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PRINCIPAL INVESTIGATOR: David Takeda

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston, MA 02215

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David Takeda

E-Mail: david_takeda@dfci.harvard.edu

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Dana-Farber Cancer Institute
450 Brookline Ave
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14. ABSTRACT

TMPRSS2-ERG is an oncogenic translocation present in approximately half of all prostate cancers that contributes to tumorigenesis. Despite being an attractive therapeutic target, transcription factors have historically been difficult to target pharmacologically. Furthermore, the exact role of ERG in mediating tumorigenesis is unknown making it difficult to develop physiologically relevant assays to measure its activity. To address these challenges, we developed a gene expression signature for ERG activity that provides a readout for ERG activity. Using a novel bead based assay to measure gene expression in a high throughput format, we used the ERG gene signature to screen a RNAi kinome library to identify kinases that modulate ERG activity. To identify novel small molecules that directly bind to and inhibit ERG, we tested 100,000 compounds using small molecule microarrays. The identified compounds were subsequently tested using our gene signature assay to discover novel compounds that inhibit ERG activity. Using our high throughput gene expression screening method, we identified a novel small molecule that inhibits the proliferation of prostate cancer cells with an ERG translocation. We identified the cellular target of the small molecule as p300/CBP and demonstrated that it functions as a novel inhibitor of p300/CBP enzymatic activity.

15. SUBJECT TERMS
prostate cancer, TMPRSS2-ERG, ERG, ETS, gene expression, high-throughput screening, small molecule microarray, functional genomics

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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. As a result, ERG is expressed at high levels in the prostate where it is not normally expressed. Several mouse models indicate a causal role in the development of prostate cancer, however, the exact role of TMPRSS2-ERG in tumorigenesis is unclear, making it difficult to design assays to target its function therapeutically. In addition, transcription factors have been historically difficult to target pharmacologically with small molecules. To address these problems, we used a gene expression signature as a readout of ERG activity allowing us to measure activity even without a detailed mechanistic understanding of ERG mediated oncogenesis. We used a novel method to measure gene expression in a high throughput format to screen shRNAs and small molecules in prostate cancer cells for perturbations that would modulate the ERG signature. These results will provide new insights into ERG function as well as potential leads for therapeutic intervention.

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 – completed June 2017)

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 – completed June 2016)

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 – completed January 2017)

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, 25% completed)

6d. Measure transcription of ERG targets by quantitative PCR (months 39-42, completed June 2017)

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, completed June 2017

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, completed July 2016)

7b. Assay development for surface Plasmon resonance with purified ERG protein (months 31-36, completed July 2016)

7c. Perform thermal shift and surface Plasmon resonance on compounds and determine binding constants (months 37-42, completed July 2016)

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, 80% 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:

To validate candidate kinases from the genetic shRNA screen, we cloned shRNAs targeting each candidate kinase into a lentiviral infection system and generated virus. We

subsequently measured ERG dependent invasion and anchorage independent growth following transduction of prostate cancer cells.

2) Specific objectives

- Validate candidate kinases in cell-based assays
- Determine mechanism of inhibition following suppression of validated kinases

3) Results and conclusions/other achievements

We generated an ERG gene signature by suppressing ERG in the TMRSS2-ERG positive VCAP prostate cell and measuring changes in the gene expression. Using a novel high throughput bead based gene expression method, referred to as L1000, we screened a library of shRNAs targeting 808 kinases, with an average of 5 shRNAs per kinase, in an arrayed format. To identify shRNAs that modulated the ERG gene signature, we used 5 different metrics referred to as summed score, weighted summed score, naïve bayes, K-nearest neighbor, and support vector machine which have been used previously for gene expression high throughput screening. We identified 34 kinases for further validation studies.

Initial validation of the putative kinases consisted of measuring the expression of two genes known to be regulated by ERG in VCAP cells, ARGHDIB and PLA1A, following suppression of each kinase. Individual shRNAs targeting the 34 kinases were cloned into a lentiviral expression system for transduction of VCAP cells. Gene expression was subsequently determined by QPCR. We validated that 12 our of the 34 candidate kinases suppressed the downstream targets of ERG and were chosen for follow up cell based assays specific for ERG function.

