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TITLE: The role of the paralogs CBP and p300 in androgen receptor function and prostate cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Multiple lines of evidence suggest the importance of the paralogs EP300 and CBP in advanced prostate cancer, and drug development efforts are underway to create efficacious small molecule inhibitors of the proteins. A greater understanding of the behavior of EP300 and CBP including how they relate to the Androgen Receptor in prostate cancer is critically needed, especially given ongoing clinical trials attempting to target these proteins in advanced prostate cancer, often in combination with or after the administration other anti-androgen therapies such as enzalutamide. This grant proposes to define the cistrome of EP300 and CBP in prostate cancer models, delineate the functional differences between CBP and EP300 through chemical and genetic perturbations, and determine the impact of mutations within the bromodomain and acetyltransferase domains of EP300 and CBP as well as clinically-identified mutations on protein function and drug response

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. INTRODUCTION:

Based on multiple lines of evidence suggesting the importance of the paralogs EP300 and CBP in advanced prostate cancer, various inhibitors are being designed against these proteins, some of which are in clinical trials. This proposal aimed to obtain a detailed mechanistic understanding of the unique and overlapping functions of these proteins and the impact of their inhibition in prostate cancer, including contexts in which these proteins are mutated.

2. KEYWORDS:

Prostate cancer, EP300, CBP, androgen receptor

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Define the cistrome of EP300 and CBP
Major task 1: Define the cistrome of EP300 and CBP

- Subtask 1: Determine CBP and EP300 cistrome in cell models. 40%, limited by lab closure and antibody optimization.
    - Multiple commercially available antibodies tested for Western blotting, ultimately chose one each that seemed to be specific for EP300 or CBP but not both
    - Western blotting optimization included gel concentration optimization, testing nitrocellulose vs PVDF membrane for transfer, semi-dry vs wet transfer, testing optimal transfer time and switch to LICOR imaging reagents.
    - After extended testing, unable to reliably ChIP-Western EP300 and CBP, though had a few potential successes
    - Attempted ChIP-seq of EP300 and CBP, but immunoprecipitation efficiency was low as evidenced by very low number of called peaks
    - Optimization attempted by trialing multiple protocols with various buffers, double fixation, nuclear protein isolation prior to chromatin immunoprecipitation to try and enrich for the nuclear fraction of these proteins
- Subtask 2: Relate cistrome to RNA-expression data. RNA-expression data for models has been generated in charcoal-stripped (no androgens) and dihydrotestosterone exposure

Major task 2: Compare EP300 and CBP cistrome to AR cistrome

- Subtask 1: Generate any additional AR ChIP-seq that is needed. AR cistrome generated in models in charcoal-stripped and dihydrotesterone treated conditions
- Subtask 2: Relate EP300 and CBP cistrome to AR cistrome and AR transcription. Pending major task 1 subtask 1, unable to compare AR cistrome to EP300 and CBP cistrome

Specific Aim 2: Delineate the functional differences between CBP and EP300 through chemical and genetic perturbations.
Major task 1: Identify differences in cell growth and transcriptional programs of CBP and EP300 utilizing genetic knockdown

- Subtask 1: CRISPR Knockout of CBP and EP300. 50% completed
    - CRISPR guides computationally chosen and lentiviral constructs made with confirmed viral titer
    - Infection and antibiotic selection complete, though this required significant optimization given lab issues with viral infection constructs
    - Given issues with consistent western blotting detailed above, unable to confirm knockout
    - Next step would be amplicon sequencing to confirm CRISPR DNA editing
    - After selection in LNCaP and 22Rv1 cells, multiple selected clones were unable to propagate which could suggest CRISPR editing (if successful) knocked out essential genes
    - Designed shRNA constructs and made lentiviral particles
    - Cell lines infected, but unable to confirm knockdown or knockout given Western blotting issues

- Subtask 2: Analyze cistrome after KO limited due to lack of success so far with subtask 1

