and was profiled in a high throughput bead based platform referred to as L1000. The gene signature was also vetted using prostate tumor data and we demonstrated that the gene signature is enriched in TMPRSS2-ERG positive prostate tumors, suggesting that we are capturing biology relevant to human disease. We screened a library of shRNAs targeting 808 kinases using the gene expression high throughput assay and at the conclusion of the last progress report we had identified 34 kinases for further study.

We validated the kinases identified by the query using quantitative PCR. We measured the expression of two genes regulated by ERG, ARGHDIB and PLA1A, after knockdown of each candidate kinase using 3 different shRNAs per kinase. In order to control for off target effects of shRNAs we required 2 out of the 3 shRNAs to downregulate ARGHDIB and PLA1A. Using this criterion, we identified 12 kinases as candidates that modulate ERG signaling (Figure 1).

Gene	shRNA ID	PLA1A QPCR	ARHGDIB QPCR
ZAK	3266	0.12	0.19
ZAK	3268	0.6	0.43
ZAK	194822	0.63	0.45
SGK1	196562	0.36	0.36
SGK1	40174	0.41	0.27
ADCK3	21508	0.49	0.69
ADCK3	21506	0.28	0.43
MAPK14	511	0.37	0.22
MAPK14	10051	0.32	0.51
PIK3R3	197032	0.61	0.64
PIK3R3	33290	0.58	0.49
PIK3R3	33289	0.7	0.48
MAP2K5	197077	0.41	0.48
MAP2K5	1466	1.4	1.8
TPR	60065	0.48	0.23
TPR	60066	0.51	0.69
TPR	60064	0.69	0.64
DYRK1A	2143	0.41	0.28
DYRK1A	10613	0.58	0.26
DGKI	194955	0.42	0.44
DGKI	195422	0.51	0.66
RIOK2	37504	0.72	0.42
RIOK2	37507	0.4	0.45
PRKCZ	10114	0.32	0.33
PRKCZ	10120	0.69	0.6
BRSK2	737	0.48	0.27
BRSK2	739	0.96	0.42

Figure 1: Validation of kinases from shRNA kinome screen.

VCaP cells were transduced with shRNAs targeting the indicated kinases. RNA was isolated and transcription of ERG targets PLA1A and ARHGDIB were measured by quantitative PCR using the delta delta Ct method. Values represent fraction relative to transduction with control shRNAs.

We were particularly interested to discover that protein kinase C (PKC) scored because in parallel (aim 3) we discovered that PKC inhibitors (PKCi's) could modulate the ERG signature. When we suppressed PKC with shRNAs, we discovered that ERG transcription was decreased (Figure 2).

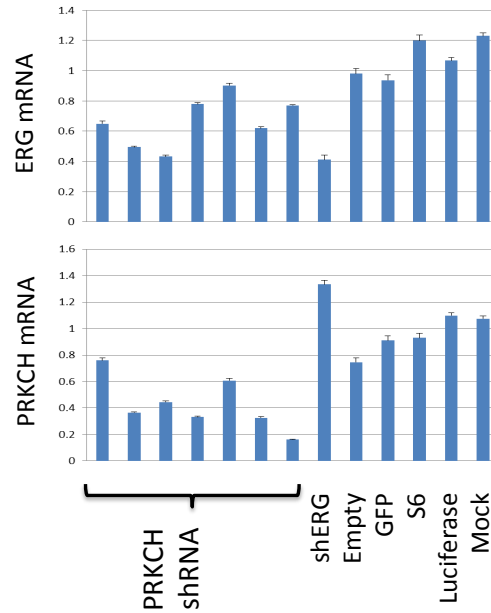


Figure 2: Suppression of PKC eta decreases ERG transcription.

VCAP cells were transduced with 8 different shRNAs targeting PKC eta (PRKCH shRNA) or control shRNAs. RNA was harvested and transcription measured by quantitative PCR using the delta delta Ct method. Results represent relative expression to average of negative control shRNAs. Top figure demonstrates decrease of ERG mRNA by shRNAs targeting PRKCH. Bottom figure confirms suppression of PRKCH using shRNAs targeting different regions of PRKCH.

Since TMPRSS2-ERG transcription is driven by the androgen dependent TMPRSS2 promoter, we tested whether suppression of PKC affects the activity of AR. Indeed, we found AR targets such as PSA were also decreased (Figure 3).