We developed two separate cell based assays for ERG function. The first is a transwell invasion assay, which measures cells' ability to invade through a matrigel membrane. We ectopically expressed ERG under a tetracycline inducible promoter in PC3 cells, which do not express ERG at baseline. Following induction of ERG by doxycycline, PC3 invade through the matrigel membrane in an ERG dependent manner. Using this

model we discovered that three kinases, ZAK, MAPK14, and MAP2K4 inhibit ERG mediated invasion of PC3 cells.

The second assay we developed to determine the effect of candidate kinases on ERG function was a transformation assay. LHSAR is a nontransformed prostate epithelial cell line LHSAR that has been immortalized with human telomerase and SV40 large T antigen. It is also engineered to express AR ectopically and forms tumors in nude mice in an androgen dependent manner when transduced with oncogenic factors such as Ras and SV40 small T antigen. Similar to the PC3 cell line we used for the invasion assay, we transduced LHSAR cells with lentivirus expressing tetracycline inducible ERG. After addition of doxycycline, cells are plated in soft agar in the presence of the synthetic androgen R1881 and demonstrate anchorage independent colony formation dependent on doxycycline (ERG induction) and R1881. We tested all 12 kinases candidates using this transformation assay. However, none of the kinases inhibited ERG mediated anchorage independent growth. It is possible that invasion and transformation are two distinct functions of ERG that are regulated by separate signaling pathways and our screen was only able to identify those kinases that affect invasion. Alternatively, our screen suppressed kinases individually and inhibition of multiple kinases may be required to affect ERG mediated transformation. For future experiments it would be interesting to perform a screen where multiple signaling pathways are inhibited simultaneously.

In conclusion, using a gene expression high throughput genetic screen we identified 3 kinases that suppress ERG dependent transcriptional activity and invasion in prostate cancer cells. We propose that they may represent potential new drug targets for ERG positive prostate cancers and our future experiments are to determine the mechanism by which these kinases inhibit ERG function. We are planning to undertake proteomic studies where we profile phosphorylated proteins in ERG positive versus negative cell lines following suppression of kinases by shRNA or knockout using CRISPR/Cas9 technology.

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

1) Major activities

High throughput gene expression profiling of a chemical library identified several compounds that perturbed the ERG gene signature. We identified one compound that showed significant activity against ERG transcription and proliferation of prostate cancer cells. We demonstrate that the mechanism is through inhibition of ERG transcription by inhibiting the histone acetyltransferase (HAT) p300/CBP. Despite being important for enhancer activation by acetylating histone H3 K27 acetylation (H3K27Ac), there are currently very few compounds that target p300/CBP enzymatic activity. We demonstrate that p300/CBP inhibition can target ERG dependent prostate cancer proliferation and identified a novel p300/CBP inhibitor.

2) Specific objectives

- Target identification of small molecule from chemical screen
- Determine mechanism of action of novel small molecule

3) Results and conclusions/achievements

The compound identified as DOS16 was found to inhibit the ERG signature in a dose dependent manner as measured by the L1000 gene expression assay. We confirmed the ability of DOS16 to inhibit transcription of ERG targets by QPCR. Figure 1 shows dose dependent inhibition of two ERG targets ARGHDIB and PLA1 by DOS16. Interestingly, we also found that transcription of ERG itself was also inhibited by DOS16 suggesting that it may inhibit AR dependent transcription of ERG. To confirm inhibition of AR activity, we also measured KLK3 and FKBP5, which are two known targets of AR. Transcription of both KLK3 and FKBP5 were also inhibited by DOS16 in a dose dependent manner suggesting that DOS16 inhibits the activity of AR.