Major task 2: Identify effect of BRD and HAT treatment. 80% complete

- Subtask 1: Treat cell lines with BRD and HAT inhibitors: Completed
    - Purchased CCS1477 (EP300 selective bromodomain inhibitor) from Selleck and got significantly different IC50 in cell viability assay compared to published results
    - Worked with medicinal chemistry collaborators locally to obtain synthesized CCS1477, A485 (histone acetyltransferase inhibitor) as well as EP300 and CBP-specific degrader compounds
    - Identified IC50 for all compounds in cell line models
- Subtask 2: Determine effect of BRD or HAT treatment on cistrome, RNA expression and chromatin accessibility. 30% completed
    - RNA-seq of cell line models +/- androgen +/- CCS1477 completed
    - List of candidate genes differentially expressed under each condition generated
    - Given inability to reliably generate cistrome data to date, the remainder of this aim was unable to be completed

Specific Aim 3: Determine the impact of mutations within the bromodomain and acetyltransferase domains of EP300 and CBP as well as clinically-identified mutations on protein function and drug response.

Major task 1: Introduce mutations seen in patient cohorts into cell line models and assess their impact on cellular phenotype

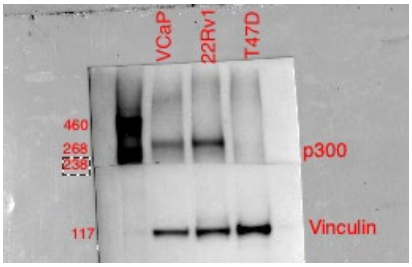
- Subtask 1: Have optimized Western blot conditions for EP300, CBP and AR. Have designed overexpression constructs. Have not yet introduced ovexpression constructs into cell line models, this is planned during no cost extension period.
- Subtask 2: Functional mutagenesis screen planned but not executed given high cost in the context of being unable to validate the results of such a screen (inconsistent Western blotting, unable to ChIP-seq reliably)
- Subtask 3: Pending subtask 1.

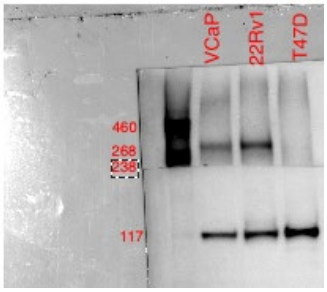
What was accomplished under these goals?

(1) Major activities and specific objectives

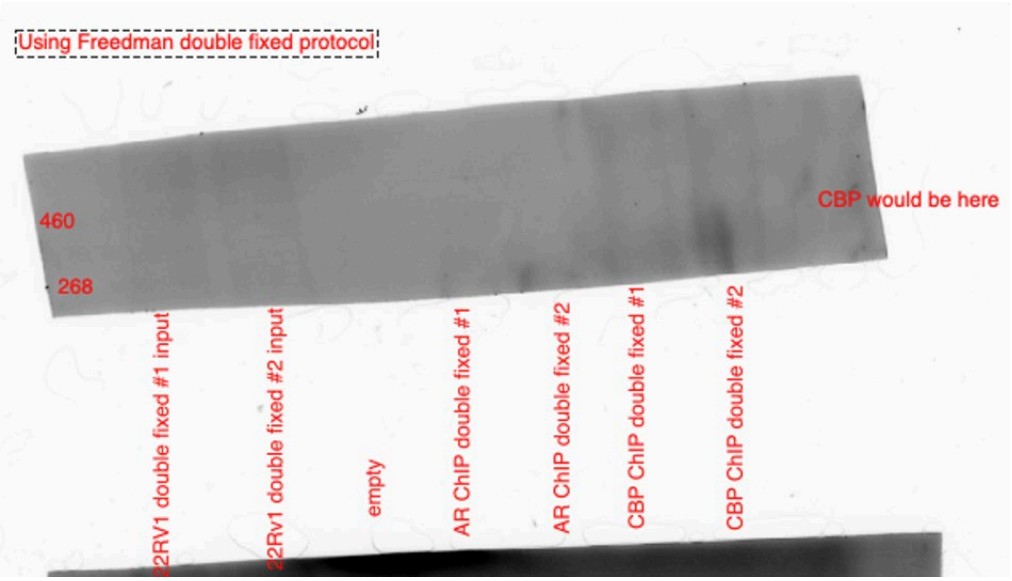
Within the timeframe of this grant, all three Aims were attempted with detailed troubleshooting, with mixed success. The most significant effort was directed towards optimization of western blotting and ChIP-seq.

