RNA Epitranscriptomic editing Screening Identifies Functional m^{6}A Sites in Prostate Cancer

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Background: Epitranscriptomic modifications such as N6-methyladenosine (m^{6}A) are increasingly recognized as critical regulators of cancer biology. While individual m6A regulators and sites have been linked to tumor progression, a systematic functional interrogation of m6A modifications in prostate cancer has been lacking.

Methods:
We developed an RNA epitranscriptomic-editing screening platform by combining dCasRx-*METTL3* with a pooled sgRNA library of 12,000 guides targeting 2,120 clinically relevant m^{6}A sites derived from prostate tumors and cell lines. This high-throughput approach enabled transcriptome-wide assessment of functional m^{6}A modifications.

Results:
Our screening identified 360 sgRNAs with significant effects on cell proliferation, with validation confirming the reproducibility of key hits. Among these, *CHD9* emerged as the top candidate, where m^{6}A modification at its last exon enhanced protein translation through recognition by YTHDF1/3 readers. Functional studies revealed that CHD9 acts as a tumor suppressor: silencing or demethylation accelerated cell proliferation and tumor growth in vitro and in vivo, while higher *CHD9* expression correlated with favorable prognosis in patient cohorts.

Conclusions:
This study represents the first transcriptome-wide functional screening of m^{6}A modifications in prostate cancer, uncovering *CHD9* as a novel tumor suppressor regulated at the epitranscriptomic level. Our findings establish RNA epitranscriptomic-editing as a powerful tool for systematically identifying functional RNA modifications, with broad implications for targeting cancer vulnerabilities.

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Conflicts of Interest:
The authors disclose no conflicts.