Identification of miR-30b-3p and miR-30d-5p as direct regulators of Androgen Receptor Signaling in Prostate Cancer by complementary functional microRNA library screening

Binod Kumar[1], Salar Khaleghzadegan[1], Brian Mears[1], Koji Hatano[1], Tarana A. Kudrolli[1], Wasim H Chowdhury[1†], David B. Yeater[1], Charles M. Ewing[1], Jun Luo[1], William B. Isaacs[1,2], Luigi Marchionni[2], **Shawn E. Lupold[1,2*]**

[1]The James Buchanan Brady Urologic Institute and Department of Urology, Johns Hopkins School of Medicine, Baltimore, MD;
[2]The department of Oncology, Sidney Kimmel Comprehensive Cancer Center, School of Medicine, Johns Hopkins University, Baltimore, MD.

Background: The Androgen Receptor (AR) plays a key role in prostate biology and in the progression of prostate cancer (PCa) to castration resistance. The role of microRNAs (miRNAs) in aberrant AR signaling have not been fully characterized.
Methods: Here we screened a library of 810 miRNA mimics to identify miRNAs that alter AR activity in complementary functional assays including protein lysate microarray (LMA) quantification of AR and PSA protein levels, AR transcriptional reporter activity, and AR-positive PCa cell viability. Candidate AR-regulating miRNAs were verified through AR transcriptional reporter and cell viability assays. MiRNA binding sites were found within the AR 3'-untranslated region (UTR) and within the AR and AR-V7 coding regions. MiRNA activity was characterized by western blotting, 3'-UTR reporter assay, and AR-GFP and AR-V7-GFP reporter assays.
Results: Screening uncovered miR-30 family members as direct AR inhibitors. Inhibition of endogenous miR-30b-3p and miR-30d-5p enhanced AR expression and androgen-independent cell growth. Droplet digital RT-PCR quantification of miR-30c-5p and miR-30d-5p revealed significantly reduced levels in metastatic castration resistant PCa (CRPC), when compared to healthy prostate tissues. MiR-30d-5p levels were inversely correlated with AR activity, as measured by PSA mRNA, in metastatic CRPC.
Conclusions: Collectively these studies provide a comprehensive evaluation of AR-regulating miRNAs in PCa and uncover miR-30b-3p and miR-30d-5p as direct inhibitors of AR expression and activity in PCa cells.