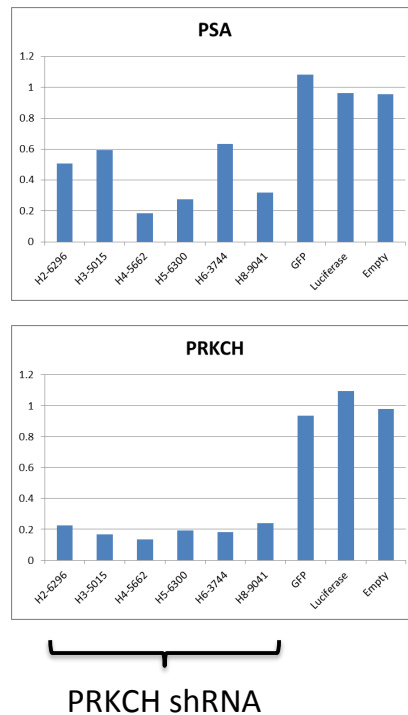


Figure 3: Suppression of PKC eta decreases PSA transcription.

Cells were transduced with 8 different shRNAs targeting PKC eta (PRKCH shRNA) or control shRNAs. RNA was harvested and transcription measured by quantitative PCR using the delta delta Ct method. Results represent relative expression to average of negative control shRNAs. Top figure demonstrates decrease of PSA mRNA by shRNAs targeting PRKCH. Bottom figure confirms suppression of PRKCH using shRNAs targeting different regions of PRKCH.

In addition to identifying a pathway potentially amenable to therapeutic targeting, these results validate our gene expression signature strategy to identify modulators of ERG activity and currently we are validating the additional kinases that scored in our RNAi screen.

Specific Aim 2 - Chemical approach to identify small molecules that directly bind to and inhibit TMPRSS2-ERG activity.

1) Major activities

We purified sufficient amounts of recombinant ERG protein to perform biophysical assays. We developed a thermal shift and surface plasmon resonance assay to measure direct ERG binding to small molecules. We further tested candidate compounds in the gene expression assay in prostate cancer cells.

2) Specific objectives

- Develop and validate biophysical assays including thermal shift and surface plasmon resonance to measure direct binding of ERG to candidate compounds identified from SMM screen
- Assess activity in cells by using gene expression profiles of candidate compounds at different doses.

3) Results and conclusions/achievements

At the conclusion of the last progress report we had identified 182 compounds from a small molecule microarray screen using ERG from cell lysates or purified ERG. We had begun efforts to develop biophysical assays to measure ERG binding as well as perform gene expression assays using the compounds at different doses.

In order to perform biophysical assays we need larger quantities of protein compared to the amount we generated using 293T cells for screening by SMMs. As described in our last progress report. We were able to identify a nearly full length construct that could be expressed at high levels in bacteria. We used an N-terminal histidine tag to purify ERG expressed in E. coli with Ni-NTA agarose beads. The protein was eluted using imidazole and buffer exchanged using standard methods. This protein was used in a thermal shift assay by combining protein with fluorescent dye in the presence or absence of compound. As the temperature is increased the dye is able to bind to hydrophobic residues of the unfolded protein which increases the fluorescent signal. We used DNA as our positive control as ERG contains a DNA binding domain. We also used a second method to validating binding referred to surface plasmon resonance (SPR). In both thermal shift and SPR we used DNA as our positive control as ERG contains a DNA binding domain. After optimization of buffer conditions we were able to measure binding affinities using DNA as the positive controls (Figure 4).

Figure 1 consists of two vertically stacked plots. The top plot shows the raw ITC data as a series of heat flow spikes (μcal/sec) over time (min) from 0 to 80. The baseline is around 0.00 μcal/sec, with a significant increase in heat flow starting around 30 minutes. The bottom plot shows the integrated heat per injection (kcal mol⁻¹ of injectant) versus the molar ratio from 0.0 to 2.0. The data points are black squares, and a red sigmoidal fit curve is overlaid. The fit curve starts at approximately -2.5 kcal mol⁻¹ at a molar ratio of 0.5 and rises to 0.0 kcal mol⁻¹ at a molar ratio of 1.0. A text box in the bottom right corner of the plot provides the following data: Date: 10/02/12, 12C12N; Stock: 100 μM; [12C12N] = 1.000E-04; n: 0.541; ΔH: 5475 J/mol; K: 7.49E5 ± 1.00E+05; pH: 8.00; ΔH: 110.4 kJ/mol; ΔG: 23.0 kJ/mol.

