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TITLE: Dissecting Translational Dynamics and Lineage Plasticity in Prostate Cancer

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CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Center, Seattle, WA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer often acquires androgen receptor (AR) independence during treatment via switching to a neuroendocrine phenotype. Whether AR inhibition triggers this lineage plasticity program through translation control remains elusive. Protein synthesis is an important step of gene expression and but how prostate cancer cells regulate mRNA-specific translation is unknown. This study investigates the molecular mechanism underlying phenotype switching in prostate cancer from the translation perspective.

15. SUBJECT TERMS
Phenotype switching, neuroendocrine prostate cancer, androgen receptor pathway, mRNA translation, tRNA, 5' UTR

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Prostate cancer often acquires androgen receptor (AR) independence during treatment via switching to a neuroendocrine phenotype. Whether AR inhibition triggers this lineage plasticity program through translation control remains elusive. Protein synthesis is an important step of gene expression and but how prostate cancer cells regulate mRNA-specific translation is unknown. This study investigates the molecular mechanism underlying phenotype switching in prostate cancer from the translation perspective.

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

Phenotype switching, neuroendocrine prostate cancer, androgen receptor pathway, mRNA translation, tRNA, 5' UTR

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

What were the major goals of the project?

Specific Aim 1: Delineate the role of Arg-TCT-1-1 in the development of lineage plasticity and restoration of enzalutamide sensitivity in NEPC.

Major Task 1: Determine how Arg-TCT-1-1 regulates AR activity.

Major Task 2: Determine if Arg-TCT-1-1 is necessary for AR signaling activity in a lineage specific or independent manner.

Major Task 3: Determine Arg-TCT-1-1 tRNA levels in adenocarcinoma versus NEPC patients.

Specific Aim 2: Interrogate the functional topology of prostate cancer oncogene and tumor suppressor 5' UTRs.

Major Task 1: Identify and characterize the functional 5' UTR topology of prostate cancer-associated genes.

Major Task 2: Investigate the function of cancerous 5' UTR cis-regulatory structures through their interactions with trans-acting factors.

What was accomplished under these goals?

Specific Aim 1: Delineate the role of Arg-TCT-1-1 in the development of lineage plasticity and restoration of enzalutamide sensitivity in NEPC.

Major Task 1: Determine how Arg-TCT-1-1 regulates AR activity.

Arg-TCT-1-1 tRNA overexpression in NEPC resulted in increased AR activity (**Fig. 2**) through enhanced translation of AR co-regulators (**Fig. 1**).