As shown below by Western blotting, at times we were able to successfully blot for EP300 and CBP, but the quality of results was not consistent. Below is an example of successful western blotting.

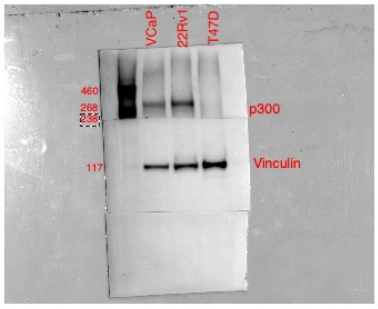




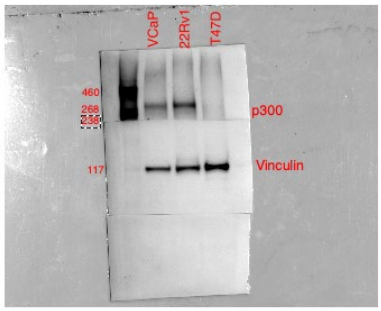
Below is a more typical example of poorer quality results with the same antibody (but different batch)

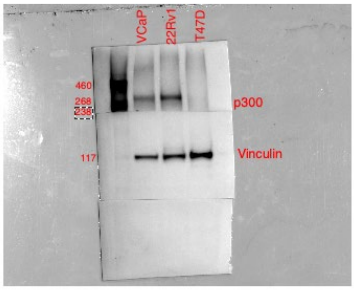


We were unable to get a reassuring result with ChIP-western (chromatin immunoprecipitation for either EP300 or CBP followed by Western blotting with the same antibody to confirm appropriate pull down).

