Long Non-Coding RNAs Linking AR Activity and DNA Damage in Prostate Cancer

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Background and Methods: Androgen receptor (AR) signalling is a key driver of prostate cancer (PC) growth, even in advanced 'castrate-resistant' prostate cancer (CRPC). We thus hypothesised that miRs may modulate AR activity in lethal CRPC, and represent novel therapeutic targets. A high-throughput miR inhibitor screen in hormone-responsive and -resistant PC cell lines stably expressing a luciferase-based AR reporter identified eighty miR inhibitors to significantly alter AR activity. AR signalling also regulates the DNA damage response (DDR) and tumour-suppressor genes, *BRCA1, BRCA2* and *p53*. Reciprocally, *BRCA1 and BRCA2* enhance ligand-dependent AR transactivation. Pathway analysis of AGO-PAR-CLIP-seq-identified AR-modulatory miR targets revealed enrichment of DNA replication and repair factors. AGO-PAR-CLIP-seq identified two highly-abundant, evolutionarily-conserved long non-coding RNAs (lncRNAs) as AR-modulatory miR-346 targets in multiple PC cell lines. lncRNA-1 maintains mitosis, DNA repair, and chromosomal integrity by tethering and repressing Pumilio proteins, PUM1/2, whose activity increases turnover of DNA repair factors. We hypothesised that miR-346 and its targeted lncRNAs may link AR activity and DDR in PC.

Results: Inhibition of miR-346 or -361-3p significantly reduced AR activity, mRNA and protein levels, and target gene expression by up to 90%, with opposing effects observed for miR overexpression. MiR-346/-361-3p inhibition increased apoptosis, dramatically reduced cell growth and inhibited PC EMT, migration and invasion. Inhibition of AR-modulatory miRs showed additive effects with AR silencing or Enzalutamide, suggesting potential combinatorial applications for PC treatment. Silencing of miR targets (e.g. tumour suppressors, ARHGDIA and TAGLN2), phenocopies effects of miRs, demonstrating physiological relevance. MiRs were found to directly and inversely correlate with AR status and target genes expression, respectively, in patient tumours.

AGO-PAR-CLIP identified a miR-346 binding site in lncRNA-1 immediately adjacent to a PUM response element (PRE). MiR-346 reduced levels (but not localisation) of lncRNA-1, an effect rescued by miR-346 inhibitor and abrogated by miR-346 binding site mutation. In addition, significant overlap was observed between DDR factors downregulated by PUM1/2 and AGO2-PAR-CLIP-identified miR-346 targets. Indeed, qRT-PCR revealed direct and indirect (via lncRNA-1) miR-346 regulation of both PUM-target and non-PUM target DDR transcripts, suggestive of miR-346 regulation of DDR both independent from, and attributable to, lncRNA-1. Both miR-346 overexpression and lncRNA-1 silencing significantly induced DNA damage (phospho-γH2AX) and loss of MCM4 DNA replication factor, with opposing results for lncRNA-1 overexpression. lncRNA-1 significantly decreased 22RV1 PC cell growth, and significantly correlated with p53 mutation status and decreased survival in PC patients.

Conclusions: AR-regulatory miR-346 targetting of lncRNAs induces DNA damage through hyper-activitation of PUM proteins and may have implication for DDR-targetting PC therapeutics.

Conflicts of Interest: None

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