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Identification of Androgen Receptor-Specific Enhancer RNAs

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14. ABSTRACT

The major goal of this application is to determine whether prostate cancer cells express enhancer RNAs in response to androgen treatment such that these enhancer RNAs may serve as novel biomarkers for prostate cancer diagnosis and prognosis. There are two Tasks in this application. First, we will perform global run-on assay and deep sequencing to identify AR-specific enhancer RNAs. Second, we will validate them and then determine their functional significance. The success of this study may lead to novel clinical applications such as identification of biomarkers or therapeutic targets for prostate cancer, especially for castration resistant prostate cancer.

15. SUBJECT TERMS
lncRNA, eRNA, biomarker

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Table of Contents

Introduction

The androgen receptor (AR) is a nuclear receptor transcription factor required for normal prostate development and prostate cancer pathogenesis. AR is a very important druggable target. For instance, androgen deprivation therapy (ADT) is a frontline treatment for advanced prostate cancer. However, despite initial response, patients become resistant to ADT due to castration resistance. Extensive studies have suggested that multiple factors contribute to castration resistance. We hypothesize that long non-coding RNAs (lncRNAs) is one of the important factors, especially those AR specific enhancer RNAs (eRNAs), contributing to castration resistance. In this application, we proposed to determine whether we can identify such AR specific eRNAs.

There are two major tasks for this project:

Task 1. Perform global run-on assay and deep sequencing to identify AR-specific enhancer RNAs

Task 2. Validation and functional significance of AR specific eRNAs

We have finished Task 1 so far. Major findings are reported below

Body

Profiling identified several potential eRNAs that are induced by androgen.

As newly discovered molecules, eRNAs are poorly characterized. Little is known whether they are dysregulated in prostate cancer. Thus, our first step was to characterize these new molecules by profiling. Results indicate that a number of eRNAs are upregulated in response to androgen treatment. Bioinformatics analysis further narrows down three interesting eRNAs and their sequences are shown below.

AR-eRNA-#1 (117 bp)

caccattacacaggactgcctttcaggaaggttctctgccactggaaaagggtacagcttttctagagggcaatagg
tgatttctattaaaatatcaaagacacactccttgcccctt

This eRNA is localized to chr14:24408289-24408405. Literature search indicates that it is homologous to AS1eRNA that may enhance another lncRNA called *DHRS4-AS1*

AR-eRNA-#2 (504 bp)

```
>hg19_dna range=chr18:54746000-54746503
tgggaaaaagacctgcagacggcctgccaggggccccccggctctgtgtg
cccaggtgaggccttaggaggaggcagcagtgggtgtcggggaggggatt
caggcctcctgggctgtccagggctttagccaaccatcaatgggcgaaac
agagccgctatcctgacttccacatacaagcattagaaatctcctcttgt
acaaaggaagaccatatcccaaccatacatgtgcacttgactcaatggct
gtgctatcaagtgggagggacatccagccctgggtccccccggtcctagc
catctgtatgtactaaggacactgtctctcagagcctgcaaacatgccat
ccctgcaatacccatgagtcactcaccttctcagaggagccttttctggc
aattctgttgaatgttgccccaaccctgtattccctacacactgcctgct
ttattttctctatagctgtggtcacaattggacggactgtatattttact
tatt
```

Bioinformatics analysis mapped this sequence at the upstream of the potential promoter of Linc-RoR. Linc-RoR is known for its role in stem cell maintenance. Our lab has also demonstrated that Linc-RoR is a repressor for p53 in response to DNA damage. Furthermore, Linc-RoR increases Myc mRNA stability through interaction with hnRNP I and AUF1. Thus, identification of this potential eRNA that may regulate Linc-RoR will provide new insight into lncRNA regulation in prostate cancer, further implying that Linc-RoR might be also controlled by androgen.

AR-eRNA-#3 (468 bp)

```
>hg19_dna range=chr19:51354315-51354782
tgcctcccaacactgttcagccagagccttccacccttgtctgcaggaca
gtctcaacgttccaccattaaatacttcttctgtcacatcctgcttattt
atgcctaaccaaggttctaggtcccgatcgactgtgtctggcagcactcc
actgccaaacccagaataaggcagcgctcaggatcccgaaggggcatggc
tggggatcagaacttctgggtttgagtgaggagtgggtccaccctcttga
atttcaaaggaggaagaggctggatgtgaaggaactgggggagggaaagt
gtcagttccgaactcttaggtcaatgagggaggagactggtaaggtccca
gctcccgaggtactgatgtgggaatggcctaagaatctcatatcctcagg
aagaaggtgctggaatcctgaggggtagagttctgggtatatttgtggct
taaggctctttggcccct
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Bioinformatics analysis indicated that this sequence is homologous to KLK3e which is a known androgen inducible eRNA. Of interest, KLK3e has been shown to positively regulate Kallikrein-related peptidase 3 (KLK3), which codes for prostate-specific antigen (PSA); PSA is a well-known AR-regulated gene.

Together, these results suggest that there exist a group of AR-regulated eRNAs which may impact prostate tumorigenesis. Thus, further characterization of these eRNAs will provide a better understanding of AR-mediated gene regulation and castration resistance, and thus it will help design a better strategy for prostate cancer therapy.

Future work

We will focus on these three candidate eRNAs to perform experiments as stated in Task 2 of SOW. In brief, we will first verify their expression by qRT-PCR. Based on their expression in response to androgen, we will determine whether they are differentially expressed in androgen sensitive and androgen insensitive (i.e., castration resistant) cell lines such as LNCaP and 22Rv1 cells. Finally, we will then determine whether they play a role in castration resistance.