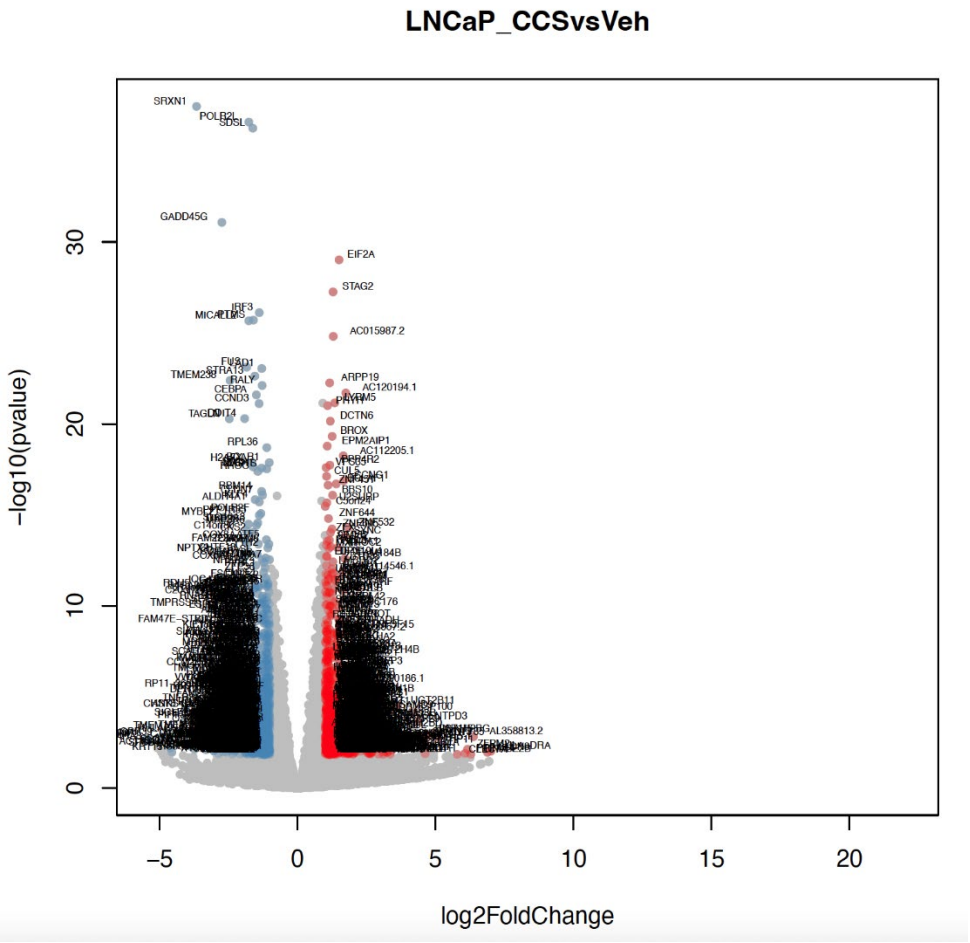


There were some potentially promising knockdown results with CRISPR guide infection (sg2), but these did not result in knockout, and Western results were not consistent.





We generated RNA-seq data for two cell lines models in the presence of androgen and/or the bromodomain inhibitor CCS1477. Based on a priori knowledge of androgen regulated genes in each model, the data from LNCaP looked best after differential expression analysis, and we now have candidate genes that are direct candidates in this line model.



Given concerns about our ability to accurately assess EP300 and CBP protein levels by Western blot, we could not justify the expense of the mutagenesis screen within the timeframe of this grant.

The common and divergent roles of EP300 and CBP remains of significant clinical interest given multiple agents targeting these proteins are in clinical development. We intend to continue troubleshooting this work and pursuing the remaining aims on an ongoing basis.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Overall, this grant provided critical salary support to pursue clinically relevant prostate cancer research and facilitated my transition from clinical and postdoctoral fellow to research faculty. I now hold an investigator position at Dana-Farber Cancer Institute, and continue to work on this and other translationally relevant projects in prostate cancer. More specifically, this work has provided significant training in troubleshooting chromatin immunoprecipitation, ATAC-seq, drug treatment, and analysis of sequencing data from these experiments. I am very grateful for this opportunity and support. Preliminary results from this work were also presented at the 2023 AACR conference.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Poster presentation (Abstract 4759) at the 2023 AACR annual conference

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

This is the final report, however, as mentioned above the aims of this grant remain clinically important. We therefore plan to continue to work on understanding the roles of EP300 and CBP in prostate cancer.

4. IMPACT:

Nothing to report

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
No significant changes in approach beyond covid19 pandemic delays. Detailed troubleshooting attempts are described above.

Actual or anticipated problems or delays and actions or plans to resolve them
Our laboratory space was closed for several months during the covid19 pandemic, and re-opened slowly requiring shift work. Additionally, there were delays in obtaining various lab supplies and drug compounds due to the pandemic. This resulted in significantly impaired ability to address the planned major objectives within the initially proposed timeframe. To counter this, we pursued a no cost extension. Ultimately, technical challenges potentially related to antibody quality or the size/concentration of the proteins of interest limited our ability to fully execute the aims despite dedicated effort.

Changes that had a significant impact on expenditures
No travel to conferences were supported by this grant.

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

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.
  Nothing to report

  Books or other non-periodical, one-time publications.
  Nothing to report

  Other publications, conference papers and presentations.
  2023 AACR abstract #4759 – presented as poster

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Alok Tewari
Project Role: Principal Investigator
Research Identifier (ORCID ID): 0000-0003-2617-7499
Nearest person month worked: 6.6 CM
Contribution to Project: Principal Investigator overseeing the design and reporting of the research

Name: Kiran Mirpuri
Project Role: Research Technician (not supported by this grant)
Nearest person month worked: 6 CM
Contribution to project: Research technician who assisted in cell culture and labotaory assays
Funding Support: Myles Brown (Grant mentor) research funds

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

I have transitioned to an independent investigator position with salary support from my employer.
I have obtained internal research support funding at Dana-Farber, but no other external grants in the last reporting period.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: