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ChIP-seq Data Reveals an Association between Androgen Receptor Protein and NORAD Promoter

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BACKGROUND

Androgens and Androgens Receptor (AR) in Prostate Cancer

- Prostate cancer is the most common cancer that occurs in men [1].
- Androgens and AR are primary factors of the disease progression
  - Androgens trigger prostate cancer cells to divide + multiply.
  - AR is activated by binding to androgens.

- To suppress the cancer, androgen deprivation therapy can block androgen synthesis [2].
- The cancer can cause AR mutation
  → survive without binding to androgens [3]

Non-Coding RNA Activated by DNA Damage (NORAD)

- Highly abundant + conserved long non-coding RNA
- Promotes metastasis + cell proliferation of prostate cancer cells [4]
- Reduces response to chemotherapy
  → death rate of cancer patients ↑

Key observation

Castration of mice → The expression of **NORAD** ↑ and **AR** ↑
(C. Fletcher, personal communication)

Hypothesis:

NORAD expression may be regulated by **AR**

Aim:

- To investigate an interaction and relationship between NORAD and AR protein

MATERIALS AND METHODS

- RIP-seq, RNA-seq, and ChIP-seq data of LNCaP cell line

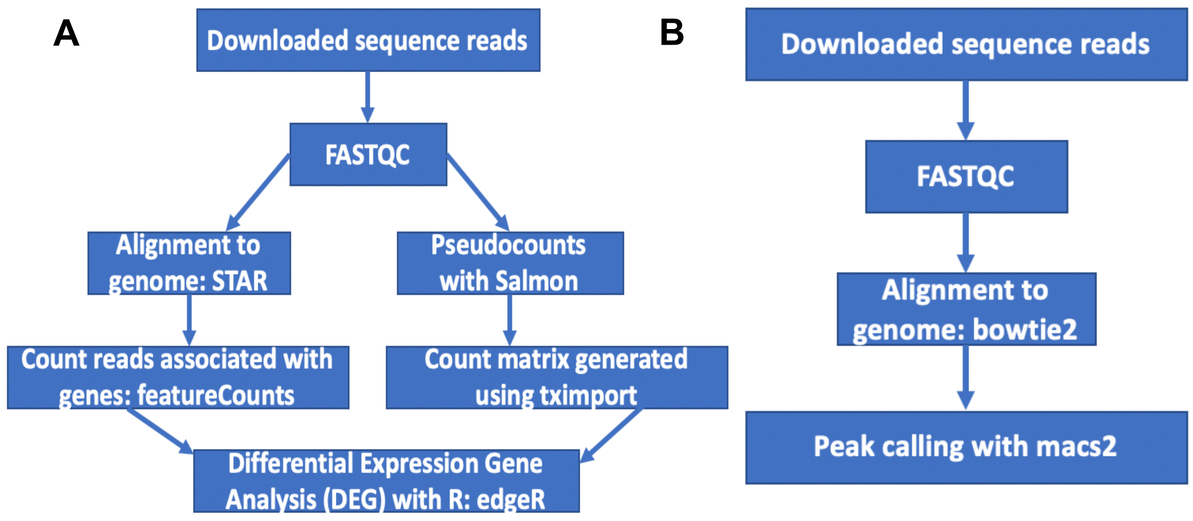

Figure 1. Data analysis pipelines. (A) Analysis pipeline for RIP-seq and RNA-seq data. **(B)** Analysis workflow for ChIP-seq data.