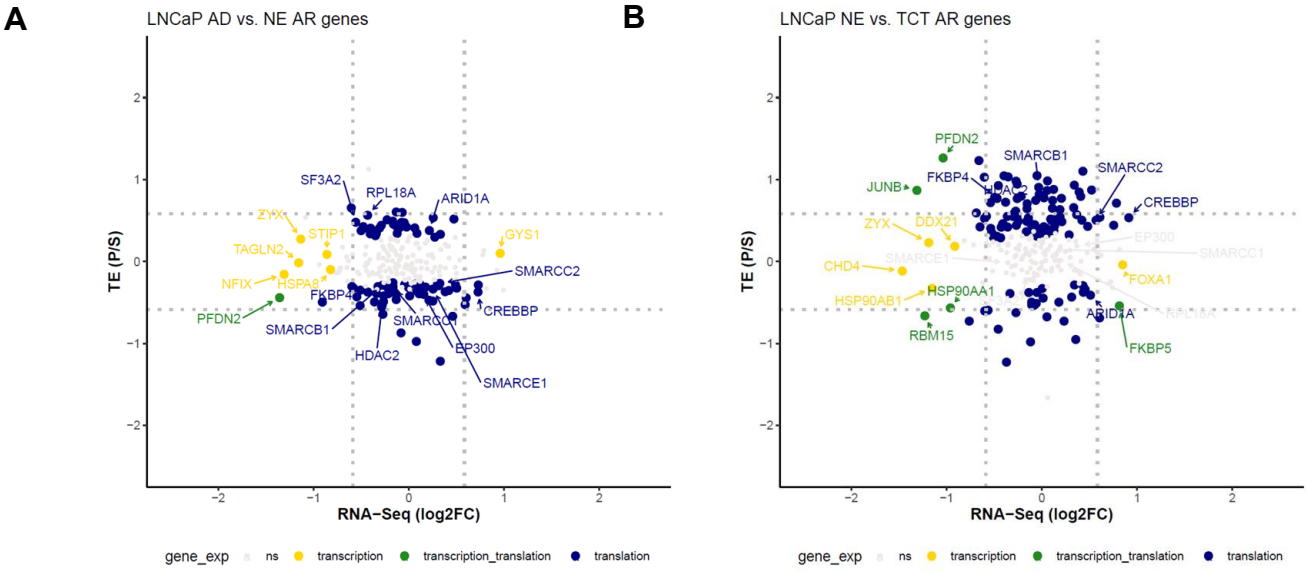

Fig 1. Changes in the expression of AR co-regulator genes are shown in scatter plots. Transcriptional changes (mRNA expression levels) are visualized along the x-axis and translational efficiency which was calculated based on a ratio of polysome to 80s monosome fractions (P/S) is shown along the y-axis. Transcriptional changes are marked as yellow, translational changes as navy, and co-transcriptional translational changes as green. **A.** Changes in the AR co-regulator genes between the AD (adenocarcinoma) and the NE (neuroendocrine) states. **B.** Changes in the AR co-regulator genes between the NE (neuroendocrine) and the TCT (NE overexpressing Arg-TCT-1-1 tRNA) states.

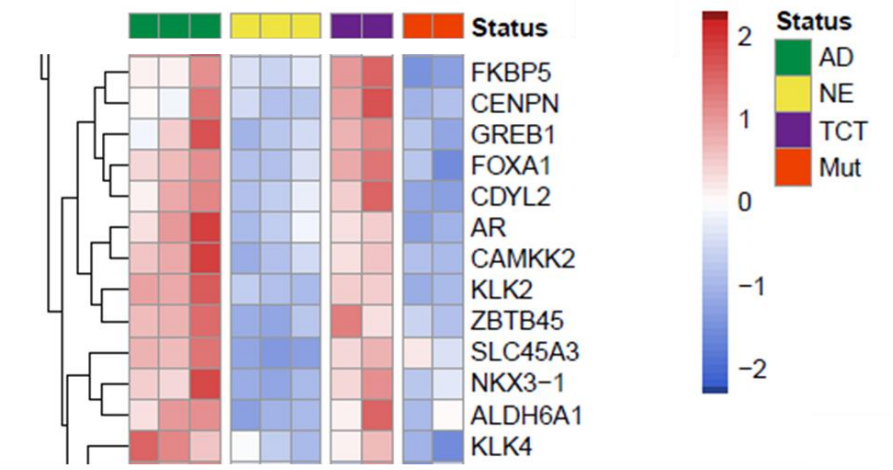

Fig 2. A heatmap of RNA-seq data demonstrates that a subset of AR target genes (including AR gene) is elevated transcriptionally in the TCT group (NE overexpressing Arg-TCT-1-1 tRNA). However, the Mut group (NE with mut tRNA overexpression) does not show transcriptional changes in these genes.

Major Task 2: Determine if Arg-TCT-1-1 is necessary for AR signaling activity in a lineage specific or independent manner.

To be done in Year 2

Major Task 3: Determine Arg-TCT-1-1 tRNA levels in adenocarcinoma versus NEPC patients.

