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INVITED RESEARCH HIGHLIGHT

Prostate Cancer

Long and noncoding RNAs (lnc-RNAs) determine androgen receptor dependent gene expression in prostate cancer growth *in vivo*

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Hyperactive androgen receptor (AR) activity remains a key determinant of the onset and progression of prostate cancer and resistance to current therapies. The mechanisms governing castrate resistant prostate cancer are poorly understood, but defining these molecular events is essential in order to impact deaths from prostate cancer. Yang *et al.* demonstrate that two lnc-RNAs known to be overexpressed in therapy resistant prostate cancer, PRNCR1 (also known as PCAT8) and PCGEM1, bound to the AR to enhance ligand-dependent and ligand-independent AR gene expression and proliferation of prostate cancer cells.1 The sequence of these interactions involved the binding of PRNCR1 to the acetylated AR and a subsequent association of DOT1L, which was required for the sequential recruitment of the lncRNA PCGEM1 to the AR amino terminus, which in turn was methylated by DOT1L.

The AR is a nuclear receptor gene superfamily member that is activated by androgens (5 α-dihydrotestosterone). Ligand-dependent activation of the AR induces cytoplasmic translocation of the receptor to the nucleus and the subsequent formation of multiprotein complexes that coordinates gene expression. The AR is a determinant of reproductive cell function and also governs functions in nonreproductive tissues, including the muscle and brain. The AR undergoes posttranslational modification by phosphorylation, acetylation, and sumoylation.[2] The AR binds and is regulated by both histone acetylates (p300, p/CAF) and histone deacetylases.[3] AR acetylation[3] has been shown to serve as a key node for diverse signaling pathways, including regulation by long noncoding RNAs in the current studies. DHT and other ligands, such as bombesin, induce AR acetylation.[4] The AR acetylation site also determines AR recruitment into chromatin in response to a subset of AR phosphorylation events[5] and determines the interaction with corepressors, including BRCA1, Sirt1,[6] and the NCoR-like repressor DACH1.[7] Sirt1, which inhibits AR positive prostate cancer cell growth, deacetylates the AR at these acetylated residues.[6] Furthermore, these AR acetylated residues play a critical role in histone methylation and MEKK1-dependent apoptosis of prostate cancer cells, consistent with an intermolecular signaling cascade within the AR.[8] Thus, these acetylated AR residues serve as a key platform for interaction with a broad array of key signaling pathways. AR acetylation thus serves as a molecular switch to determine human prostate cancer cellular contact-independent growth and cellular apoptosis.[9]

Noncoding RNAs are categorized as small (< 200 bp) which includes microRNAs, and long noncoding RNAs (> 200 bp) which range from 200 to 200 000 nucleotides in length. The estimated number of lncRNAs in the human genome range from 7000 to 23 000 and noncode database has collected 33 800 lncRNAs. Unlike miRNA, lncRNAs conservation is modest across species.[10] The majority of lncRNAs are transcribed by RNA polymerase II/III. The lncRNA subtypes are based on genomic location (inter, exonic, overlapping, and antisense). The mechanisms by which lncRNA function include decoys function (either sequestering RNA or protein), regulation of RNA processes (splicing and translation), interactions with tumor suppressor signaling, and serving as a flexible scaffold for chromatin modifying complexes.[11] A dominant function of lncRNAs involves epigenetic regulation of target genes through coupling, with histone modifying or chromatin remodeling complexes, including the polycomb repression complex (PRC) 1 and the PRC2 complexes. PRC2 complexes are linked to regulation of ANRIL, HOTAIR, H19, KCNQ10T1, and XIST. There appears to be a pool of lncRNAs bound to PRC2, as approximately 20% of all lncRNAs bind PRC2 for a function that currently remains unclear. LncRNA bind chromatin remodeling proteins through specific motifs. HOTAIR binds LSD1/co-REST1, AIR binds G9A, and KCNQ10T1 interacts with PRC2, G9a, and DNMT1. The scaffold function of lncRNAs allows them to tether multiprotein complexes to coordinate specific biologic functions. Enhancers for target genes associate with lncRNAs. Enhancer activity may be regulated through chromosomal looping, as was found through HOTTIP. Tumor suppressor signaling of lncRNAs occurs via p53 (MEG2) and either the CDKN2a or the CDKN2B locus, which are affected by several LncRNA.

