

**Title: A non-enzymatic role for METTL3 as an Androgen Receptor co-regulator that promotes prostate cancer proliferation**

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**Background:** Most treatment for prostate cancer (PCa) focuses on targeting the Androgen Receptor (AR), the main oncogene responsible for driving most tumors. Despite these therapies targeting AR, most patients still succumb to AR-driven disease. One avenue garnering attention for targeting AR-driven, therapy-resistant disease is targeting epigenetic regulators that promote AR-activity; however, the importance of epitranscriptomic regulators, like those that modify mRNAs, is not well understood. Here, we identify a new role for the key catalytic subunit of the RNA N6-methyladenosine (m<sup>6</sup>A) transferase complex, Methyltransferase-like-3 (METTL3), as a transcriptional coregulator of AR.

**Methods:** Cell viability and m<sup>6</sup>A ELISA were assayed after METTL3 knock down or pharmacologic inhibition by STM2457 in non-transformed and cancer cell lines, with or without wild-type or catalytic inactive mutant overexpression. Biochemical co-immunoprecipitation and Nanobit-split luciferase assays were utilized to determine AR and METTL3 protein-protein interaction, and chromatin immunoprecipitation sequencing was utilized to assay genomic occupancy of AR and METTL3 under androgen starved and stimulated conditions with METTL3 knock down.

**Results:** METTL3 is upregulated in prostate tumors compared to normal tissue, and METTL3 protein is elevated in AR-expressing cell lines. Depletion of METTL3 significantly reduces proliferation of cancer cells and has no effect on the growth of non-transformed prostate epithelial cells, despite decreasing global m<sup>6</sup>A levels on mRNA. The catalytic activity of METTL3 is dispensable for the growth of both non-transformed and PCa cell lines, as pharmacologic inhibition of METTL3 does not inhibit proliferation, despite the reduction of global m<sup>6</sup>A on mRNA. Knock down of METTL3 has an additive effect with AR-antagonism by the anti-androgen enzalutamide in AR-positive PCa cells. Overexpression of both wild-type and catalytically inactive METTL3 mutants enhances cell viability and rescues cells in which METTL3 is knocked down. Finally, we report on direct interaction between AR and METTL3, their co-localization on chromatin, and reduced AR-cistromic occupancy within cells with METTL3 knockdown.

**Conclusions:** Together, these findings identify a non-enzymatic role for METTL3 in supporting AR-driven transcriptional programs and PCa proliferation. Ultimately, we suggest METTL3, through its interaction with AR, is an attractive therapeutic target for PCa.

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