Long non coding RNA (lncRNA) and Prostate Cancer

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LONG NON CODING RNA (LNCRNA) AND PROSTATE CANCER

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1 INTRODUCTION

A recent paper on the understanding of several long non-coding RNAs in the case of androgen resistant prostate cancer has raised the hopes of many to begin understanding the function of these epigenetic players in the control of malignant cells. To date, very few lncRNAs have been determined no less understood functionally. This brief note focuses on them in the context of PCa. We examine the AR case of PCa, then the basics of lncRNAs, and then spend some time examining the recent work and its implications. Finally we present some overall observations.

Epigenetic factors have become more important in understanding cancer dynamics. Much of the earlier work looked at the genetic fabric as the sole if not dominant element in cancer development. Mutations, translocations, and the like have been considered as the driving factors. Then came the introduction of miRNAs and methylation. We have examined these in some detail and at first considered them as noise in the process. However that has become an incomplete if not erroneous assumption. These epigenetic factors have silenced or activated genes, whereas the genes themselves have remained untouched.

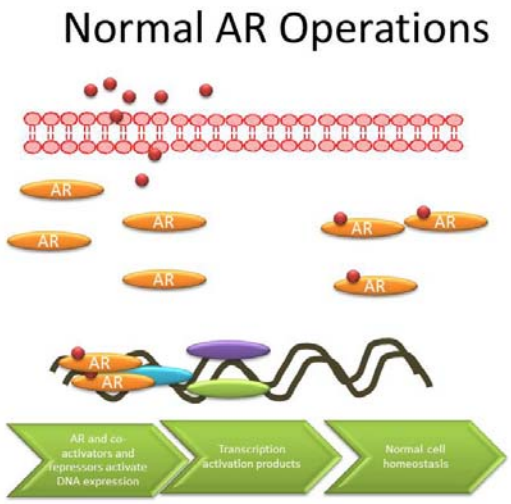
Now come lncRNA, another epigenetic factor which can block gene expression or activate them. The epigenetic factors can be carried from cell to cell and yet can be deactivated by certain types of therapeutics. The bad news is that epigenetic factors exert strong influences. The good news is that oftentimes the epigenetic factors can be more readily controlled. However since epigenetic factors are now so pervasive they may have significant unintended consequences.

Finally we use this specific example of lncRNA as a way to introduce a new epigenetic factor. We must be able to ascertain their presence, manage their effects, and ultimately incorporate them into the models we have developed.

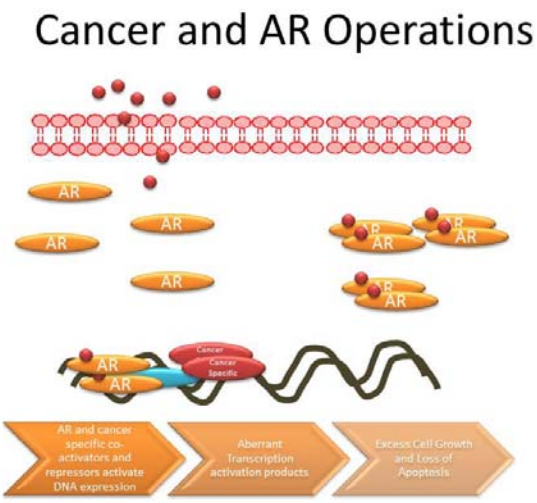
2 ANDROGEN PATHWAY

Prostate cancer can be controlled if not cured if the cancer is detected and removed in a controlled area. However when the cancer begins to spread it lives off of androgens for a long while, and by eliminating the androgens we can in turn "starve" the cancer cells. However the cells manage to find alternative paths to existing without the androgens and this becomes what is termed androgen resistant (AR) prostate cancer, PCa, or ARPCa.

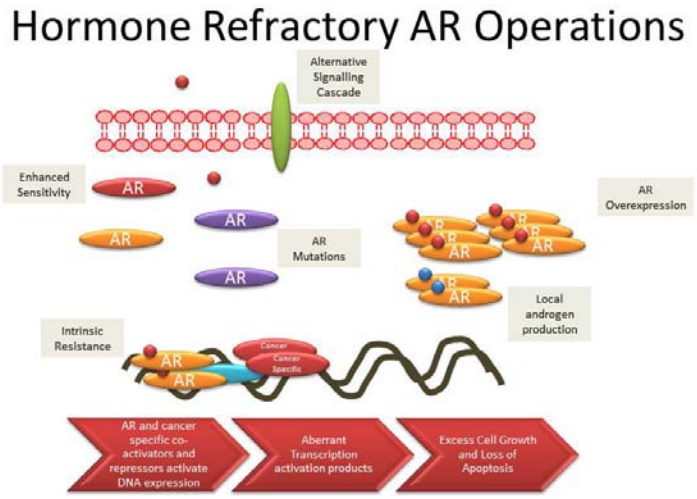
Normal operations of the prostate cell are shown in the Figure below. They result in normal cell homeostasis, namely basal and luminal cells reproducing as needed and in a normal manner.



The next step is a cell becoming cancerous. This we depict below. The result is excess cell growth and loss of apoptosis. Yet the driver is still the androgens entering the cell and driving the process through the AR.



Finally we have the AR independent growth as shown below. The assumption is that mutations occur that result in the ability to activate the AR functions leading to uncontrolled cell growth without the androgen exogenously being provided. In the recent paper in Nature that we shall discuss the growth change and control is now linked to epigenetic elements, namely the lncRNAs.



This gives one a simple introduction to androgen dynamics. We shall now expand this to include epigenetic effects.

3 LONG NON-CODING RNA

Long non-coding RNA, lncRNA, are the long RNAs recently discovered, most of which whose function is yet unknown, which can actually control gene transcription. The lncRNAs range from 200 to well over 100,000 nucleotides. In Weinberg's latest edition of Cancer he presents about one page only to lncRNAs, and such is an example of their newness and lack of understanding[1].

We know that there are over 25,000 genes expressible in the human genome but that these genes comprise about 2% of the total DNA. The question always has been; what does the rest of the DAN do, if anything? lncRNA may be one of many answers to this question.

3.1 LNCRNA OVERVIEW

We begin with a brief summary of some of the details of lncRNA. From the recent book by the Kovalchuks, the authors state that lncRNA have several functions:

1. Regulation of expression of neighboring genes
2. Blocking of splicing proteins-coding genes using antisense transcripts
3. Interaction with proteins making them more or less capable of fulfilling specific functions
4. Serving as precursors for smaller ncRNAs.

Kornienko et al present an excellent overview of these functions and we summarize here in their words some key elements of them:

Regulation of transcription is considered to be interplay of tissue and developmental-specific transcription factors (TFs) and chromatin modifying factors acting on enhancer and promoter sequences to facilitate the assembly of the transcription machinery at gene promoters. With a growing number of lncRNAs implicated in transcriptional gene regulation, this view may need refinement to include networks of tissue and developmental-stage specific lncRNAs that complement known regulators to tightly control gene expression and thereby organism complexity.

Transcriptional regulation by lncRNAs could work either in cis or in trans, and could negatively or positively control pc gene expression. lncRNAs work in cis when their effects are restricted to the chromosome from which they are transcribed, and work in trans when they affect genes on other chromosomes.

They continue:

lncRNAs can inhibit general protein-coding (pc) gene expression in trans

(a) by preventing transcription factor (TF) activity (7SK lncRNA) or

[1] Weinberg, 2013, p 26.

(b) by inhibiting RNAPII binding to DNA (B2 lncRNA). Xist lncRNA is transcribed from the X inactivation center (XIC) and inactivates a whole chromosome in cis

(c) by recruiting epigenetic modifiers (EM). lncRNAs can regulate specific genes, acting in trans like HOTAIR

(d) or in cis like HOTTIP

(e) by directly recruiting epigenetic modifiers to certain genomic loci.

In both cases the lncRNA binds EMs via a specific sequence or structure and targets them to promoter regions via DNA/RNA interaction elements to affect expression of the respective pc gene. Transcription of a lncRNA through a pc gene promoter or a cis-regulatory element (RE) affects pc gene expression in cis independent of the lncRNA product (f) by mechanisms discussed in the text. Both DNA strands are shown as separate boxes to indicate lncRNA transcription over the pc gene promoter in the antisense orientation.