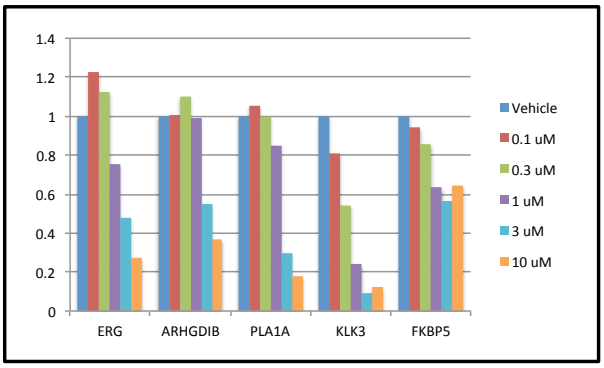

Figure 1: DOS16 inhibits AR dependent transcription of ERG. VCAP cells were treated with indicated concentrations of DOS16 and transcription measured by QPCR.

In order to determine the effect on proliferation, we treated cancer cells with different concentrations of DOS16 and measured viability by Cell Titer Glo (Figure 2). We discovered that the AR positive prostate cancer cells, VCAP and LNCAP, were sensitive to DOS16 with an IC50 in the high nM to low uM range. Interestingly, there was no effect on the proliferation of the AR negative prostate cancer cell lines DU145 and PC3 supporting the hypothesis that the compound specifically targets AR. In addition, DOS16 did not have any effect on the proliferation of the bladder caner cell line RT114.

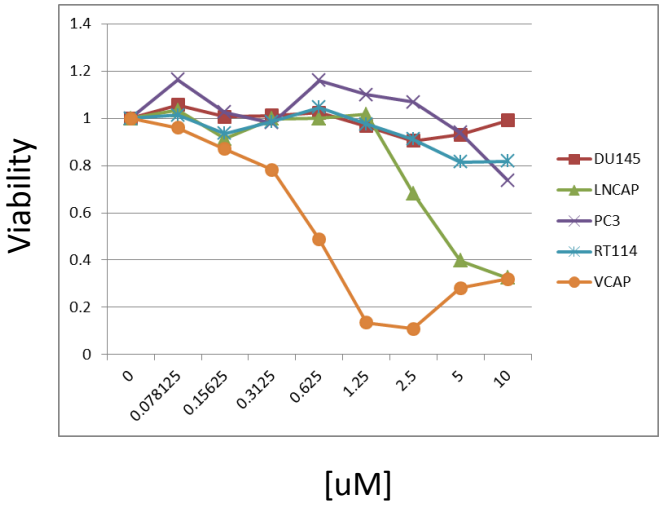

Figure 2: DOS16 inhibits proliferation of AR dependent prostate cancer cell lines. Cancer cell lines were treated with the indicated concentrations of DOS16 and proliferation measured after 5 days by Cell Titer Glo.

The chemical structure of DOS16 contains three stereocenters. Therefore to test whether the activity of DOS16 shows any stereospecificity, we measured transcription of ERG and AR targets following treatment of all 8 possible stereoisomers. (Figure 3). Interestingly, only two of the stereoisomers showed activity (SSS and SSR). Both active isomers contained the S stereocenter at the first two positions. The stereospecificty of DOS16 suggests that it may bind a specific cellular target.

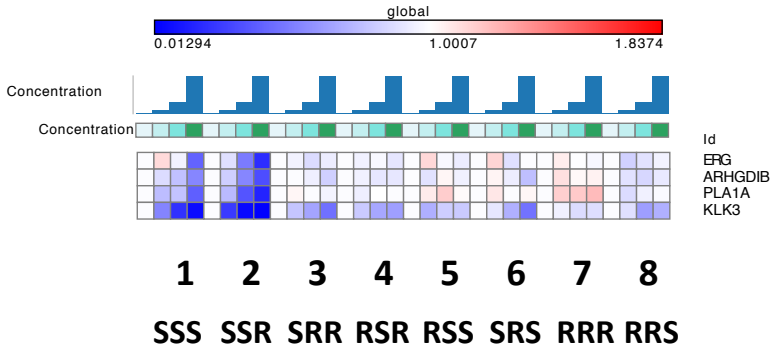

Figure 3: DOS16 shows stereospecific activity. VCAP cells were treated with eight possible stereoisomers of DOS16 at multiple concentrations and transcription measured by QPCR.

