

A MicroRNA Approach to Directly Induce Potent DNA Damage in Transcriptionally-Hyperactive Prostate Cancer Cells, and Delivery via Biocompatible 'Nanogels'

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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, extensive transcription-dependent DNA damage in PC cells (including AR-null and p53-deficient models) - the first report of direct microRNA-induced DNA damage. MiR-346 sensitises PC cells to DNA-damaging drugs including PARP/ATR/ATM/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. MiR-346 is efficiently turned over by a genome-protective lncRNA, NORAD, through a process called target-directed microRNA decay (TDMD). 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, HOXB13, NKX3.1, and importantly, AR, resulting in target transcript downregulation. Notably, >90% of genes contained miR-346-induced DSBs. ChIRP-mass spectrometry identified TOP1, TOP2B, XRCC1, BPTF, MED23 as nuclear miR-346 protein interactors in PC cells, with key roles in chromatin remodelling, DNA repair, RNA methylation and basal transcription. Consistent with this, GRO-seq reveals miR-346 transcription induction at 4h, but repression at 72h. Importantly, pharmacological inhibition of TOP2 and BPTF revealed their requirement for miR-346-induced DSBs, and mutagenesis studies show that miR-346 induction of DNA damage requires the miR's seed region and 5' and 3' nucleotides. Overall, 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, consistent with ATAC-seq observations that miR-346 alone is unable to alter chromatin accessibility alone.

We recently showed that nanogels (biocompatible crosslinked-polymeric nanoparticles) can outperform transfection reagents for PC cell delivery of miR-346, resulting in potent induction of DNA damage and proliferative inhibition. They have numerous benefits over LNPs including scalability, modular design, uniformity, flexible chemistry for incorporation of targeting moieties and environmentally-friendly single-step aqueous synthesis. Importantly, gradual degradation of redox-responsive structural elements within cells avoids drug endosomal retention. We are currently investigating their efficacy in PC explants.

In conclusion, we 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 nanogel-encapsulated miR-346 therapeutics.

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