Thus the lncRNAs have become an interesting target for examination especially as we learn more about why certain cancers return after targeted pathway control. lncRNAs are one of the many epigenetic elements which make understanding the process of cancer development and metastasis so complex.

3.2 FUNCTIONS OF LNCRNA

lncRNA are complex in their function and are being discovered at a rapid pace. We present herein some details that may assist in gaining a better understanding of how they operate and how they are classified. The lncRNAs have many functions. Although they do not encode into proteins they have the ability to interfere and facilitate many other intra-nuclear processes. It must be remembered that this is still a work in progress, the understanding of lncRNAs.

We rely upon some of the recent summary literature which describes these newly observed entities in some detail. From Kornienko et al we have an overview of classification:

Transcriptional regulation by lncRNAs could work either in cis or in trans, and could negatively or positively control gene expression. lncRNAs work in cis when their effects are restricted to the chromosome from which they are transcribed, and work in trans when they affect genes on other chromosomes.

Thus the classification of cis and trans is a critical distinction. In addition they may activate or suppress, and do so directly or via agents. The authors then proceed to define cis and trans. They state as follows:

Regulation in trans: *Some significant examples of lncRNAs that act in trans are those that can influence the general transcriptional output of a cell by directly affecting RNAPII activity*

…Regulation in trans can also act locus-specifically. While the ability of lncRNAs to act locus-specifically to regulate a set of genes was first demonstrated for imprinted genes where lncRNA expression was shown to silence from one to ten flanking genes in cis

Regulation in cis: *In contrast to trans-acting lncRNAs, which act via their RNA product, cis-acting lncRNAs have the possibility to act in two fundamentally different modes:*

(i) The first mode depends on a lncRNA product.

(ii) The second mode of cis regulation by lncRNAs involves the process of transcription itself, which is a priori cis-acting

The authors describe several mechanisms for its action. We report their comments as follows which are mechanisms by which lncRNA transcription silences gene expression**.**

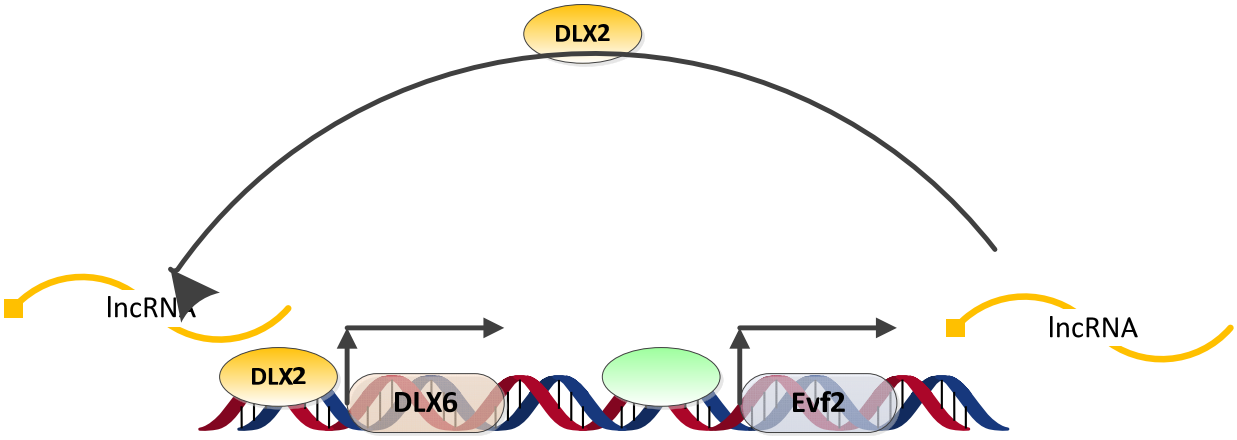
Mechanism	*Description*
Transcription-mediated silencing, also referred to as 'transcriptional interference' (TI)	This defined here as a case in which the act of transcription of one gene can repress in cis the functional transcription of another gene.
Mechanisms by which lncRNA transcription silences gene expression	Transcription-mediated silencing, also referred to as 'transcriptional interference' (TI), is defined here as a case in which the act of transcription of one gene can repress in cis the functional transcription of another gene.
Transcriptional interference acting by promoter nucleosome repositioning	DNA in the nucleus is organized into chromatin with the organizational scaffold consisting of nucleosomes, each with two copies of H3, H4, H2A and H2B histones. Nucleosomes can be densely packed, interfering with protein-DNA interactions, or relaxed, facilitating these interactions. The transcription process, which generates single-stranded DNA as RNAPII progresses along a gene locus, can directly affect nucleosome positioning.
Transcriptional interference acting by promoter histone modifications	Promoter associated nucleosomes carry post-translational histone tail modifications that reflect the activity state of the promoter and also influence accessibility of DNA binding factors involved in transcription.
Transcriptional interference acting by promoter DNA methylation	In mammalian genomes DNA methylation is generally associated with silent CpG island promoters, but the majority of CpG island promoters remain methylation free independent of their expression status.
Transcriptional interference in the absence of chromatin changes at the silenced promoter	In addition to RNAPII acting as a carrier of chromatin modifying enzymes, other TI models predict that RNAPII from one promoter traversing across another promoter can interfere with its activity without introducing chromatin changes.
lncRNA transcription creating a permissive chromatin environment	Enhancers are genetic elements that bind transcription factors facilitating transcription machinery assembly at nearby promoters.
lncRNA transcription and locus activation	Other examples indicate that lncRNA transcription activates gene expression by blocking access of repressor complexes to chromatin.