We also tested analogs of DOS16 with different substituents that were taken from the DOS library (Figure 4). We found that substitution of the R1 position with any of the substituents tested resulted in loss of activity. However, substitution at the R2 position had no effect on activity. These results suggest that the R1 substituent is critical for activity.

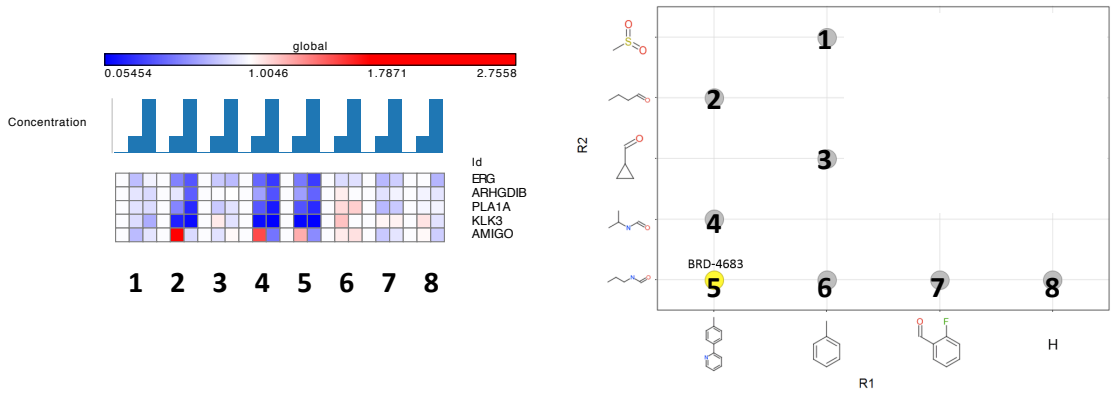

Figure 4: R1 substituent is important for activity of DOS16 . VCAP cells were treated with DOS16 analogs and transcription of ERG and AR targets measured by QPCR.

To identify the cellular target of DOS16, the compound was covalently bound to agarose beads and used to affinity purify proteins from cellular extracts followed by mass spectrometry. We found that p300 and CBP interacted with DOS16 but not to the stereoisomer control. Treatment of prostate cancer cells with DOS16 results in rapid decrease in H3K27 and H3K18 acetylation after 1-2 hours (Figure 5). Acetylation of other lysine residues of histone H3 such as H3K9 were unaffected, suggesting that DOS16 is selective for p300/CBP, which is known to specifically acetylate H3K27 and H3K18.

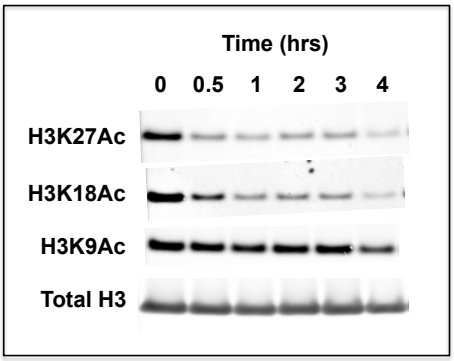

Figure 5: DOS16 inhibits H3K27 and H3K18 acetylation.
Immunoblotting of lysates from VCAP cells treated with 5 uM DOS16 at indicated time points.

To confirm specificity for p300/CBP, we genetically suppressed p300 and CBP using RNA interference (RNAi). Identical to treatment with DOS16, we found that suppression of p300 and CBP resulted in decreased H3K27 and H3K9 acetylation but had no effect on H3K9 (Figure 6).

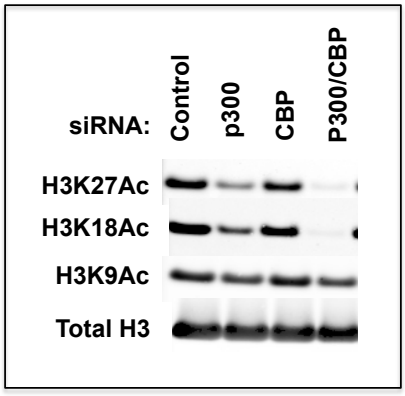

Figure 6: siRNA of p300 and CBP inhibits H3K27 and H3K18 acetylation.
Immunoblotting after transfection of VCAP cells with indicated siRNAs.