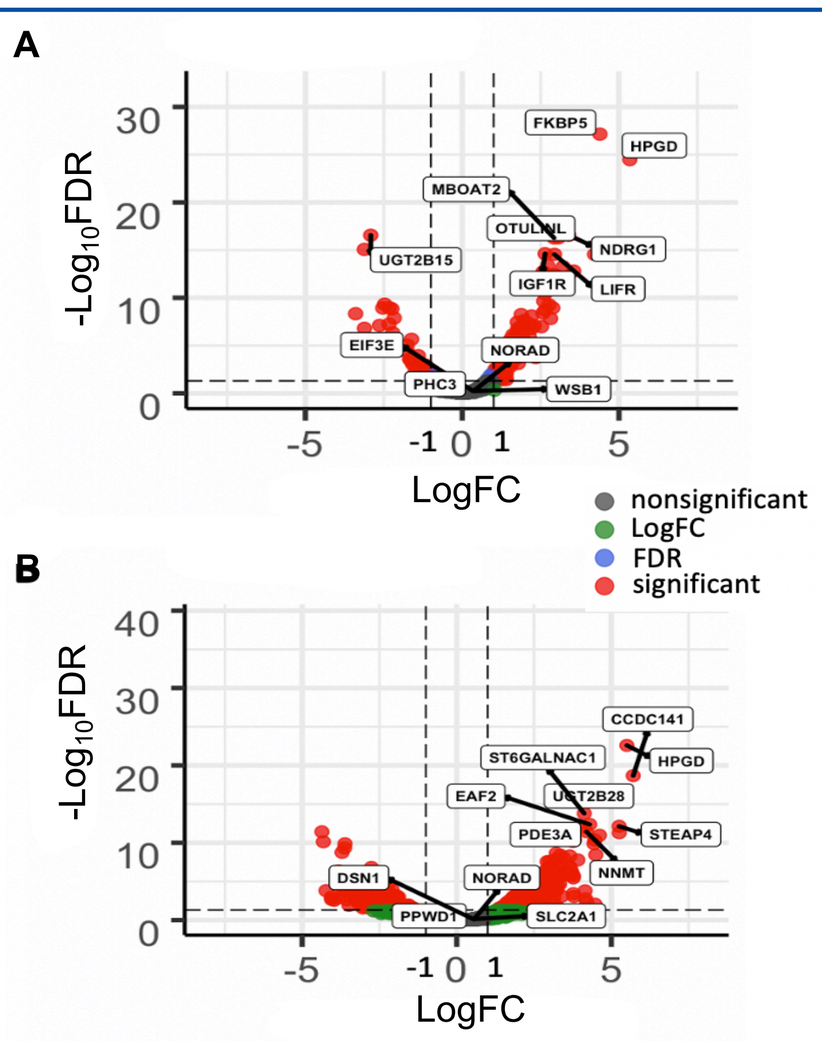

Figure 3. DEG analysis from RNA-seq data using STAR and Salmon aligners.
The log ratio of expression levels between untreated and treated androgen samples (x axis) is plotted against –logFDR. Significantly differentially-expressed genes between the two conditions based on the specified criteria of FDR < 0.05 and |log2FC| >= 1.0.

(A) A volcano plot depicting DEG analysis using STAR. NORAD was not significant (FDR = 0.59, logFC = 0.36)

(B) Volcano plot representation of DEG analysis using Salmon. NORAD was not considered significant as FDR = 0.74 and logFC = 0.56.