They continue as follows:

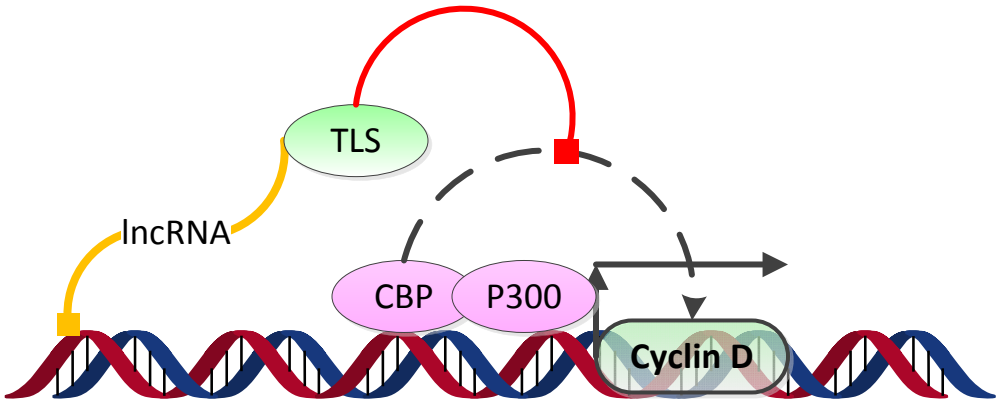
Modes of action include cotranscriptional regulation (e.g., through either the interaction of factors with the nascent lincRNA transcript or the act of transcribing through a regulatory region), regulation of gene expression in CIS or in TRANS through recruitment of proteins or molecular complexes to specific loci, scaffolding of nuclear or cytoplasmic complexes, titration of RNA-binding factors, and pairing with other RNAs to trigger posttranscriptional regulation.

The two latter mechanisms are illustrated in the cytoplasm (where they are more frequently reported) but could also occur in the nucleus. Additional mechanisms will presumably be proposed as additional functions of lincRNAs are discovered.

The following are two examples of how lncRNA may either activate or suppress gene transcription. Case 1 is an activation shown below.



Case 2 is a suppression mode of operation as shown below.