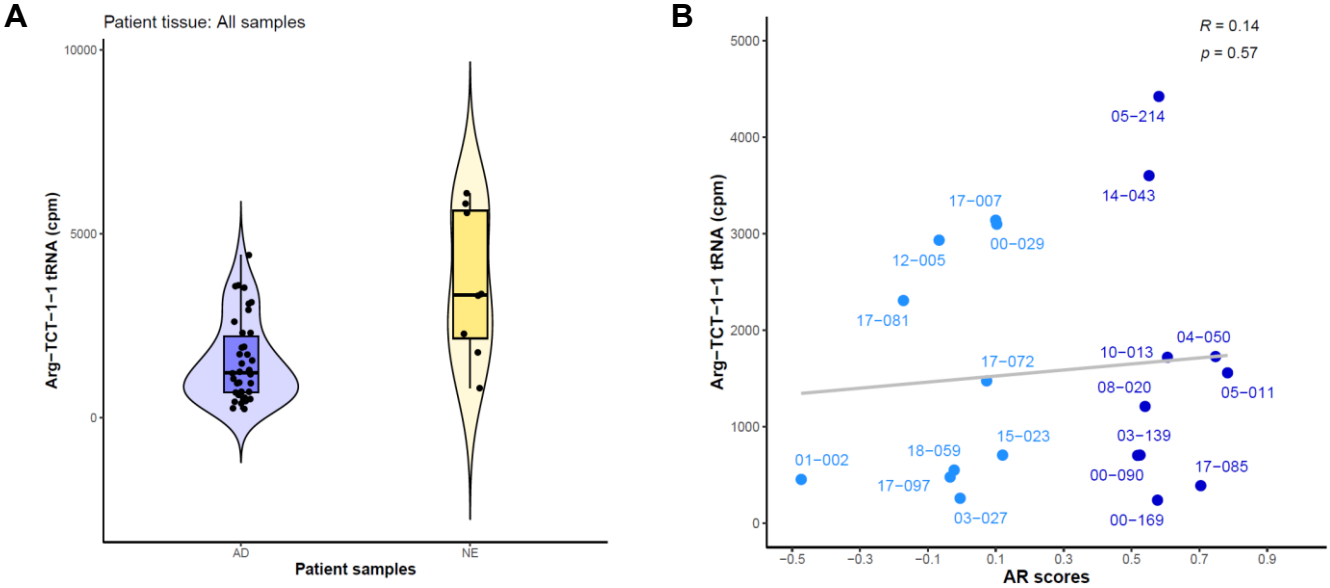

Fig 3A. tRNA-seq of patient tissues indicates that there is no association between the disease states (AD and NE) and Arg-TCT-1-1 tRNA expression. **B.** Among AD patient samples, there is a weak correlation (R = 0.067) between the AR scores (calculated based on the RNA-seq analysis of AR target genes) and the expression of Arg-TCT-1-1 tRNA. The AR-high group (blue) has a slightly higher Arg-TCT-1-1 tRNA levels compared with the AR-low group (light blue).

There is no correlation between histological subtypes of prostate cancer and Arg-TCT-1-1 tRNA levels (**Fig. 3A**). However, among the adenocarcinoma samples, there is a weak correlation between the AR score and Arg-TCT-1-1 tRNA expression (**Fig. 3B**).

Specific Aim 2: Interrogate the functional topology of prostate cancer oncogene and tumor suppressor 5' UTRs.

Major Task 1: Identify and characterize the functional 5' UTR topology of prostate cancer-associated genes.

The screen identified 5' UTR regions of prostate cancer-associated genes that are important for cell viability (**Fig 4**).

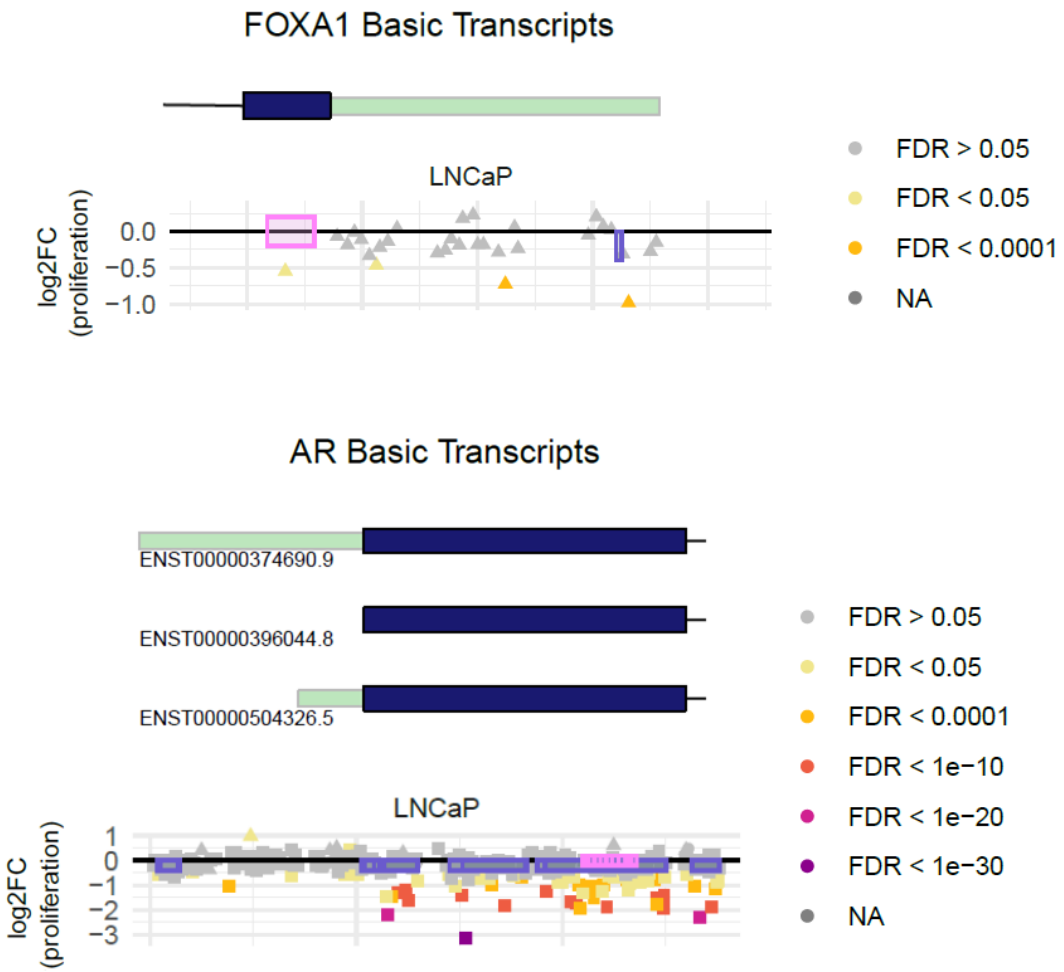

Fig 4. 5' UTR CRISPR screen in LNCaP reveals unexplored 5' UTR elements that are critical for prostate cancer cell growth and proliferation. The colored points below the transcripts indicate sgRNAs with significant effects on cell proliferation (FDR < 0.05). In the examples above, both FOXA1 and AR genes have 5' UTR sgRNAs (green segments in the mRNA transcripts) that are critical for cell proliferation. sgRNAs targeting the coding region (navy segments in the mRNA transcripts) are used as positive controls as these genes are known to be important for prostate cancer cell growth and proliferation.

Major Task 2: Investigate the function of cancerous 5' UTR cis-regulatory structures through their interactions with trans-acting factors.

To be done in Year 2

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

The project provided learning opportunities for undergraduate interns by performing basic molecular biology experiments under my guidance. The training helped them gain both theoretical knowledge and lab techniques.

The project allowed me to attend the annual Prostate Cancer Foundation (PCF) conference in 2023, where I interacted and shared ideas with expertise in the prostate cancer field.

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

Nothing to Report

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.

In Year 2, I will complete Specific Aim 1 – Major Task 2 and Specific Aim 2.

4. **IMPACT:** D*escribe 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:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What was the impact on other disciplines?
Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What was the impact on technology transfer?
Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What was the impact on society beyond science and technology?
Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

5. CHANGES/PROBLEMS:
The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No. The application for Not Human Subject research was approved 2/1/2023. OHRO Cadaver activity approval received 4/25/2023.

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:** *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."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*

- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Yeon Soo Kim
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-9677-374X
Nearest person month worked: 12

Contribution to Project: Dr. Kim has performed all the work on this project.
Funding Support: N/A

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

Yeon Soo Kim:
This DOD Fellowship was added to “Current”.

Andrew Hsieh:
The following previously active awards have moved to Past: Kleberg Foundation, Emerson Collective
The following pending proposals are awarded (moved to Current section): DOD-AMRA HT9425-23-1-1055 (CA220034), PNW SPORE Pilot. HB Pilot (in Kind)
NIH R37: the end date has been extended to 11/30/2025.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
QUAD CHARTS:
N/A

9. APPENDICES:

N/A