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TITLE: Mechanisms and Therapeutic Targeting of Nuclear Shape Instability in Lethal Prostate Cancer

PRINCIPAL INVESTIGATOR: Michael R. Freeman, PhD

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

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Michael Freeman, PhD

Email: michael.freeman@cshs.org

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CEDARS-SINAI MEDICAL CENTER
8700 Beverly Blvd.
Los Angeles, CA 90048-1804

8. PERFORMING ORGANIZATION REPORT NUMBER

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

USA MED RESEARCH ACQ ACTIVITY
820 OHANDLER ST
FORT DETRICK MD 21702-5014

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14. ABSTRACT

This project is attempting to determine the molecular mechanisms that promote nuclear shape instability (NSI) and that produce extracellular vesicles (EVs) containing chromosomal DNA. We will determine whether EVs from prostate cancer cells contain genomic DNA. We will test the hypothesis that a novel protein driver of metastasis in drug-resistant prostate cancer, ONECUT2, will produce NSI, whether inhibiting this protein using a novel class of small molecules we developed can reverse this process, whether EVs with nuclear content are associated with aggressive prostate cancer, and test for ONECUT2 activity in blood using a novel approach. In year 1 we showed that DNA is associated with small-, medium- and large-sized EVs, suggesting that information about tumor phenotype can be derived from EVs. We have obtained evidence that ONECUT2 can operate as a pioneer factor to open chromatin. We have used in vitro models to replicate the 'very small nuclei' (vsn) phenotype seen in circulating tumor cells from patients with advanced prostate cancer, suggesting that the vsn phenotype reflects nuclear shape instability (NSI). We obtained evidence that chromatin structure in the ONECUT2 gene body may not reflect expression of the ONECUT2 gene, as originally proposed. These studies are uncovering novel connections between nuclear structure deficits and features of aggressive prostate cancer. No change in plans are anticipated for year 2.

15. SUBJECT TERMS
Prostate cancer

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1. INTRODUCTION:

This project is attempting to determine the molecular mechanisms that promote nuclear shape instability (NSI) and that produce extracellular vesicles (EVs) containing chromosomal DNA. We will determine whether EVs from prostate cancer cells contain genomic DNA. We will test the hypothesis that a novel protein driver of metastasis in drug-resistant prostate cancer, ONECUT2, will produce NSI, whether inhibiting this protein using a novel class of small molecules we developed can reverse this process, whether EVs with nuclear content are associated with aggressive prostate cancer, and test for ONECUT2 activity in blood using a novel approach.

2. KEYWORDS:

Castration-resistant prostate cancer, extracellular vesicles, EVs, nuclear shape instability, NSI, metastasis, DNA, chromatin

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major task 1: Investigate the molecular mechanisms of nuclear shape instability (NSI) that promote shedding of large extracellular vesicles (L-EVs) containing DNA. Timeline 1-18 months.
Subtask 1.1: Determine whether NSI gives rise to L-EVs that contain DNA.
Subtask 1.2: Determine whether the formation of L-EVs containing DNA is modulated by ONECUT2 (OC2).
Subtask 1.3: Determine whether NSI is targetable by next-generation OC2-targeting.

Major task 2: Determine the correlation between circulating L-EVs containing DNA and PC progression in mouse models and patient outcome in men. 12-36 months.
Subtask 2.1. Correlate the presence of circulating nuclear-derived EVs to PC malignancy and metastasis in preclinical mouse models of NSI.

Major task 3: Determine whether the number of circulating L-EVs containing DNA can predict disease status and progression. 12-36 months.
Subtask 3.1. L-EVs and S-EVs will be isolated using refined methodologies as well as quantified and characterized using qNano, flow cytometry, Atomic Force Microscopy in the blood of PC patients.
Subtask 3.2. Determine whether L-EV-containing genomic DNA is associated with either disease status or disease progression using four different cohorts of PC patients.

