AD_____

Award Number: W81XWH-16-1-0567

TITLE: A Master Regulator of Aggressive Prostate Cancer Variants

PRINCIPAL INVESTIGATOR: Michael Freeman, PhD

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center
Los Angeles, CA 90048

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2017	Annual	30 Sep 2016 - 29 Sep 2017

4. TITLE AND SUBTITLE

"A Master Regulator of Aggressive Prostate Cancer Variants"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0567

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Michael Freeman, PhD

E-Mail: michael.freeman@cshs.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, CA 90048

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Treatment of PC by androgen suppression is known to promote the emergence of aggressive variants that are AR-independent. In a study funded by this grant and published in Nature Medicine in December 2018 (Rotinen, You et al. Nat Med 24:1887-1898, 2018, PMID: 30478421) we identified the developmental transcription factor ONECUT2 (OC2) as a master regulator of AR networks in metastatic castration-resistant prostate cancer (CRPC). We showed that OC2 acts as a survival factor in metastatic CRPC (mCRPC) models, suppresses the AR transcriptional program by direct regulation of AR target genes and the AR licensing factor FOXA1, and activates genes associated with neural differentiation and progression to lethal disease. OC2 appears active in a substantial subset of human prostate adenocarcinoma and neuroendocrine tumors. Inhibition of OC2 by a newly identified small molecule, CSRM617, suppresses metastasis in mice. These findings suggest that OC2 displaces AR-dependent growth and survival mechanisms in many cases where AR remains expressed, but where its activity is bypassed. We also demonstrated that OC2 is also a potential drug target in the metastatic phase of aggressive PC.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The main goal of the project was to examine the role, and understand the mechanism of action, of the developmental transcription factor ONECUT2 (OC2) in prostate cancer. OC2 emerged from an unbiased computational model of highly active transcription factors and their associated network relationships in castration-resistant prostate cancer (CRPC). At the time we applied for funding for this project in 2015, OC2 had not been linked to prostate cancer in the literature, and links between OC2 and cancer overall more broadly was very limited. The computational model we developed predicted that OC2 was active in a significant percentage of CRPCs and that it was networked in some way to the androgen receptor (AR) and the histone-lysine methyltransferase EZH2, both known to be master regulators operating in CRPC. The project represented an attempt to understand the role, if any, of OC2 in aggressive prostate cancer and the emergence of CRPC, and to identify mechanisms of action that could potential reveal novel therapeutic targets.

KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Prostate cancer (PC), castration-resistant prostate cancer (CRPC), androgen receptor (AR), AR-indifferent PC, metastasis, network, chromatin, promoter, enhancer, gene expression, targeting, small molecule, metastasis-suppression.

ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

- *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

  The major goals were:

  Specific Aim 1: Determine the mechanism and functional consequences of OC2 interaction with chromatin, and of androgen receptor (AR)-OC2 interactions at chromatin, in prostate cancer cells.

  Specific Aim 2: Determine the functional and clinical consequences of OC2 activation in prostate cancer in vivo.

What was accomplished under these goals?

- All of the tasks and subtasks defined in the SOW were accomplished. The central findings of the study are summarized in the next bullet.
- Treatment of PC by androgen suppression is known to promote the emergence of aggressive variants that are AR-independent. In a study funded by this grant and published in Nature Medicine in December 2018 (Rotinen, You et al. Nat Med 24:1887-1898, 2018, PMID: 30478421) we identified the developmental transcription factor ONECUT2 (OC2) as a master regulator of AR networks in metastatic castration-resistant prostate cancer (CRPC). We showed that OC2 acts as a survival factor in metastatic CRPC (mCRPC) models, suppresses the AR transcriptional program by direct regulation of AR target genes and the AR licensing factor FOXA1, and activates genes associated with neural differentiation and progression to lethal disease. OC2 appears active in a substantial subset of human prostate adenocarcinoma and neuroendocrine tumors. Inhibition of OC2 by a newly identified small molecule, CSRM617, suppresses metastasis in mice. These findings suggest that OC2 displaces AR-dependent growth and survival mechanisms in many cases where AR remains expressed, but where its activity is bypassed. We also demonstrated that OC2 is also a potential drug target in the metastatic phase of aggressive PC.