In parallel with the biophysical assays we tested these compounds in our gene expression signature assay in VCAP cells at different doses of drug. We hypothesized that true hits would show a dose dependent change in gene signature. We used our novel high throughput gene expression assay and tested the compounds at 8 different doses. We determined a score for the gene signature in order to quantify the effect on gene expression at each concentration of drug. Based on these results, we selected 16 compounds for further testing by quantitative PCR. Interestingly 2 compounds affected ERG targets in a dose dependent manner with one compound having an IC₅₀ value in the low micromolar range. (Figure 5a). Preliminary experiments also demonstrated that this novel compound inhibits the proliferation of VCAP cells as predicted if ERG activity is suppressed (Figure 5b).

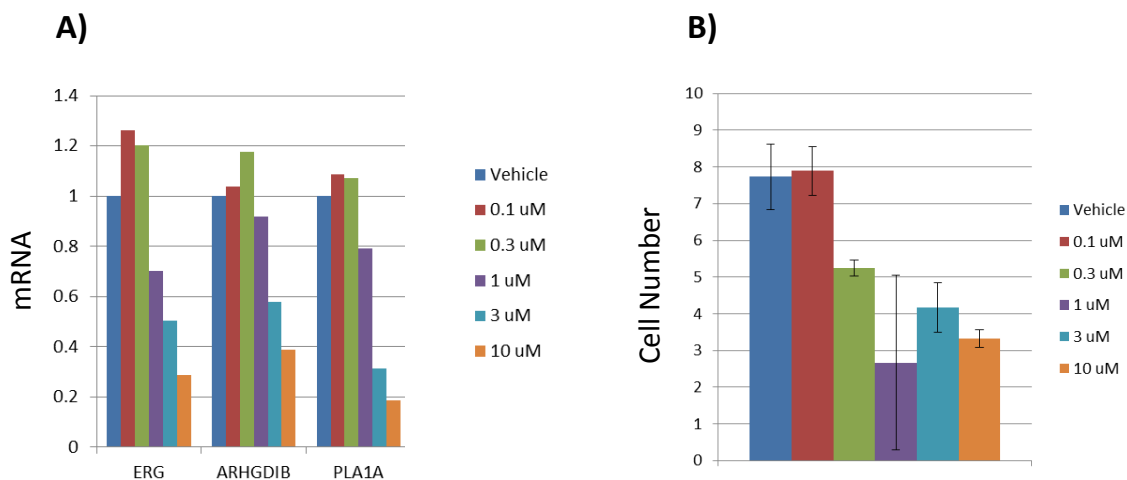


Figure 5: Novel small molecule inhibits ERG activity and proliferation of VCAP cells.

A) VCAP cells were treated with increasing concentrations of novel small molecule for 24 hours. RNA was harvested and transcription measured by quantitative PCR. ERG and two downstream targets, ARHGDIB and PLA1A, are inhibited by the novel compound. B) VCAP cells were treated with increasing concentrations of the small molecule and cell number measured 5 days later using Vi-Cell counter.

Specific Aim 3. Repurpose FDA approved drugs that inhibit TMPRSS2-ERG activity.

1) Major Activities

We validated the effect of PKCi using cell based assays. We are attempting to determine the mechanism by which PKCi inhibit ERG activity.

2) Specific objectives

- Validate PKC as modulating ERG activity
- Determine the mechanism by which PKC inhibitors inhibit ERG activity

3) Results and conclusions/achievements

As discussed in the last progress report, we screened approximately 1800 compounds with known molecular mechanisms in order to identify novel ERG modulators and potentially repurpose drugs that are either FDA approved or commercially available. At the conclusion of our report we generated 10,000 profiles, and discovered that treatment with several PKC inhibitors modulated ERG activity. We confirmed that the PKC inhibitors identified inhibit transcription of ERG in VCaP cells and decreased proliferation of VCaP cells versus an ERG negative cell line.

Since the androgen regulated TMPRSS2 promoter drives ERG expression we tested whether PKC inhibitors affect AR signaling. Interestingly, we found that they inhibited the upregulation of AR regulated genes including TMPRSS2 (Figure 6).

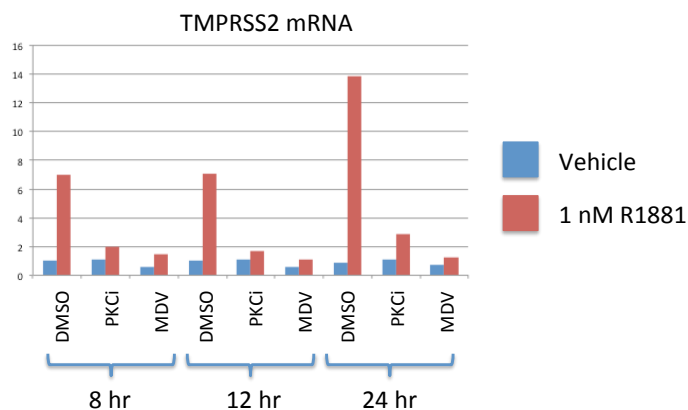


Figure 6: PKCi inhibits upregulation of TMPRSS2 by androgen.

TMPRSS2 mRNA was measured by quantitative PCR at the indicated time points after treatment with PKCi in the absence or presence of the synthetic androgen R1881.

MDV3100 was used as a positive control.

The mechanism of inhibition differs from MDV3100 which is the most potent antiandrogen currently used clinically. Whereas the inhibition of AR activity by MDV3100 can be overcome with excess androgen, the effect of PKC inhibition on AR activity is unaffected by increasing concentrations of androgen (Figure 7).

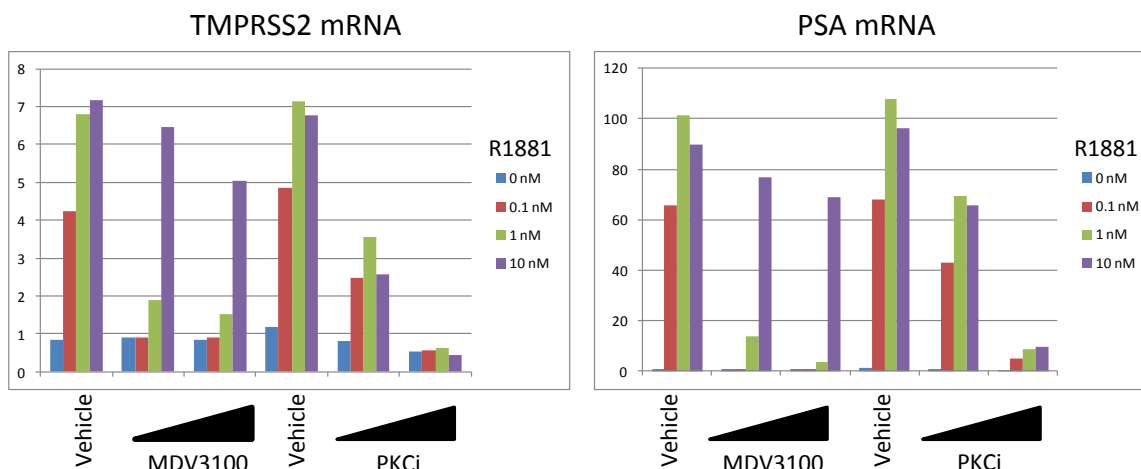


Figure 7: PKC inhibition cannot be overcome with increasing concentrations of androgen.

TMPRSS2 and PSA transcription was measured by quantitative PCR after treatment with increasing concentrations of MDV3100 or PKCi in the absence or presence of increasing concentrations of the synthetic androgen R1881.

These results provide rationale for potentially combining MDV3100 and PKC inhibitors as they inhibit AR by two different mechanisms and may prevent development of resistance.

In order to further elucidate the mechanism of PKC inhibition on androgen upregulation of ERG, we intend to perform AR chromatin immunoprecipitation followed by next generation sequencing to determine if AR binding is affected by PKC inhibition.

What opportunities for training and professional development has the project provided?

I continued to gain clinical experience in genitourinary oncology where 10% of my effort was dedicated to clinical work. Over the past year I participated in clinical research meetings, a subset of which were dedicated to design of new trials. I experienced first hand how trials are initially designed based on preclinical data. In addition I observed the importance of having clinicians with basic research experience in designing different research portions of a trial, such as collection of samples for

molecular analyses. Through these meetings and working alongside other clinical researches I formulated relationships that will be valuable for my future career in translating observations in the laboratory to meaningful treatments for patients.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Specific Aim 1 - Genetic approach to identify kinases essential for TMPRSS2-ERG activity in prostate cancer cells.

We will complete validation candidate kinases that modulate the ERG signature using shRNA and new CRISPR technology as an orthogonal approach.

