Elucidating the function of 3'UTR somatic mutations in advanced prostate cancer

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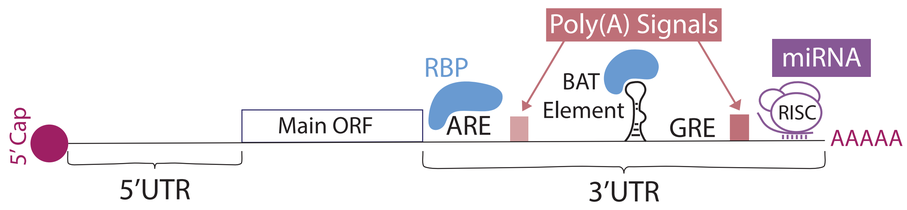
ABSTRACT

Metastatic, castration-resistant prostate cancer (mCRPC) is an advanced form of prostate cancer with a high mortality rate due to a current lack of treatment options. While much is already known about how mutations in protein-coding sequences across the genome affect prostate cancer, somatic mutations occurring in the 3' untranslated regions (3'UTRs) of genes are largely unstudied. The 3'UTR controls post-transcriptional gene expression through recruitment of trans-acting factors such as RNA-binding proteins (RBPs) and microRNAs (miRNAs), which themselves are known to be oncogenes and tumor suppressors in many cases. To better understand the role of 3'UTR mutations across prostate cancer, we have created a database of 3'UTR somatic mutations in 185 patients with mCRPC, discovering 14,497 single-nucleotide mutations throughout the 3'UTRome. In order to functionally assay these variants, we have developed a massively parallel reporter assay (MPRA) able to determine the effect of thousands of patient somatic mutations on post-transcriptional gene expression. In this approach using polysome profiling, we are able to measure whether each of 6,892 patient mutations found in recurrently mutated 3'UTRs affect steady-state transcript level and translation efficiency. This deep functional assessment of thousands of 3'UTR mutations allows us to uncover patterns in mutation functionality, including their association with known and unknown RNA motifs. Investigation into how the resultant gene expression changes from 3'UTR mutations affect prostate cancer pathogenesis, such as cancer growth or response to treatment, is also underway. This work represents an unprecedented view of the extent to which disease-relevant 3'UTR mutations affect translation efficiency, and cancer phenotypes, expanding the boundaries of functional cancer genomics and potentially uncovering novel therapeutic targets in previously unexplored regulatory regions.

REGULATORY ROLES OF THE 3'UTR



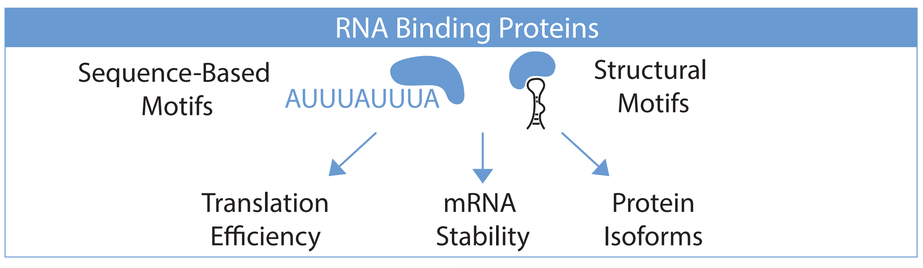
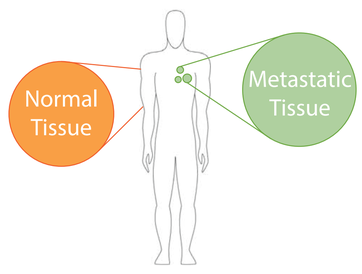
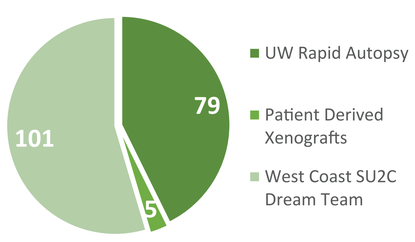


Figure adapted from: Schuster and Hsieh (2019) Trends in Cancer

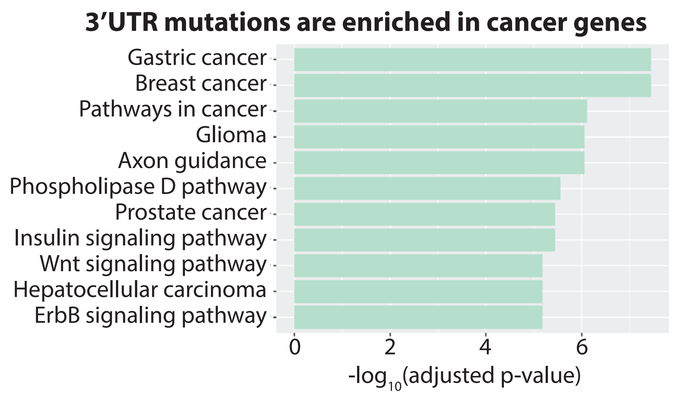
PATIENT SEQUENCING

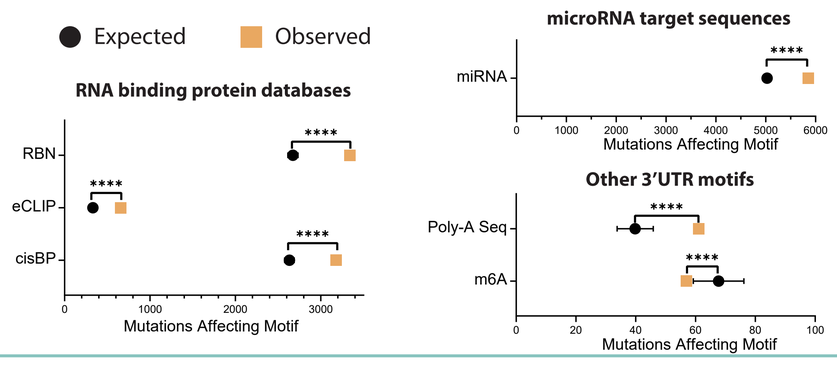




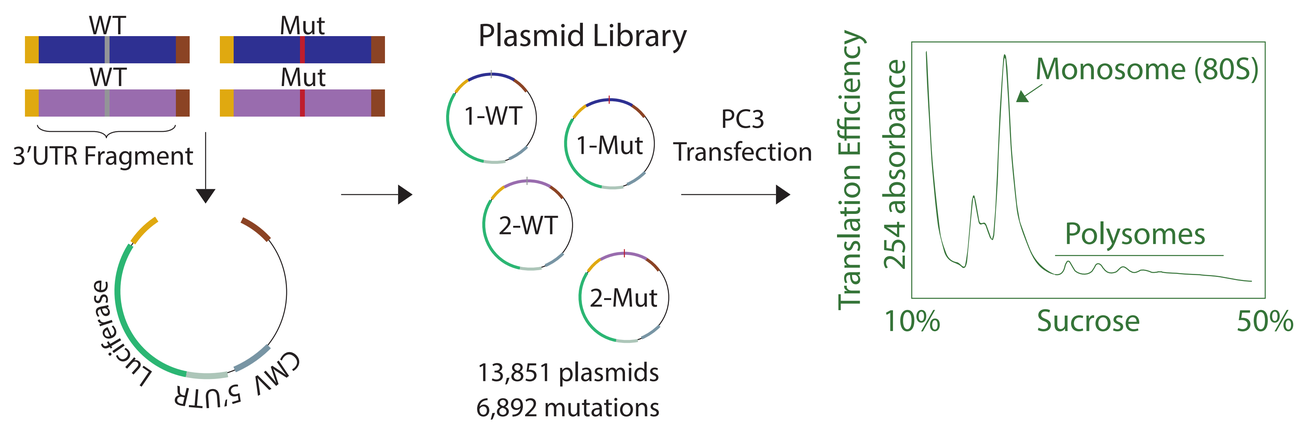
Patient samples from UW TAN (Rapid Autopsy), UW Lucap (PDX), and West Coast SU2C (Quigley et al. Cell 2018).

