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

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CONTRACTING ORGANIZATION:
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6. AUTHOR(S)
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5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

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**
					USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	7	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

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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 aims 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
Major task 1: Define the cistrome of EP300 and CBP

- Subtask 1: 10%, limited by lab closure and antibody optimization. Difficulties with antibody validated by recent publication (Welti et al Cancer Discovery) requiring custom antibodies. Currently pursuing custom antibody optimization as well as trying to optimize using commercially available
- Subtask 2: RNA-expression data for models has been generated

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

- Subtask 1: AR cistrome generated in models
- Subtask 2: Pending major task 1 subtask 1, have not been able to compare AR cistrome to EP300 and CBP cistrome

Specific Aim 2
Major task 1: Identify differences in cell growth and transcriptional programs of CBP and EP300 utilizing genetic knockdown

- Subtask 1: CRISPR KO has not been successful in models, suggesting that these genes may be essential to growth when depleted by CRISPR. Currently pursuing inducible shRNA based approaches
- 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

- Subtask 1: Have conducted cell line treatments with BRD and HAT inhibitors that are commercially available through Selleck. However, IC50 in cell line models are quite different than what has previously been published. I am currently troubleshooting this, and may need to try and obtain CCS1477 (BRD inhibitor) directly from the company that produces it if there are issues with the generic formulation made by Selleck.
- Subtask 2: Pending successful completion of Subtask 1

Specific Aim 3
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 for the next year of the award
- Subsask 2: Functional mutagenesis screen planned for next year.
- Subtask 3: Pending subtask 1.

What was accomplished under these goals?

To date, much of the proposed work has been limited by covid19-related lab closure and limited access to materials. The following experiments have been successfully completed:

- Optimized western blotting for EP300 and CBP
- AR cistrome and RNA expression data in models

Current work is focused on:

- Troubleshooting EP300 and CBP ChIP-seq (especially CBP as published antibodies for this are quite limited)
- Drug treatment optimization given calculated IC50 are off by approximately 1 log from published IC50s in literature
- Creating mutant overexpression lines
- Designing functional mutagenesis screen

What opportunities for training and professional development has the project provided?
This work has provided training for the principal investigator in chromatin immunoprecipitation, ATAC-seq and analysis of sequencing data generated by these experiments. It has also provided significant experience in troubleshooting various issues related to both these experiments (cell lysis conditions, sonication, western blotting) as well as those related to QC of drug inhibition experiments.

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

4. IMPACT:

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

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, in the next period will continue to work on all intended objectives, and may require no cost extension subsequently in order to ensure that all work is completed.

Changes that had a significant impact on expenditures
No travel to conferences

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.
Nothing to report

- **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

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?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES