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Science–Business eXchange

lncRNA meets the androgen receptor

By Chris Cain, Senior Writer

Although many long noncoding RNAs are upregulated in cancer, relatively few have been shown to functionally contribute to disease.[1] Now, a U.S. team has found that two lncRNAs act directly on the androgen receptor and are required for castration-resistant prostate cancer growth. The findings provide new targets for the disease and illustrate a previously unknown mechanism of action for lncRNAs.[2]

Extensive genome profiling studies have identified hundreds of lncRNAs associated with cancer, including prostate cancer.[3–5] Among the best-studied examples is prostate cancer antigen 3 (PCA3), a prostate-specific lncRNA that is overexpressed in patients with prostate cancer.

Based in part on this work, Gen-Probe Inc. developed Progensa, a prognostic assay that is marketed in the U.S. and Europe to measure levels of PCA3 to determine the need for a repeat biopsy in men suspected of having prostate cancer. Gen-Probe was acquired last year by diagnostics company **Hologic Inc.**

In contrast to these correlative studies, there are few functional studies of prostate cancer–specific lncRNAs or cancer-associated lncRNAs in general.[1] Indeed, even the function of PCA3 remains unclear.

One reason for the lack of functional studies is that the prevalence of lncRNA expression has only been appreciated in the past few years. Another reason is the difficulty of characterizing RNA, which is generally less stable than protein and can be hard to isolate for biochemical analysis.

Now, a team from the **University of California, San Diego** and **The University of Texas MD Anderson Cancer Center** has used a suite of immunoprecipitation techniques and knockdown studies to show how two lncRNAs functionally contribute to prostate cancer growth.

The group honed in on prostate cancer non-coding RNA 1 (PRNCR1), which is encoded in a region of the genome associated with susceptibility to prostate cancer, and prostate-specific transcript 1 (PCGEM1), whose expression had previously been associated with an increased risk of prostate cancer.

RNA immunoprecipitation identified proteins associated with each of these lncRNAs and showed that both associate with the androgen

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receptor (AR) in prostate cancer tissue. Additional biochemical pull-down and mass spectrometry experiments found multiple proteins associated with the lncRNAs, including histone methyltransferase DOT1L (DOT1L) and the RNA-binding protein pygopus homolog 2 (PYGO2).

To probe the functional role of PRNCR1 and PCGEM1, the team turned to cultured prostate cancer cell lines. In cell lines sensitive to dihydrotestosterone, individual small hairpin RNA against the lncRNAs decreased gene activation by AR compared with control shRNA.

In castration-resistant prostate cancer (CRPC) cell lines, in which the lncRNAs were shown to be overexpressed, knockdown of either lncRNA also decreased gene activation by AR compared with no knockdown.

Thus, PRNCR1 and PCGEM1 are required for both androgen-dependent and androgen-independent activation of target genes by AR.

Additional knockdown and immunoprecipitation experiments led the team to propose a pathway to explain how PRNCR1 and PCGEM1 contribute to AR activity.

First, AR binds PRNCR1 directly at specific acetylated residues. Then DOT1L is recruited, which methylates a specific lysine residue on the receptor. The methylated receptor is then bound by the PCGEM1 lncRNA, which binds and recruits the PYGO2 protein. This complex, including PYGO2, enhances looping of chromatin regions bound by AR and thus promotes the expression of AR target genes.

To firm up the link between these lncRNAs and a functional role in disease, the team tested the relevance of PCGEM1 and PRNCR1 function to prostate cancer growth *in vivo*. To do this, the researchers generated a CRPC cell line with inducible shRNA knockdown of PCGEM1 or PRNCR1 and used it to make xenograft mouse models of prostate cancer.

In this model, knockdown of either lncRNA decreased tumor growth compared with no knockdown.

Results were published in *Nature*. The study was led by Liuqing Yang and Chunru Lin, assistant professors of molecular and cellular oncology at MD Anderson.

The work started while both were postdocs in the lab of Michael Rosenfeld, a corresponding author on the manuscript, a professor of medicine at UCSD and a **Howard Hughes Medical Institute** investigator.

Expanding the scope

Claes Wahlestedt, associate dean and director of the Center for Therapeutic Innovation and a professor of psychiatry and behavioral sciences at the **University of Miami Miller School of Medicine**, told *SciBX* that the methods used to map this pathway could serve as a model for how to dissect lncRNA function.

"In this paper the authors utilized many innovative and powerful methods to deeply characterize the function of PRNCR1 and PCGEM1. They performed RNA immunoprecipitation, chromatin isolation by RNA purification, protein pull-down using biotinylated RNA, chromatin

immunoprecipitation and chromosome conformation capture," he said. "These techniques represent part of the gold-standard strategy for functionally characterizing lncRNAs and could be applied to study the function of many other disease-associated lncRNAs."

Howard Chang, a professor of dermatology at the **Stanford University School of Medicine** and an HHMI early career scientist, agreed. "Certainly many other lncRNAs have been found to have altered patterns of expression in many kinds of human cancers and other disease states. They would benefit from functional analyses of this type," he said.

Chang, whose lab is focused on understanding lncRNA function, wrote a news summary that was published alongside the article in *Nature* and highlighted the work.[6]

Arthur Krieg, president, cofounder and CEO of **RaNA Therapeutics Inc.**, told *SciBX*, "I believe this study is going to stimulate many other groups to look closely at the lncRNA expressed in various disease states for similar associations that could be therapeutically relevant."

RaNA is developing short oligonucleotides that disrupt the ability of lncRNAs to recruit polycomb repressive complex 2 (PRC2) and thus repress target genes.

The key next step for Yang and Lin's team is to further establish the contribution of PRNCR1 and PCGEM1 in patients with prostate cancer.

Lin said that her team is now developing locked nucleic acids (LNAs) targeting PRNCR1 and PCGEM1 and plans to test the LNAs in additional mouse models of prostate cancer. She also plans to look at the expression and functional contribution of these lncRNAs in a wide range of CRPC samples at various stages of disease progression.

Wahlestedt agreed that the lncRNAs might make for attractive targets but said developing oligonucleotide therapeutics for solid tumors remains a challenge.

"In my opinion, targeting the two lncRNAs may have very promising clinical applications, but the main problem remains how to target lncRNAs. [Small interfering] RNA and antisense oligonucleotides, although very efficacious *in vitro*, have certain limitations when utilized *in vivo*," he told *SciBX*.

Enzon Pharmaceuticals Inc. had an AR-targeting LNA antisense oligonucleotide, EZN-4176, in Phase I trials to treat prostate cancer. Last December, Enzon said that based on the data, it would suspend clinical development of the program to conserve cash. The compound was in development in collaboration with **Santaris Pharma A/S**.

Wahlestedt wanted to see more work done to understand how *PRNCR1* and *PCGEM1* are regulated in prostate cancer cells because an alternative approach could be to identify small molecules that reduce their expression.

Lin said her team is continuing to study how the lncRNAs are regulated.

The findings are unpatented.

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