LANDSCAPE OF 3'UTR MUTATIONS IN PROSTATE CANCER



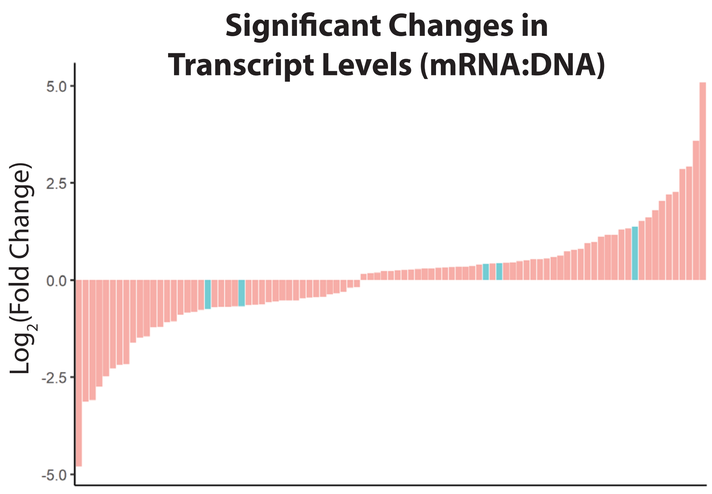


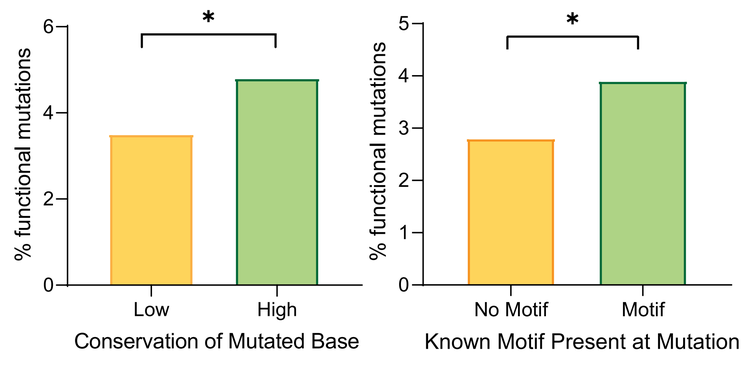
A MASSIVELY PARALLEL REPORTER ASSAY OF PROSTATE CANCER 3'UTR MUTATIONS

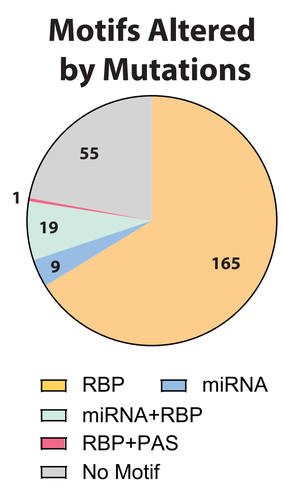


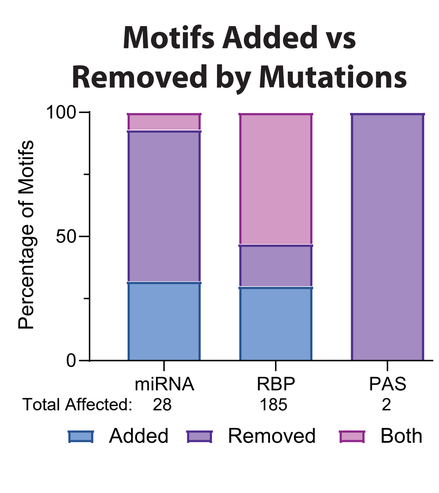
A massively parallel reporter assay to test the effects of 6,892 mutations on translation efficiency and transcipt levels using polysome profiling. A plasmid library is transfected into PC3 prostate cancer cells and polysome profiling used to separate total mRNA from monosome and polysome-bound mRNA to determine changes in post-transcriptional gene expression.

