

AI-Guided Functional Interrogation of Rare Mutations Reveals Hidden Drivers of Therapy Resistance in Prostate Cancer

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Background: Cancer genome sequencing has identified millions of somatic mutations, yet functional interpretation remains dominated by recurrent alterations found across many patients. Individually rare mutations are therefore frequently deprioritized, although they collectively represent a substantial fraction of the cancer mutational landscape and may converge on common genes and pathways. We hypothesize that rare mutations comprise a hidden class of functional drivers that promote prostate cancer progression and resistance to androgen receptor (AR)-targeted therapy.

Methods: We developed ATHENA, an AI-assisted framework that integrates whole-exome sequencing, RNA sequencing, functional impact prediction, and clinical information to prioritize candidate functional mutations. ATHENA was first evaluated in an isogenic prostate cancer progression model derived from LNCaP cells. Candidate mutations were reconstructed using adenine and cytosine CRISPR base editors and tested under androgen-deprived conditions. We are extending this strategy to metastatic castration-resistant prostate cancer patient datasets, followed by large-scale pooled base-editing screens under androgen deprivation plus enzalutamide. Functional hits will be analyzed at the mutation, gene, pathway, and network levels and subsequently challenged in genetically diverse Yale Urology Prostate Organoid (YUPO) models.

Results: ATHENA identified a refined set of high-confidence, transcriptionally supported candidate mutations acquired during prostate cancer progression. In a pilot analysis of metastatic patient samples, ATHENA recovered 94% of mutations reported in cBioPortal while identifying a substantially larger repertoire of transcriptionally supported variants that were absent from the published mutation catalog. For functional feasibility testing, guide RNAs were designed for 17 candidate genes; 16 of 17 successfully generated the intended base substitutions. Precise editing of representative TP53(Y234H) and SMARCD3(R140W) mutations was confirmed by Sanger sequencing. Functional screening identified four single-nucleotide substitutions that consistently enhanced growth under AR-targeted selective pressure, supporting the feasibility of systematic mutation-level discovery.

Conclusions: These studies establish an integrated AI-plus-base-editing platform for directly testing patient-derived rare mutations in prostate cancer. By shifting discovery from recurrence of individual mutations to functional convergence at the gene and pathway levels, this approach may reveal previously unrecognized drivers of therapy resistance and provide a generalizable framework for interpreting the long tail of rare mutations across cancers.

Funding Acknowledgements: National Cancer Institute/National Institutes of Health: 1K22CA299598-01 (SD); Department of War: HT9425-26-1E119 (SD); Prostate Cancer Foundation: 25YOUN22 (SD); National Cancer Institute/National Institutes of Health: R37CA258730, R01CA288820, R01CA292949 P. Mu; Prostate Cancer Foundation: 25CHAL05, 17YOUN12 P. Mu.

Conflicts of Interest Disclosure Statement: No authors have conflict of interest to disclose.