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

Claire E. Fletcher, Wei Yuan, Lin Deng, Damien A. Leach, Ieva Eringyte, Antje Neeb, Ines Figueiredo, Pasquale Rescigno, Sean McGuire, Xavier Gidrol, Eric Sulpice, Stephanie Combes, S. George Zhao, Felix Feng, Ian Mills, Johann De Bon, and Charlotte L. Bevan

1 Imperial Centre for Translational and Experimental Medicine, Department of Surgery & Cancer, Imperial College London, UK
2 Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, Sutton, UK
3 Department of Radiation Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA
4 Université Grenoble Alpes, CEA, INSERM, BIG, BGE, Grenoble, France
5 Departments of Urology and Radiation Oncology, University of California San Francisco, CA, USA
6 Nuffield Department of Surgical Sciences, University of Oxford, John Radcliffe Hospital, Oxford, UK

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 IncRNA. 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.

No conflicts of interest are reported.

The authors gratefully acknowledge funding from The Prostate Cancer Foundation, Prostate Cancer UK, Movember and The Rosetrees Trust.