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) inhibitor screening in AR-reporter CRPC cell lines revealed that miR-346 inhibition reduces 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. These include 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.

AGO-PAR-CLIP and *in silico* analyses identified ten miR-346-binding sites in NORAD, several immediately adjacent to PUM1/2 binding elements. 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), which was rescued by NORAD. Interestingly, quantification revealed numbers of NORAD miR-346 binding sites to far exceed endogenous miR-346 copies in PC cells. Further, two 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. We propose that 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.

Since NORAD represses DNA damage and promotes DNA replication fidelity, we proposed that it could inhibit early PC development, but also reduce response to DNA-damaging therapeutics (e.g. chemotherapy, PARP inhibitors) in CRPC. Thus miR-346 would represent a DNA damage-sensitising agent. Indeed, miR-346 significantly increased efficacy of PARPi and Carboplatin in cell proliferation assays. In further support, a robust NORAD activity score (NAS) significantly correlated with DNA damage repair across multiple PC patient cohorts. Low NAS associated with increased patient survival, likely representing improved efficacy of chemotherapy in DNA repair-deficient patients.

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.

No conflicts of interest are reported.

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