A Novel MicroRNA-Based Mechanism of DNA Damage in Prostate Cancer Requires Interaction with Topoisomerases and Basal Transcription Machinery

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Agents that can induce irreparable DNA damage in PC cells may represent highly-effective therapeutics in the context of DNA repair-deficient mCRPC. We previously showed that microRNA-346 (miR-346) induces rapid and extensive transcription-dependent DNA damage in PC cells (including AR-null and p53-deficient models) - the first report of direct microRNA-induced DNA damage.

Of therapeutic relevance, high miR-346 levels are associated with improved PC patient survival. Further, miR-346 sensitises PC cells to DNA-damaging drugs including PARP, ATR, ATM and DNA-PKc inhibitors and chemotherapy, as well as inducing *in vivo* tumour regression as a monotherapy. Importantly, miR-346 does not induce DNA damage in non-malignant prostate cells. In what we have identified as a novel genome protection mechanism, miR-346 is efficiently turned over by a genome-protective lncRNA, NORAD, through a process called target-directed microRNA decay (TDMD). This is so effective that NORAD silencing increases mature miR-346 levels several thousand-fold, whilst wild-type but not TDMD-mutant NORAD rescues miR-346-induced DNA damage. This is important since NORAD is strongly correlated with adverse PC clinical outcomes and is reduced in mCRPC compared to matched primary tumours. This reduction represents a potential "Achilles' heel", exploitable through miR-346 therapeutic delivery.

To further characterize miR-346 mechanism of action, we performed INDUCE-seq genome-wide mapping of DNA double-strand breaks (DSBs): miR-346-induced DSBs are enriched at promoters (but not enhancers) bound by some of the most highly-transcriptionally active transcription factors in PC cells, including c-Myc, FOXA1, HOXB13, NKX3.1, and importantly, AR, resulting in target transcript downregulation. Notably, >90% of genes contained miR-346-induced DSBs, which were particularly enriched within the *BRCA2*-containing region of chromosome 13. ChIRP-mass spectrometry was used to identify nuclear miR-346 protein interactors in PC cells and results validated by RNA-IP. Top miR-346 interacting proteins (TOP1, TOP2B, XRCC1, BPTF, MED23) have key roles in DNA unwinding, chromatin remodelling, DNA repair, RNA methylation and basal transcription/ mediator complex function. Importantly, pharmacological inhibition of TOP2 and BPTF revealed their requirement for miR-346-induced DSBs. Consistent with this, miR-346 is unable to alter chromatin accessibility in isolation. Thus two mechanisms of miR-346-induced DNA damage were identified:

1) miR-346 drives transcriptional hyperactivation, R-loop formation and replication stress, leading to checkpoint activation, cell cycle arrest and apoptosis
2) miR-346 binds chromatin remodelling and repair factors to prevent repair of transient DSBs formed during active transcription and DNA replication to relieve torsional stress.

In conclusion, we have identified miR-346 as a potential novel PC therapeutic that may be particularly effective in the context of decreased NORAD observed in advanced PC, and in transcriptionally-hyperactive cancer cells. We are currently performing pre-clinical efficacy studies of prostate-targeted miR-346 therapeutics.

The authors gratefully acknowledge funding from Prostate Cancer Research (UK) and Prostate Cancer Foundation.

No conflicts of interest are reported.