Now from Nie et. al. we have the following summary of lncRNAs. This is but a short list of what is currently known.

lncRNA Name	*Function/Characterization*
AIR	Imprinted, monoallelically expressed from the paternal allele, interacts with histone methyltransferase G9a
AK023948	Antisense transcribed from the intron of SIR-like adaptor gene (SLA), significantly downregulated in most of papillary thyroid carcinoma
ANRIL	Antisense transcript of INK4n/ARF/INK4a and p15/CDKN2B, required for the PRC2 recruitment to and silencing p15INK4b tumor suppressor gene
BACE1AS	Antisense transcript for beta-secretase-1, directly implicated in the increased abundance of Abeta 1-42 in Alzheimer's disease
CCND1/	Transcribed from 5' end of Cyclin D1 gene, induced by DNA damage and
CUDR	Upregulated in drug-resistant human squamous carcinoma, regulates drug sensitivity, cellular transformation and apoptosis
Cyclin D1	binding to TLS protein, leading to allosteric changes and repression of Cyclin D1 and anti-sense transcripts of tie-1 related to vascular malformation
DHFR	Transcribed from upstream of DHFR gene, regulates DHFR expression by forming triple helix with the promoter and disassociating pre-initiation complex
Evf-1	Activates transcriptional activity by directly influencing Dlx-2 activity
Evf-2	An alternatively spliced form of Evf-1 activates transcriptional activity by di-
GAS5	Growth arrest-specific transcripts, controls apoptosis and cell cycle, down-regulated in breast cancers
H19	Imprinted at the lgf2 locus; controls igf2 expression *in cis,* implicated in both tumor suppressors and oncogenes
HAR1	REST target gene, decreased in the neurons of Huntington's disease
HOTAIR	Intergenic transcript of HoxC locus, gene silencing in trans through interacting with PCR2 and LSD1 complex, involved in breast cancer metastasis
HOTAIRM1	Antisense intergenic RNA myeloid 1, transcribed antisense to the HOXA genes, plays a role in the myelopoiesis through modulation of gene expression in the HOXA cluster
KCNQ1OT1	Tissue-specific imprinted genes within the Kcnq1 domain, interacting with both PRC2 and G9a leading to gene silencing in a lineage-specific manner
KRAS1P	Transcript of KRAS pseudogene, overexpression of KRAS1P 3'-UTR, increases KRAS mRNA abundance and accelerates cell growth
LincRNA ROR	Expressed in the induced pluripotent stem cells (iPSCs), involved in the conversion of lineage-committed cells by interacting with reprogramming complexes
MALAT-1/NEAT2	Expressed in many cancers, regulates alternative splicing of pre-mRNA and promotes cell motility through transcriptional and post-transcriptional regulation of motility related gene expression
MEG3	Imprinted transcripts, highly expressed in human pituitary, stimulates p53-mediated transactivation and suppresses tumor growth in the absence of p53
Myc	Antisense transcript to myc gene, may be targeting the sense transcripts for immediate degradation
P15AS	Antisense transcript of p15, highly expressed in leukemia, epigenetically silences the tumor suppressor gene p15 directly influencing Dlx-2 activity
p21NAT	Antisense to cdkn1a/p21, requires Ago1 for epigenetic silencing of Cdkn1a/p21 p21
PCGEM1	Prostate tissue-specific and prostate-associated, overexpressed in prostate cancers, regulates cell proliferation and apoptosis, promotes colony formation
PTENP1	Transcript of PTEN tumor suppressor pseudogene, PTENP1 3'-UTR exerts a tumor suppressive function by acting as a decoy for PTEN-targeting miRNAs
SRA-1	Alternative splicing of SRA-1, loss of coding frame, an increased expression is associated with tumor metastasis
TERRA	Telomeric UUAGG repeat-containing RNA, inhibits telomerase activity, also regulates Xist and HOTAIR
Tie-1AS	Expressed temporally and spatially in vivo with its native gene tie-1, binds tie-1 mRNA, regulating tie-1 transcripts; imbalance of sense

Tsix	Antisense transcript to Xist , prevents Xist stabilization and inhibits the interaction between Rep A and PRC2, silencing Xist expression
TUG1	Ubiquitously expressed in human and mouse cell types and tissues, involves eye development, upregulated by p53, repressed cell proliferation via bound to PRC2
UCA1	Urothelial carcinoma-associated transcript, upregulated in bladder carcinoma and embryo, influences cell growth and promotes invasion
VL30-1	A mouse noncoding retroelement RNA, binds and releases PSF from a proto-oncogene, thus activating Rab23 proto-oncogene transcription
Xist	Mosaic expression, spreads on Xi *in cis*, interacts with BRCA1, correlated with breast cancer, cervical, ovarian, and testis tumors
Zeb2NAT	Antisense to Zeb2, regulates splicing of the IRES-containing intron of Zeb 2, involved in EMT

A similar result is from Ulitsky and Bartel which shows the number of identified lncRNAs determined by a number of investigators. The numbers go from just over 3,000 to almost 15,000. The functions of these lncRNAs is still less well understood than for example the miRNAs. They do however play a significant role in epigenetic control, especially in cancer dynamics.

