**EPIGENETIC MECHANISMS TARGET RARG NETWORK TO REDIRECT ANDROGEN RECEPTOR ENHANCER USAGE IN PROSTATE CANCER**

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Epigenetic mechanisms in prostate cancer (PCa) alter androgen receptor (AR) enhancer access resulting in re-wired AR signaling. Reduced RARg expression is common in PCa, and we sought to understand how miR-96 targeting of RARg impacts AR signaling, cell fates and promotes PCa progression. In HPr1AR, LNCaP and 22RV1 cells we undertook; miR-96 mimic RNA-Seq and mass spectrometry; biotinylated miR-96 to capture binding sites (IMPACT-Seq (identification of MiRNA REs by pull-down and alignment of captive transcripts— sequencing)); RIME (Rapid immuno- precipitation mass spectrometry) to test RARg interactions; RAg Cut and Run ChIP-Seq; viability, invasion and apoptosis assays to test phenotypic consequences.miR-96 regulated 1032 mRNA and 891 proteins in LNCaP cells with fewer in HPr1AR and 22RV1, including RARg and coregulators such as TACC1, and were enriched for miR-96 motifs (e.g. LNCaP RNA NES 5.6, p.adj=0.0005; LNCaP protein NES 2.6, p.adj=0.005) as well as Androgen and MYC networks, and response to Tretinoin (retinoid). IMPACT-seq identified 777 significant miR-96 recognition elements (MREs) in LNCaP cells (log OddRatio > 1, p.adj > 0.1), and fewer in HPr1-AR cells ~ 50% were shared. miR-96 bound and regulated targets were common. For example, TACC1 contains MREs, was significantly miR-96 downregulated (-1.6 fold mRNA; p.adj=1.2e-8; - 1.7 fold protein; p.adj = 0.0025), and RARg and TACC1 physically interact. TACC1 dependent/Independent RARg RIME revealed ~400 proteins like important coregulators (AKR1C2, CTBP1, SMARCD2, TRIM24 and MDC1), chromatin-remodelers (ARID2 and PHF6), m6A regulators (METTL3) and PCa drivers (NSD2) and AR. The binding of these proteins with RARg is enhanced in presence of TACC1 and dynamically regulated by CD437. RNA-Seq in normal 22Rv1 cells treated with miR-96 Inhibitor and ENZA confirmed a strong additive transcriptional response to the combination of miR-96 Inhibitor and ENZA raise the possibility of using miR-96 inhibitor nanoparticles in combination with ENZA. We addressed the question of how restoring expression of RARg/TACC1 complex impacted AR genomic binding. We established clones of 22Rv1 cells with a GFP-tagged RARg (and mock transfected controls) and transiently expressed TACC1, and undertook ChIP-Seq for RARg, TACC1, AR, H3K27ac in response to the AR ligand DHT, ENZA or CD437, combined with RNA-Seq in the same conditions. RARg expression results in a significant overlap with AR frequently at enhancer sites (p = 3.4e-12).Increased RARg levels increased both DHT and ENZA antiproliferative sensitivity associated with an increase in G1 and G2/M notably after 72 h. Together these findings support the concept that restoring the RARg/TACC1 complex redirects AR to active enhancers marked by H3K27ac and significantly associates with ENZA-regulated genes and increases the propensity for cells to undergo cell cycle arrest thus displaying pioneer-like functions of RARg for the AR. Also our data support the concept that miR-96, a known oncomir, evolves in its choice of binding sites in PCa progression to increasingly suppress the actions of the RARg network, which cooperates with TACC1 to promote luminal differentiation. Distortion to this network predicts overall survival in advanced PCa.