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

Nothing to report.

How were the results disseminated to communities of interest?

- The findings from the study were reported this month as a research article in Nature Medicine (Rotinen, You et al. Nat Med 24:1887-1898, 2018, PMID: 30478421).

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

- The funding period is now closed (this was a two-year funding period). Content in this technical report includes the final reporting information.

IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- We identified several of the molecular mechanisms in which OC2 intersects with and alters the AR signaling axis.
- We defined a gene signature that allows OC2 activity to be inferred in human prostate cancer tissues.
- We developed a multiplex quantitative immune-imaging method to identify cancer regions where OC2 activity is increased.
- We demonstrated that certain aggressive PC cells can be addicted to OC2.
- We demonstrated that OC2 can be targeted directly with a drug-like small molecule that suppresses established metastases of virulent human CRPC cells in mice.
- Our findings, which include analyses of thousands of human PC specimens, indicate that OC2 is activated in ≥ 30% of high grade prostate tumors and that activation of this protein can precede treatment with hormonal therapy.
- Mechanistically, our results suggest that OC2 is a proximal driver of a lethal differentiation program that emerges in aggressive PC.

What was the impact on other disciplines?

- We present evidence in our Nature Medicine study that OC2 may be involved in other malignancies, including breast cancer, gastric cancer, colon cancer, renal cell clear cell carcinoma, medulloblastoma, non-small cell lung cancer, and small cell lung cancer.

What was the impact on technology transfer?

- We identified a novel small molecule that targets a driver AR-indifferent PC and suppresses established human CRPC metastases in mice. Cedars-Sinai Medical Center has filed a patent application in which several of the authors of our Nature Medicine report are listed as inventors.

What was the impact on society beyond science and technology?

- CRPC is a disease entity for which no precision approach to medical therapy is known. The identification of OC2 as a targetable driver of aggressive PC opens new avenues for clinical translation and therapeutic development based on these discoveries.

CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

- Nothing to report

PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publication:**
  Rotinen, M., You, S., Yang, J., Coetzee, S.G., Reis-Sobreiro, S., Huang, W-C., Huang, F., Pan, X., Yáñez, A., Hazelett, D.J., Chu, C-Y., Steadman, K.A., Morrissey, C.M., Nelson, P.S., Corey, E., Chung, L.W.K., Freedland, S.J., Di Vizio, D., Garraway, I.P., Murali, R., Knudsen, B.S., and **Freeman, M.R.** (2018) ONECUT2 is a targetable master regulator of lethal prostate cancer that suppresses the androgen axis. Nature Medicine 24:1887-1898. PMID: 30478421.

- **Patent application:**

  Cedars-Sinai patent application reference 065472-000700WO00. More information available from Jason L. Moore, PhD, patent specialist, jlmoore@nixonpeabody.com

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

The participants, their affiliations, and their contributions to the study are des Rotinen, M., You, S., Yang, J., Coetzee, S.G., Reis-Sobreiro, S., Huang, W-C., Huang, F., Pan, X., Yáñez, A., Hazelett, D.J., Chu, C-Y., Steadman, K.A., Morrissey, C.M., Nelson, P.S., Corey, E., Chung, L.W.K., Freedland, S.J., Di Vizio, D., Garraway, I.P., Murali, R., Knudsen, B.S., and **Freeman, M.R.** (2018) ONECUT2 is a targetable master regulator of lethal prostate cancer that suppresses the androgen axis. Nature Medicine 24:1887-1898. PMID: 30478421.

- **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 organizations were involved as partners?**
  - Cedars-Sinai Medical Center. Most of the authors of the Nature Medicine report are at Cedars-Sinai as their primary institution.
  - University of Washington. Contribution of data and anonymized PC specimens.
  - Fred Hutchinson Cancer Center. Contribution of data and anonymized PC specimens
  - UCLA. Contribution of data.