Reference	***Data for Transcript Reconstruction***	***Genomic Features and Filters***	***Coding-Potential Filters***	***Number of lincRNAs***
Khalil et al., 2009	Chromatin marks, tiling arrays	Collection of approximate exonic regions, chromatin domain > 5kb	CSF	3,289 loci
Jia et al., 2010	cDNAs	Overlap with mRNAs allowed		5,446 transcripts
Orom et al., 2010	cDNAs	Restricted to loci >1 kb away from known protein-coding genes, > 200 nt mature length	Manual curation based on length, conservation and other characteristics of the ORFs	3,019 transcripts from 2,286 loci
Cabili et al., 2011	RNA-seq	Multi-exon only, > 200 nt mature length	PhyloCSF, Pfam	8,195 transcripts (4,662 in the stringent set)
Derrien et al., 2012	cDNAs	Overlap with mRNAs allowed (intergenic transcripts reported separately), > 200 nt mature length	Manual curation based on length, conservation and other characteristics of the ORFs	14,880 transcripts from 9,277 loci, including 9,518 intergenic transcripts

Sigova et al., 2013	RNA-seq, cDNAs, chromatin marks,	Antisense overlap with mRNA introns allowed, > 100 nt mature length	CPC	3,548 loci from embryonic stem cells, and 3,986 loci from endodermal cells

4 PCA AND LNCRNA

There is a recent paper in Nature describing how lncRNA acts in an interesting manner of providing ongoing growth capability in the case of androgen resistant prostate cancer, ARPCa. From the overview in Eureka we have:

…the study shows that two long non-coding RNAs (PRNCR1 and PCGEM1) activate androgen receptors, circumventing androgen-deprivation therapy. In their active state, these receptors turn on genes that spur growth and metastasis, making these cancers highly treatment-resistant. The study illustrates how prostate cancer can thrive, even when deprived of hormones, and provides tempting targets for new therapies.

"Androgen-deprivation therapy will often put cancer in remission, but tumors come back, even without testosterone," said contributor Christopher Evans, professor and chair of the Department of Urology at the UC Davis School of Medicine. "We found that these long non-coding RNAs were activating the androgen receptor. When we knocked them out, cancer growth decreased in both cell lines and tumors in animals."

…These prostate cancers are very aggressive and usually fatal, but their continued growth, despite being deprived of hormones, is just now being better understood. It's not unlike removing the key from a car ignition, only to have the vehicle re-start on its own. In this case, the aberrant starting mechanisms are long non-coding RNAs, a class of genetic material that regulates gene expression but does not code for proteins. Using patient samples from UC Davis, the group determined that both PRNCR1 and PCGEM1 are highly expressed in aggressive tumors…

Further investigation determined that one of these long non-coding RNAs is turning on androgen receptors by an alternate switching mechanism, like a car with a second ignition. This is critically important because many prostate cancer treatments work by blocking a part of the androgen receptor called the C-terminus. However, PCGEM1 activates another part of the receptor, called the N-terminus, which also turns on genes — with bad results. "The androgen receptor is unique, if you knock out the C-terminus, that remaining part still has the ability to transcribe genes," said Evans.

In addition, about 25 percent of these cancers have a mutated version of the androgen receptor that has no C-terminus. These receptors are locked in the "on" position, activating genes associated with tumor aggression.