Furthermore, we compared gene expression changes after drug treatment or siRNA by RNA sequencing. Differential gene expression was highly correlated confirming specific inhibition of p300 and CBP. DOS16 is also more potent and specific than the one commercially available tool compound (C646) that has been reported to inhibit p300 in vitro (Figure 7). Therefore we propose we have identified an inhibitor of p300/CBP acetyltransferases with potent cellular activity

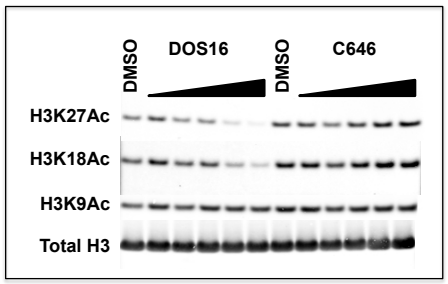

Figure 7: Comparison of DOS16 with commercially available inhibitor C646.
VCAP cells were treating with increasing concentration of indicated drug for 2 hours followed by immunoblotting.

To confirm that DOS16 binds to and inhibits p300, we performed *in vitro* assays to measure histone acetyltransferase activity using purified recombinant proteins. Purified p300 was incubated with acetyl CoA and histone H3 in the absence or presence of DOS16, and H3K27 acetylation measured by immunoblotting. DOS16 inhibited H3K27 acetylation *in vitro* in a dose dependent manner (Figure 8A). Consistent with the specificity for p300/CBP observed in cells, we found that DOS16 did not inhibit the unrelated histone acetyltransferase GCN5 (Figure 8B). As a negative control the stereoisomer of DOS16 did not inhibit H3K27Ac (Figure 8A, second row).

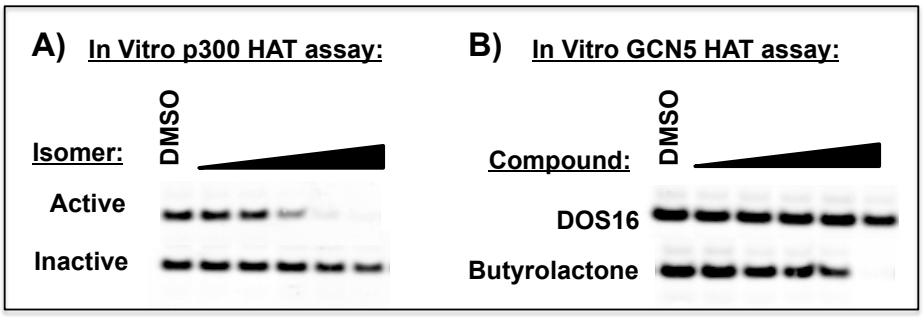

Figure 8: DOS16 inhibits p300 HAT activity in vitro. A) In vitro HAT assay with purified p300 in the presence of increasing concentrations of DOS16 (top row) or inactive isomer of DOS16 (bottom row). Activity measured by immunoblotting for H3K27Ac. B) In vitro HAT assay with purified GCN5 in the presence of increasing concentrations of DOS16 (top row). Butyrolactone was used as a positive control (bottom row). Activity measured by immunoblotting for H3K9Ac

In conclusion, we identified a novel inhibitor of p300/CBP that inhibits the ERG transcriptional program. We propose that this represents a novel means of targeting TMPRSS2-ERG prostate cancers and future work will be aimed validating these findings in *in vivo* mouse models to determine whether pharmacologic inhibition of p300/CBP could be a novel strategy for prostate cancer.

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

1) Major Activities

We identified protein kinase C inhibitors (PKCi) as modulating the ERG signature and validated their effect using cell based assays. We determined the mechanism by which PCKi inhibits ERG activity using gene expression profiling, chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq), and proteomic approaches.