3'UTR MUTATIONS CAUSE CHANGES IN TRANSCRIPT LEVELS AND TRANSLATION EFFICIENCY ACROSS PROSTATE CANCER



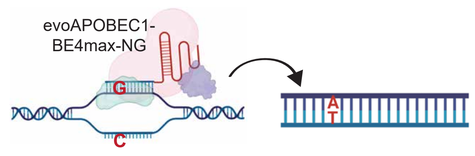


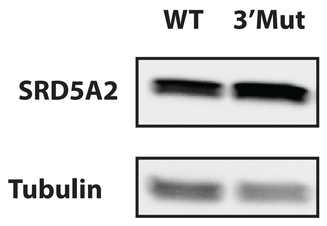


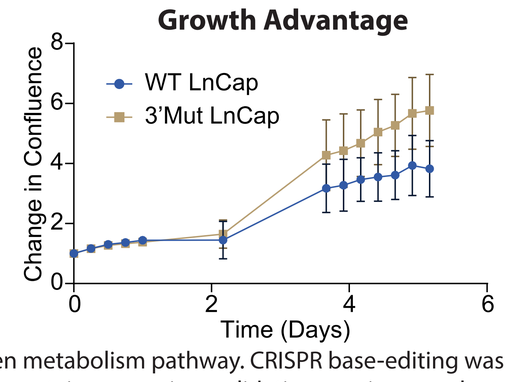


Many 3'UTR mutations significantly affect (FDR<0.1) either transcript level (93) or translation efficiency (174). Mutations in genes known to be associated with cancer are marked in blue. Functional mutations are more often in areas with high sequence conservation & known motifs (chi-squared test, *=p<0.05). Functional mutations largely alter RBP sites and remove more often than add motifs.

A PATIENT 3'UTR MUTATION IS FUNCTIONAL IN ENDOGENOUS LOCUS

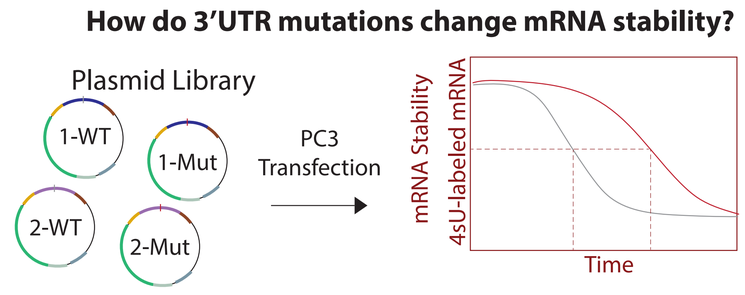


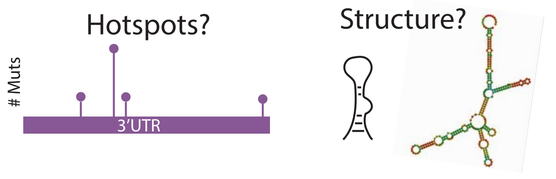


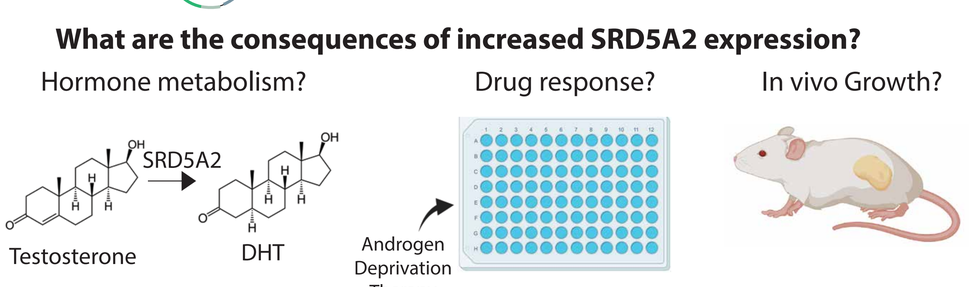


Validation of a patient 3'UTR mutation in SRD5A2, a prostate cancer-related gene in the androgen metabolism pathway. CRISPR base-editing was used to introduce mutation into the endogenous 3'UTR locus, resulting in a 1.8x- fold increase in protein expression, validating previous results from exogenous plasmid-based assays in a more complete and relevant context. This SRD5A2 3'Mutant cell line also shows increased growth.

WORK IN PROGRESS







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