

Epitranscriptomic reprogramming and enzalutamide resistance in prostate cancer

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Background: Androgen deprivation therapies (ADT) such as enzalutamide are essential in the treatment of castrate sensitive metastatic prostate cancer (PCa). These ADTs increase the overall survival in patients, however after a median time of 2-3 years resistance to these treatments arises. Novel combination therapies with enzalutamide can extend cancer free survival by preventing, delaying or reversing emergence of treatment resistance.

RNA:m6A is the most common internal epitranscriptomic modification found on mRNA. The modification is dynamically regulated, the METTL3 methyltransferase deposits the modification and the FTO and ALKBH5 demethylases remove it. RNA:m6A has roles in transcript stability, splicing and translation regulation. We and others have implicated RNA:m6A and its regulators in PCa. There is evidence that targeting RNA:m6A may represent a novel approach to treat PCa. Novel small molecule inhibitors of METTL3 are in phase I clinical trials and derivatives of drugs and candidates such as entacapone, meclofenamic acid, brequinar and bisantrene that inhibit FTO are in development.

Methods: To investigate the role of RNA:m6A methylation in ADT response and resistance, castrate sensitive (LNCaP and VCaP) and resistant (LNCaP:C42) cell lines were treated with enzalutamide in short-term (six days) and long-term (six month) experiments respectively. The effect on transcriptome-wide distribution of RNA:m6A determined using methylation RNA-immunoprecipitation coupled with RNA sequencing. The consequences of enzalutamide induced changes in RNA:m6A distribution was further investigated by short- and long read sequencing.

Results: The RNA:m6A regulators, METTL3, FTO and ALKBH5, are dysregulated in PCa. The expression of METTL3 is higher in tumour tissue compared to non-malignant tissue and FTO expression is higher in the cytoplasm in tumour tissue as compared to non-malignant prostate tissue. Knockdown or pharmacological inhibition of these RNA:m6A regulators, has shown that m6A regulates the expression and splicing of androgen receptor (AR) and androgen signalling in PCa cells. Enzalutamide resistance in PCa cell lines resulted in changes to the transcriptome-wide distribution of RNA:m6A. In both short- and long-term enzalutamide treated cells expression of AR and RNA:m6A regulators were altered. In long term treated cells global changes occurred including alterations in cellular metabolism and pathways involved in AMPK, WNT and mTOR signalling. Further work is warranted to determine the potential benefit of METTL3 and FTO inhibitors for PCa patients.

Conclusions: Targeting m6A and its regulators may provide a novel mechanism to alter androgen and androgen receptor signalling offering therapeutic potential on its own and in combination with other therapies including enzalutamide.

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