Major Task 4: Determine if OC2 gene body methylation status can be detected in L-ECs
Subtask 4.1. Test the hypothesis that the methylated OC2 gene body is detectable in L-EVs.
Subtask 4.2. Attempt to detect OC2 gene body methylation using the EPIC assay.
Subtaks 4.3 Perform a pilot experiment using the Cedars-Sinai CRPC biorepository to assess whether OC2 gene body methylation can be detected in bood.

What was accomplished under these goals?

Subtask 1.1. Completed. We were able to show in preclinical models that DNA is associated with small, medium-sized and large EVs from metastatic PC cells (Figure 1). However, we saw considerable variation in these data, suggesting that DNA is associated with all three EV classes. We observed a trend toward higher amounts of DNA in small EVs (exosomes), however these data do not support our hypothesis that L-EVs might be a richer source of DNA than other classes of EVs. data.

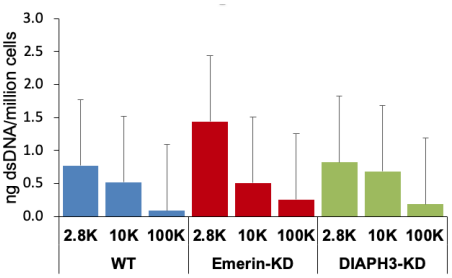

Figure 1. EVs were isolated from conditioned media of DU145 WT, Emerin-KD, or DIAPH3-KD prostate cancer cells by differential ultracentrifugation. Two populations of large EVs were isolated at 2,800 x g (2.8K) or at 10,000 x g (10K), and small EVs were isolated at 100,000 x g. EV DNA was extracted and double-stranded (ds) DNA was quantified by the Qubit dsDNA HS (High Sensitivity) assay. The amount of dsDNA in each EV population was normalized to the number of EV producing cells. Data from 3 independant experiments showed that, despite a high biological variability, large EVs from the Emerin-KD cells have a higher dsDNA content compared to WT or DIAPH3-KD cells.

Subtask 1.2. In progress. New models of OC2 activation were developed in year 1. These are being characterized by a variety of methods, including RNA-seq, ATAC-seq, immunoprecipitation followed by high resolution mass spectrometry, and a variety of imaging modalities. We *discovered* from these data that OC2 acts as a pioneer transcription factor to open chromatin widely across the genome, and we identified a series of novel functional partners based on OC2 effects on chromatin, including the neuroendocrine driver and splicing factor SRRM4 and the ETS-family oncogene ETV1.

Subtask 1.3. In progress. We have reported previously that features of NSI in preclinical models correlate with the very small nuclear (vsn) phenotype seen in circulating tumor cells (CTCs) in CRPC patients (Reis-Sobreiro et al. *Cancer Res* 2018). We have recapitulated these observations in preclinical models, showing that the vsn phenotype emerged in LNCaP and LNCaP derivative CRPC cells (C4-2B) when emerin (EMD) was silenced, and in cells that are resistant to abiraterone or enzalutamide (Figure 2). Similarly, when C4-2B cells were treated with glutamine under conditions that evoke a neuroendocrine phenotype, EMD immunolocalization was disrupted, indicating a loss of nuclear shape stability (Figure 2C). These findings link NSI to lineage plasticity and drug resistance, consistent with our hypothesis.

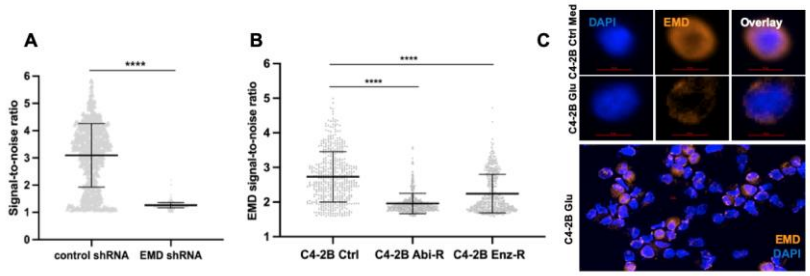

Figure 2. **A.** Reduction in emerin (EMD) expression in cells with EMD-KD. **B.** Emerin expression in human CRPC cells resistant to abiraterone (Abi-R) and enzalutamide (Enz-R). **C.** Disruption of EMD subcellular localization in cells induced to undergo NE differentiation in glutamine (Glu) containing medium.

