MIR-346 INTERACTION WITH LONG NON-CODING RNA, NORAD, REVEALS A NOVEL GENOME PROTECTION MECHANISM AND MODULATES RESPONSE TO DNA-DAMAGING THERAPEUTICS IN ADVANCED PROSTATE CANCER

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Androgen receptor (AR) signalling is a key prostate cancer (PC) driver and drug-target, even in advanced 'castrate-resistant' disease (CRPC). High-throughput microRNA (miR) screening in AR-reporter CRPC cell lines revealed that miR-346 enhances AR 3'UTR stability and expression (WT and variant), proliferation, migration/invasion, represses EMT and increases apoptosis. Pathway analysis of AGO-PAR-CLIP-seq-identified miR-346 targets revealed enrichment of DNA replication/repair factors, including NORAD (NOn-Coding RNA Activated by DNA Damage), a highly-abundant, evolutionarily-conserved lncRNA. NORAD maintains mitosis, DNA damage repair (DDR), and chromosomal integrity by sequestering PUM1/2, whose activity increases turnover of DDR factors, and through formation of a TOPO2-containing complex critical for genome integrity. **We hypothesised that miR-346:NORAD interaction modulates DDR in PC.**

MiR-346 overexpression reduced NORAD activity by both decreasing NORAD levels and blocking NORAD:PUM1/2 interaction, leading to downregulation of PUM1/2 DDR targets. Functionally, miR-346 overexpression dramatically and dose-dependently induced DNA damage (phospho-γH2AX and 53BP1 foci), rescued by NORAD. Numbers of NORAD miR-346 binding sites are ten-fold higher than endogenous miR-346 copies in PC cells, and extended-complementarity miR-346 sites in NORAD drive target-directed miR-346 decay (TDMD). Indeed, siRNA-mediated NORAD silencing resulted in 2000-fold increase in miR-346 levels. Thus under steady-state conditions, **NORAD drives TDMD of miR-346 as a critical yet undescribed genome-protection mechanism**. When miR-346 levels increase, binding 'spreads' to NORAD regions with weaker, seed-only complementarity to repress NORAD:PUM2 interaction, increasing DNA damage. **MiR-346 also induces rapid DNA damage (<1h) and R-loop formation independently of NORAD through direct association with DNA.**

Since NORAD represses DNA damage and promotes DNA replication fidelity, we proposed that it could inhibit early PC development, but reduce response to DNA-damaging therapeutics (chemotherapy, PARP inhibitors). Thus miR-346 would represent a DNA damage-sensitising agent. Indeed, **miR-346 significantly increased efficacy of PARPi and Carboplatin** *in vitro*, and high NORAD levels and activity were associated with significantly reduced patient survival. Further, a robust NORAD activity score (NAS) significantly correlated with DDR across multiple PC patient cohorts. **MiRs that bind NORAD, and correlate with NORAD expression and activity *and* DDR are dysregulated in chemo-resistant vs -responsive mCRPC patient plasma**. Of note, PTEN and MIR346 are located 3MB apart on chr10, and 85% of PC patients have matching PTEN and MIR346 CN status, suggesting they may be co-lost in PC. Evidence supports links between NORAD and AR signalling – NORAD levels are correlated with AR levels/activity in mCRPC, and elevated following castration of mice harbouring patient-derived xenografts.

In conclusion, NORAD acts as a 'Guardian of the Genome' through TDMD of the potent DNA damager, miR-346. NORAD:miR-346 interaction modulates response to chemotherapy and PARPi, and alters activated T-cell infiltration. Since DDR and immune activation are major pathways driving therapy response, this may have important implications for PC treatment selection and patient stratification.

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