Specific Aim 2 - Chemical approach to identify small molecules that directly bind to and inhibit TMPRSS2-ERG activity.

We will complete the thermal shift and SPR assays on the SMM candidates. We will obtain analogues of compounds that show physical binding and/or are confirmed to modulate the ERG signature in order to test for stereospecificity as well as assess the chemical substituents required for activity. We will prioritize those compounds that show stereospecificity for proteomic study. Those compounds will be coupled to beads and used in pulldown experiments to identify the molecular target.

Specific Aim 3. Repurpose FDA approved drugs that inhibit TMPRSS2-ERG activity.

We will perform ChIP-seq of AR in the presence of PKC inhibitors to elucidate the mechanism by PKCi's inhibit AR dependent transcription of ERG.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

About half of all prostate cancers are known to harbor a genetic mutation that fuses a gene known as ERG to the regulatory region of the gene TMPRSS2. The TMPRSS2-ERG fusion results in ERG becoming aberrantly activated in prostate cells, which contributes to the development of cancer. However, despite being an attractive and logical therapeutic target, there are currently no drugs that target ERG activity. ERG belongs to a group of proteins known as transcription factors, which have been historically difficult for drug development because they lack the well characterized active sites of enzymes in which to fit small molecule inhibitors. To address these challenges, we developed a method to measure gene expression patterns in a high throughput format and generated a gene “signature” that differentiates between cells that have active TMPRSS2-ERG activity versus cells in which its activity is suppressed. By using a gene signature as a surrogate for biological activity, we have developed an accurate readout for TMPRSS2-ERG activity. Using this method, we can then use novel techniques in genetics and chemistry to target TMPRSS2- ERG in prostate cancer.

We inhibited the activity of 800 kinases, molecules commonly involved in signaling pathways, and measured its effect on our ERG signature. Preliminary results have identified approximately 12 kinases for further follow up. Unlike transcription factors, kinases are more amenable to drug development as they have well defined activities and active sites. We are currently testing the role of these kinases in regulating ERG activity both to understand ERG biology and as leads for potential therapeutics. To identify novel small molecules that directly bind to and inhibit ERG activity, we tested 100,000 compounds using a recently developed technique of drug screening called small molecule microarrays. In order to validate these compounds we developed a method to synthesize large quantities of the ERG protein in bacteria, which can then be used in binding assays. We have successfully developed two binding assays for this purpose: thermal shift and surface plasmon resonance. Using these two assays in combination with our ERG signature assay we are attempting to confirm and optimize small molecules that could serve as leads for the development of a novel class of drugs for prostate cancer.

The process of transitioning a drug from the laboratory to FDA approval is a long and costly process typically taking years and costing an estimated \$1.8 billion per drug. Therefore there has been great interest in repurposing approved drugs for new indications. We have assembled and tested a panel of FDA approved drugs in our gene signature assay and identified multiple drugs that can modulate the ERG signature. We discovered that PKC inhibitors, which are commercially available and have been used in clinical trials modulates the ERG signature. We are currently attempting to identify the molecular mechanism by which PKC inhibitors exert its activity.

What was the impact on other disciplines?

Our method can take advantage of the growing body of gene expression data obtained in the laboratory as well as from human tissue. Using our strategy, any biologically relevant gene signature could be used to identify either genes or drugs that modify a particular phenotype. As genome sequencing has begun to identify putative targets, genome scale functional methods will be needed to advance targets into drugs.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS:

Changes in approach and reasons for change

In aim 1, we will plan to validate hits using CRISPR-Cas9 technology as an orthogonal system. The CRISPR-Cas9 system was not available at the time of the original proposal but has proven to be a powerful genetic method to study gene function.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

PRODUCTS

Publications, conference papers, and presentations

Nothing to report.

Journal publications

Nothing to report.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

Websites or other internet sites

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other products

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	<i>David Takeda</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>PI</i>
Nearest person month worked:	<i>12</i>
Contribution to Project:	<i>Performed experimental work and data analysis.</i>
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Organization Name: Broad Institute

Location of Organization: Cambridge, MA

Partner's contribution to the project (identify one or more)

- Facilities: RNAi screening libraries, chemical screening libraries, screening platform
- Data analysis: Computational support with data analysis, chemical biology with assistance in medicinal chemistry

SPECIAL REPORTING REQUIREMENTS

Collaborative Awards:

Nothing to report

Quad Charts:

Nothing to report

APPENDICES

Nothing to report