2) Specific objectives

- Determine the mechanism by which PKC inhibitors (PKCi's) inhibit ERG activity
- Identify AR cofactors that are affected by PKCi treatment

3) Results and conclusions/achievements

By performing our high throughput gene expression profiling method on VCAP cells treated with a panel of FDA approved and bioactive molecules, we discovered that 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.

To determine which subset of genes are regulated by PKCi, we performed global gene expression profiling by Affymetrix microarrays in VCAP prostate cancer cells. As a positive control for AR regulated genes we used the AR antagonist MDV3100. Figure 9 shows that as expected MDV3100 reverses the effect of R1881 (compare columns 2 and 3). Interestingly, PKCi inhibited a subset of androgen induced genes (column 4). Furthermore PKCi treatment had no effect on androgen suppressed genes.

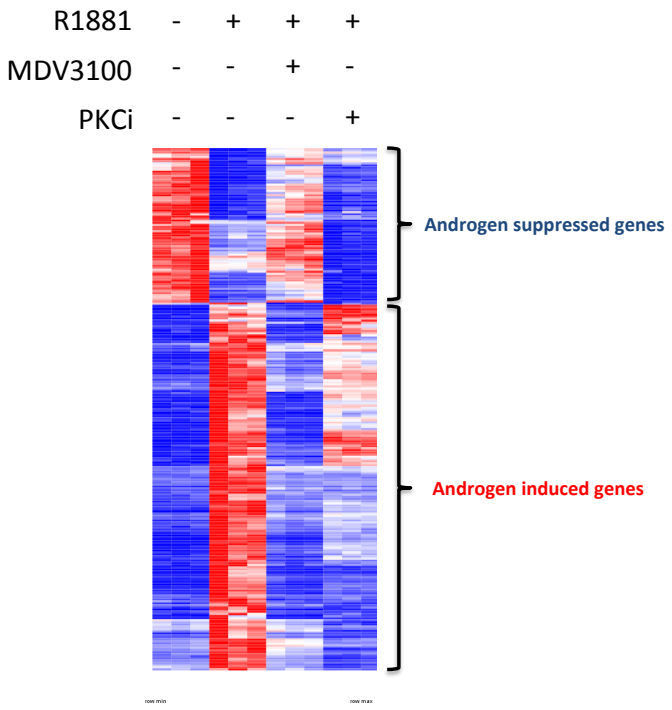

Figure 9: PKCi inhibits a subset of androgen induced genes. VCAP cells were treated with the indicated compounds and expression profile measured by Affymetrix microarray.

In order to further elucidate the mechanism of PKC inhibition on AR activity, we performed AR chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) to determine if AR binding is affected by PKC inhibition. We discovered that PKCi had no effect on genomewide binding by AR (Figure 11). AR is still able to bind to chromatin suggesting that the mechanism of PKCi occurs after AR binding to chromatin.

Figure 11: Treatment with PKCi does not affect AR binding. ChIP-seq was performed after treatment with PKCi. Treatment with MDV3100 was used as a positive control. Representative tracks at well characterized AR binding loci show no effect on AR binding peaks following PKCi treatment.

The ChIP-seq results suggest that PKCi inhibits the action of a cofactor of AR that is recruited to chromatin after AR binding. Therefore we attempted to use a proteomics approach to identify cofactors that bind to AR in the absence or presence of PKCi. We performed immunoprecipitation of AR from VCAP cells after treatment with PKCi or DMSO control followed by denaturing electrophoresis and silver staining to identify protein bands specific to one condition. However we did not see any changes in protein patterns in the presence or absence of PKCi. It is possible that the levels of the cofactor is below detection by silver staining. Future experiments are aimed at generating a stable cell line using CRISPR/Cas9 that has endogenous AR tagged with HA and V5 to improve immunoprecipitation of AR complexes. We intend to combine an optimized immunoprecipiation strategy with quantitative mass spectrometry by SILAC to identify cofactors that interact with AR in the absence or presence of PKCi.