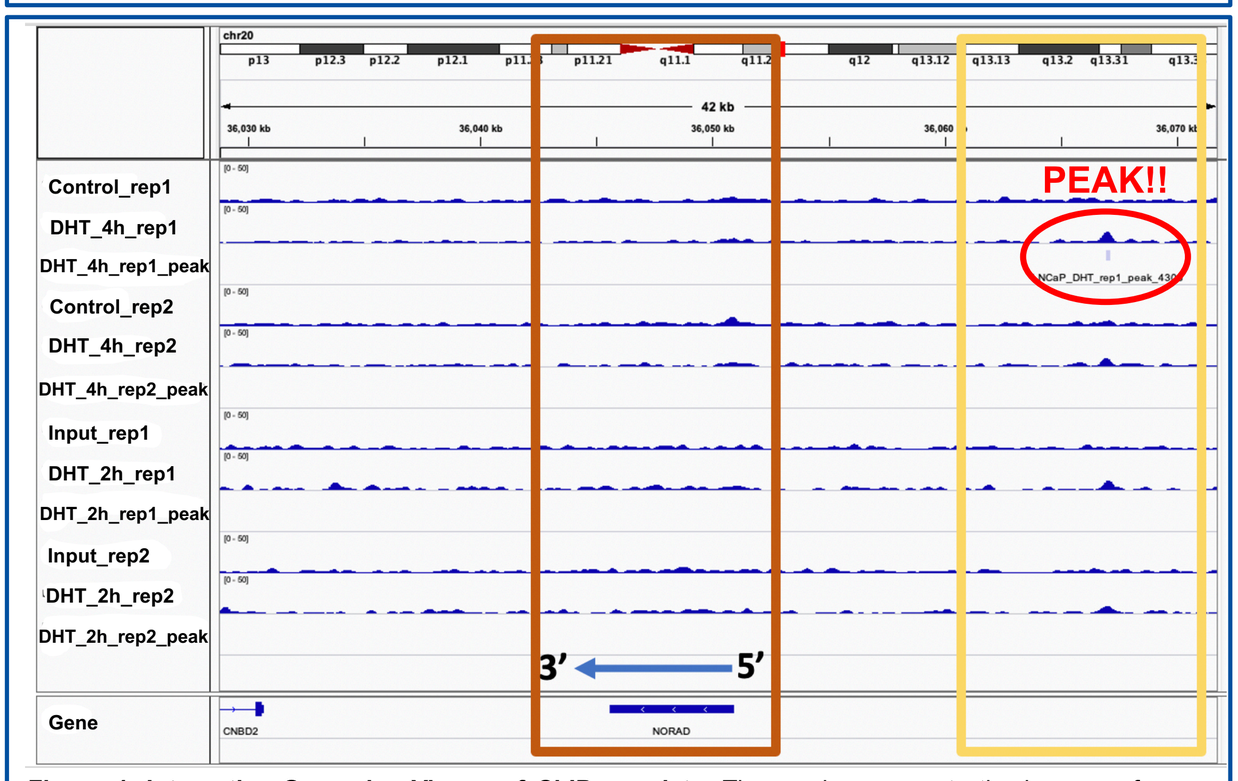

Figure 4. Integrative Genomics Viewer of ChIP-seq data. The x-axis represents the human reference genome, whereas the y-axis represents the number of sequence reads from the samples that match the human genome. Peak calling analysis was conducted using macs2. Mapped reads of treatment (DHT_2h and DHT_4h) and control (Control and Input) samples were compared in order to identify peak regions that aligned with the human genome. Unlike DHT_2h, DHT_4h samples were treated with 10 nM DHT for four hours, whereas DHT_2h samples were treated with 100 nM DHT for two hours. DHT_4h_rep1_peak, DHT_4h_rep2_peak, DHT_2h_rep1_peak, and DHT_2h_rep2_peak represent peaks that indicate AR binding sites in the DNA sequence when FDR < 0.05. One peak was identified in the DHT_4h_rep1 sample (red circle); no other peaks were identified (yellow box)

RESULTS

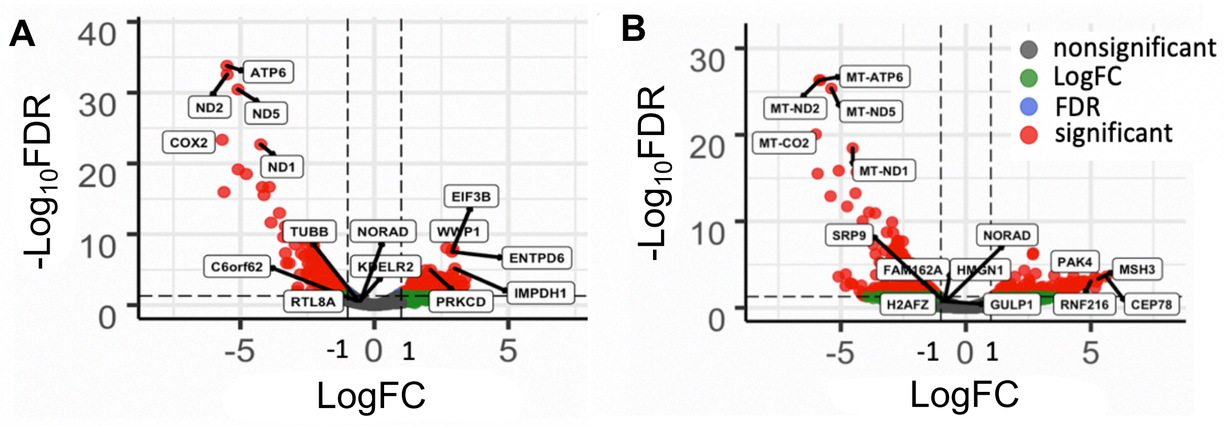

Figure 2. DEG analysis from RIP-seq data using STAR and Salmon aligners. The log ratio of expression levels between anti-AR and anti-IgG samples (x axis) is plotted against –logFDR. **(A)** A volcano plot depicting DEG genes using STAR. NORAD was not significant (FDR = 0.25, logFC = -0.52). **(B)** Volcano plot representation of DEG analysis using Salmon. NORAD was also not significant (FDR = 0.18, logFC= -0.81). Significantly differentially-expressed genes between the two conditions based on the specified criteria of FDR < 0.05 and |log2FC| >= 1.0.

CONCLUSION

- There are no supporting evidences from RIP-seq and RNA-seq data that NORAD is associated with AR protein
- ChIP-seq in LNCaP showed a peak at the area of NORAD promoter
  → **possible AR binding upstream of NORAD**

Next Step

Future work:

- To clarify AR binds to the NORAD promoter
  - ChIP-PCR for AR association with NORAD promoter and enhancer regions across a timecourse of androgen treatments and in different prostate cancer cell lines.

REFERENCES

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4. H. Zhang, H. Guo, Long non-coding RNA NORAD induces cell proliferation and migration in prostate cancer. J Int Med Res 47, 3898-3904 (2019).