Given the diverse potential mechanisms by which lncRNAs function, it is perhaps not surprising that important correlations have been found between specific lncRNAs and prostate cancer.[12] One of the early studies on lncRNAs and prostate cancer identified DD3, now known as prostate cancer androgen

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3 (PCA3).[13] PCA3 now serves as a biomarker for prostate cancer diagnosis, as it is secreted in the urine. Prostate cancer gene expression marker 1 (PCGEM1) was found in prostate tissue and is overexpressed in prostate cancer. Significant interest in PCGEM1 arose from the findings that lncRNAs are seen more frequently overexpressed in African-Americans than Caucasians, and were shown to promote prostate cancer cellular growth.[14] Several lncRNAs implicated in prostate cancer are shown in Table 1,[14] together with their potential mechanism of action. Androgen responsive lncRNAs include asMYO5A.[15] AR binding sites have been identified in the noncoding genes during transcriptome analysis of prostate cancer cell lines. The lncRNA prostate cancer noncoding RNA 1 (PRNCR1) was upregulated in prostate cancer, as well as in prostate epithelial neoplasia. The knockdown of PRNCR1 reduced AR activity in reporter assays and reduced the viability of prostate cancer cells in culture. The androgen-responsive lncRNA CTBP1-AS promotes both androgen hormone dependent and castrate resistant tumor growth by directly repressing CTBP1. The prostate cancer associated intragenic noncoding RNA transcripts (PCATs) were identified in prostate tissues and cell lines. PCAT1 regulates prostate specific cellular proliferation and targets PRC2. The PCAT1 repressed target genes served to substratify patients into molecular subtypes of prostate cancer.

Given the finding that PRNCR1 and PCGEM1 were highly expressed in high risk prostate cancer, Yang *et al*.[1] sought to determine the mechanisms by which these lncRNAs function. These elegant analyses demonstrated that shRNA to the lncRNAs reduced xenograft tumor growth *in vivo,* providing strong evidence that noncoding RNAs are a functionally relevant target for prostate cancer. PRNCR1 induction of AR activity required the AR acetylation motif, consistent with prior findings that the AR site is essential for contact-independent growth of human prostate cancer cells.[9] The association of PRND1 with the AR via its acetylation site primed the AR for sequential recruitment of PCGEM1. lncRNA chromatin isolation by RNA purification tiling for PRNCR1 and PCGEM1 demonstrated broad occupancy at a genome wide level with enrichment for the AR response element. Modified histone peptide array analysis demonstrated that PCGEM1 and PRNCR1 selectively bound H3K4, H3K4me1, and H4K16ac; marks indicative of enhancers.

Castration resistant prostate cancer arises through a variety of mechanisms, including point mutations within the AR ligand binding domain, giving rise to spurious activation by other steroids and alternative AR splicing. The AR-splicing variant known as AR-V7 is important in prostate cancer progression and is the predominant form in the CWR22RV1 cells. The AR-V7 androgen ablation therapy resistant mutant activates androgen-responsive genes in the absence of ligand. It was therefore of importance that the lncRNAs were capable of activating both the full length AR and the splicing variant AR-V7. shRNA to PRNC1 or PCGEM1 reduced the AR mutant-induced transcriptional program and cellular proliferation.

A number of new types of questions arise from the studies by Yang *et al*.[1] which will no doubt lead to further important insights into the mechanism governing activity of the AR. lncRNA can serve as a flexible scaffold to recruit other protein complexes. The finding by Yang *et al*.[1] that PCGEM1 recruited pygopus2 (PYGO2), which in turn enhanced selective looping of AR-bound enhancers to target genes, may be representative of the mechanisms by which a number of other lncRNAs function. The recognition of epigenetic markers by lncRNAs suggests that lncRNAs may function as epigenetic readers, either directly or by proxy.

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