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

During the duration of this award, I maintained a clinic one morning a week seeing prostate cancer patients. I also participated in the genitourinary oncology department protocol development meetings where we discuss the design of new protocols based on preclinical evidence. By being actively involved in taking care of patients, enrolling patients on clinical trials, and discussing protocol development, I have gained experience that will be critical to achieving my career goals of translating basic science results into meaningful treatments for patients. During this past year I had the opportunity to present my results at the Dana Farber/Harvard Cancer Center monthly genitourinary seminar series.

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?

Nothing to report (final report)

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. Unlike transcription factors, kinases are more amenable to drug development as they have well defined activities and active sites. We identified three kinases, ZAK, MAPK14, and MAP2K4 that regulate ERG activity and show using cellular assays kinases that they inhibit the ability of ERG to cause invasion of prostate cancer cells. We propose that they may represent novel targets for TMPRSS2-ERG prostate cancer.

To identify novel small molecules that inhibit ERG activity, we screened a small molecule library of novel compounds using our high-throughput gene expression method. We identified a novel compound which functions by inhibiting ERG and AR transcription through inhibition of p300/CBP. Consistent with a role in AR regulation, the compound selectively inhibits the proliferation of AR dependent prostate cancer cells. Interestingly, the compound shows structural stereospecificity suggesting interaction with a specific cellular target. Using a proteomics approach we discovered that the compound

inhibits the enzymatic activity of p300/CBP. P300/CBP activity is required for acetylation of chromatin that marks activated regulatory enhancer elements. ERG and AR dependent cell lines appear particularly sensitive of p300/CBP inhibition and may represent a novel therapeutic opportunity to treat prostate cancer. Furthermore our compound is one of the first enzymatic inhibitors of p300/CBP that has been described in the current literature.

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 (PKCi), which are commercially available and have been used in clinical trials modulates the ERG signature. Using global gene expression profiling, we find that PKCi appear to inhibit a subset of AR upregulated genes including TMPRSS2. Using chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) we found that AR binding is unaffected suggesting that PKCi modulates a cofactor required for AR activity. Although we have yet to identify the cofactor, our results indicate that PKC inhibitors may have activity in prostate cancer.

What was the impact on other disciplines?

Our method is potentially generalizable to the study of any molecular process that can be characterized by a gene expression signature. The signature can be applied to identify either genes or drugs that modify a particular phenotype. These types of functional genomic studies will be important for determining the clinical relevance of the genetic alterations discovered by genomic sequencing studies. Moreover, we identified a novel inhibitor of P300/CBP. As P300/CBP is required for enhancer activation in multiple contexts beyond prostate cancer, our inhibitor could be a useful tool compound to further understand epigenetic regulation of transcription and provide an important path to developing new therapeutic classes.

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

Nothing to report.

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

Takeda DY, Pop M, Ross K, Subramanian A, Narayan R, Wassaf D, Koehler A, Golub TR, Garraway L, Hahn WC. Targeting TMPRSS2-ERG in prostate cancer using gene expression high throughput screening. 2012 Oct 25-27; Carlsbad, CA. PCF Scientific Retreat. Abstract.

Takeda DY, Pop M, Ross K, Subramanian A, Wassaf D, Koehler A, Golub TR, Garraway L, Hahn WC. Targeting TMPRSS2-ERG in prostate cancer using gene expression high throughput screening. 2012 Aug 14-18; Cold Spring Harbor, NY. Mechanisms and Models of Cancer Meeting. Abstract.

Takeda DY, Pop M, Ronco L, Feau C, Ross K, Subramanian A, Wassaf D, Koehler A, Golub TR, Garraway L, Hahn WC. Targeting TMPRSS2-ERG in prostate cancer using gene expression high throughput screening. 2013 Oct 24-26; MD. PCF Scientific Retreat. Abstract.

Takeda DY, Pop M, Ross K, Natoli T, Subramanian A, Koehler A, Golub TR, Garraway L, Hahn WC. Targeting TMPRSS2-ERG using gene expression high throughput gene expression profiling. 2016 Aug 4-6; MD. DoD PCRP IMPacT Meeting. Abstract.

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:	*David Takeda*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*PI*
Nearest person month worked:	*12*
Contribution to Project:	*Performed experimental work and data analysis.*
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