Subtask 4.1. We performed ATAC-seq on LNCaP cells stably expressing enforced OC2 and predicted that we would see open chromatin throughout the OC2 gene body, consistent with the preliminary data that DNA methylation through the gene body could report OC2 gene activity. However, we did not see a significant difference between control and OC2-expressing cells in the ATAC-seq data, which was not consistent with our overall hypothesis.

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

Nothing to report.

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?

At the moment we are proceeding along the lines of the Statement of Work, despite some findings that are in opposition to our overall hypothesis. We have made significant progress in the first year, however, including the finding that ONECUT2 operates as a pioneer factor at the level of chromatin and the identification of several proteins that appear to be cooperating partners with ONECUT2. These insights will be incorporated into our research strategy as we move ahead.

4. IMPACT:

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

Significantly, we have shown that preclinical models of aggressive prostate cancer exhibit the “very small nuclei” phenotype, which we attribute to loss of nuclear shape stability (NSI). This finding is encouraging and is consistent with our hypothesis that NSI is a feature of advanced disease.

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

Nothing to report.

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

We have had **MANY** challenges during the COVID19 pandemic, including prolonged mandatory lab and office closures, mandatory caps on lab personnel occupying the lab at any one time, major disruptions in availability of reagents, slowed delivery times even when materials were available from suppliers, and general uncertainty about the future for all personnel. These problems have substantially affected and diminished what would be our normal anticipated progress. We are working closely with our administration to minimize these issues as much as possible.

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

Significant changes in use or care of human subjects

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: Michael Freeman, PhD
Project Role: Principal Investigator
Researcher Identifier: 0000-0001-8142-3432
Nearest person month worked: 1.3
Contribution to Project: Dr. Freeman is an expert on preclinical models of prostate cancer and other urologic diseases.
Funding Support: NIH, DOD, CSMC

Name: Dolores Di Vizio, MD, PhD
Project Role: Co-Investigator
Researcher Identifier: 0000-0002-9787-6556
Nearest person month worked: 1.8
Contribution to Project: Dr. Di Vizio provides specialized expertise and technologies in all aspects of the extracellular vesicle experiments.
Funding Support: NCI, DOD, CSMC

Name: Sungyong You, PhD
Project Role: Co-Investigator
Researcher Identifier: 0000-0003-3513-1783
Nearest person month worked: 0.5
Contribution to Project: Dr. You provides expertise with bioinformatic and statistical analysis of datasets from genomics, transcriptomics, and proteomics studies, with a strong focus on data integration methods using advanced computational strategies.
Funding Support: NIH, DOD, CSMC

Name: Chen Qian, PhD
Project Role: Postdoctoral Scientist
Researcher Identifier:
Nearest person month worked: 1.2
Contribution to Project: Laboratory experimentalist
Funding Support: NCI, AUA

Name: Krizia Sagini, PhD
Project Role: Postdoctoral Scientist
Researcher Identifier:
Nearest person month worked: 3.5
Contribution to Project: Laboratory experimentalist
Funding Support: NCI

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

Dr. Freeman received a new NCI R01 this year:

1R01CA271750 Freeman/Garraway (Multi-PI)
09/2021-08/2026
NIH
Title: Mechanisms of Prostate Cancer Metastasis
Goal: The objective of this study is to understand the relationship between activation of lineage plasticity programs and genomic and chromosome instability.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

None.