Regardless of the receptor's status, PRNCR1 and PCGEM1 are crucial to prostate cancer growth. In turn, knocking out these RNAs has a profound impact on gene expression, both in cell lines and animal models. The team used complementary genetic material, called antisense, to knock out the RNAs and observe how the tumors and cells responded. In each case, there was a direct relationship between RNA activity, gene expression and cancer growth. "These long non-coding RNAs are a required component for these castration-resistant cancers to keep growing," said Evans. "Now we have preclinical proof of principle that if we knock them out, we decrease cancer growth."

Thus the lncRNAs, PRNCR1 and PCGEM1, seem to play a significant role in activating the AR genes which in turn lead to uncontrolled proliferation.

Now we consider the work directly. of Ling et al who report:

Although recent studies have indicated roles of long non-coding RNAs (lncRNAs) in physiological aspects of cell-type determination and tissue homeostasis1, their potential involvement in regulated gene transcription programs remains rather poorly understood.

The androgen receptor regulates a large repertoire of genes central to the identity and behavior of prostate cancer cells, and functions in a ligand-independent fashion in many prostate cancers when they become hormone refractory after initial androgen deprivation therapy.

Here we report that two lncRNAs highly overexpressed in aggressive prostate cancer, PRNCR1 (also known as PCAT8) and PCGEM1, bind successively to the androgen receptor and strongly enhance both ligand-dependent and ligand-independent androgen-receptor mediated gene activation programs and proliferation in prostate cancer cells.

They continue:

In addition to their relevance to disease, the current results illuminate several fundamental molecular mechanisms. PRNCR1 and PCGEM1 underscore a new role of RNA interaction with sequence-specific DNA-binding proteins — modification of transcription factor activity. The liaisons between lncRNAs and transcription factors can program stepwise chemical modifications on transcription factors, gating the successive flow of information from enhancer engagement to target-gene activation.

The insights that these findings provide into how lncRNAs can mediate enhancer–promoter looping are also intriguing. The RNA-mediated recruitment of a protein with intrinsic avidity for a promoter-associated histone mark to distantly located enhancer elements could stabilize DNA looping and promote communication over three-dimensional space. This would mean that, rather than being simple scaffolds, lncRNAs are more akin to a complex computer circuit board, linking together various disparate molecular components and dictating the logical operation of the system.

Thus we seem to have here a clear functions example of gene activation via lncRNAs. In addition Ling et al surmise a potential therapeutic target as well. Although this therapeutic insight may be useful it is not yet actionable. It is not at all clear how to control intra-nuclear lncRNAs.

In a Nature commentary on the paper the authors Schmitt and Change state:

Yang et al. report that two long non-coding RNAs (lncRNAs) — PRNCR1 and PCGEM1 — activate the androgen receptor. Interaction of PRNCR1 with this receptor at androgen-response genomic elements allows recruitment of DOT1L, an enzyme that methylates and so activates the

receptor. PCGEM1 can now bind to the active androgen receptor and recruit the enzyme Pygo2, which allows communication between this receptor and its target genes by binding to H3K4me3 chromatin marks in the genes' promoter sequences. Many androgen receptor target genes have been implicated in prostate-cancer growth.

This discovery is quite useful and insightful. It represents a powerful argument for more aggressively examining the epigenetic factors of the lncRNA. We have seen the impact of miRNA and of methylation and now this opens another powerful area.

5 OBSERVATIONS

This is one of the first papers to lay out a complete story for how the lncRNAs may control metastatic growth. This also is a key element in our growing body of knowledge of epigenetic factors and cancer.

There are several observations worth noting:

1. Classic pathway analysis totally neglects epigenetic factors. This miRNA, lncRNA, and methylation have almost been considered as noise. Must we now expand the model to directly and expressly include these factors and if so how.

2. There is a reference to dealing with the lncRNAs via a therapeutic. There are two questions here; first, what therapeutic and second as we learn from BRAF inhibitors on melanoma and in hypomethylation therapeutics in MDS there are unintended consequences. What may they be as we expand to lncRNA?

3. As we have just begun to touch the edge of the complex and as yet indeterminate number of lncRNAs, how can we deal with them holistically?

4. As one may suspect these may add to our ever growing markers for cancer diagnosis and prognosis. How will these be added?

5. Exosomes are one way to determine what is in a cell. They have been used for prostate cancer staging. Can we now target these lncRNAs in such an exosome test?

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