Epitranscriptomic Gene Regulation by N6-Adenosine-Methyltransferase in Prostate Cancer

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Recent evidence has highlighted the role of N6-methyladenosine (m6A) in post-transcriptional control of both mRNA stability and translation efficiency. The N6-adenosine-methyltransferase (METTL3) is up-regulated in prostate adenocarcinoma (PCa), leading to the hypothesis that m6A plays an important role in the regulation of gene and protein expression in PCa. In this study we have produced the first epitranscriptome map of m6A in PCa using single-nucleotide m6A mapping (miCLIP) of benign (RWPE) and PCa (LNCaP) cell lines. We then generated PCa cell lines with stable and reversible drug-inducible METTL3 shRNAs allowing for precise timing and efficiency of the knock-down. Using these cell lines we combined ribosome footprint profiling with paired total RNA-seq in order to assess the role of m6A methylation in altering gene expression and translation. m6A is prevalent in PCa with a similar distribution to other mapped cell lines. Overall miCLIP identified over 10,000 transcripts with at least one m6A in the two cell lines, with many important genes for prostate cancer exhibiting a high number of m6A sites. Analysis of the RNA-Seq and ribosome footprinting data identified many genes dysregulated with METTL3 knock-down including most significantly a kinesin family protein involved in cargo transport along microtubules. Further gene set enrichment analysis has identified androgen receptor signaling pathways as also being regulated by METTL3. These results validate m6A as a potentially important additional mechanism of gene regulation in PCa. Further, by allowing for the identification of changes in expression at the protein level previously undetectable by gene expression analysis alone, these findings may lead to the discovery of novel biomarkers or pathways as potential targets for treatment.

The authors have no conflict of interest

This project is funded by a Prostate Cancer Young Investigator Award (18YOUN06) and by a Weill Cornell Medicine Prostate Cancer SPORE Developmental Research Project.