Crucial role of noncoding RNA in driving prostate cancer development and progression

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Genomic regions without protein-coding potential give rise to a large number of long noncoding RNAs (lncRNAs), which represent close to three-times the number of protein-coding genes [1]. Studies over the past 10 years have shown that lncRNAs are implicated in a range of developmental processes and diseases [2,3]. More recently, lncRNAs have been found to be functional and clinically relevant in prostate cancer (PCa). Specifically, lncRNA *PCA3* has been approved to aid PCa diagnosis by the US FDA [4], while lncRNA *SChLAPI* has been shown to be an independent biomarker for metastatic PCa [5].

In general, the functional aspects of lncRNAs have been summarized as decoys, guides, scaffolds and enhancer RNAs [2,3]. Similar as well as unique mechanisms have been discovered in PCa. In castration-resistant prostate cancer, lncRNA *HOTAIR* has been found to interact with *AR* and block ubiquitination of *AR* by the E3 ubiquitin ligase, *MDM2* [6]. lncRNA *SChLAPI* drives PCa metastasis by binding to *SWI/SNF* chromatin-modifying complex and antagonizing *SWI/SNF* genome localization and target gene regulation [7]. lncRNA *PCA3* was recently found to form a dsRNA complex with *PRUNE2*, a tumor suppressor gene, thereby promoting *ADAR*-mediated RNA editing [8]. Finally, lncRNA *CTBPI*-

AS directly suppresses *CTBPI* transcription by guiding the *PSF-HDAC-Sin3A* complex to the *CTBPI* promoter, which abolishes the androgen-mediated gene repression program [9]. Altogether, these studies have revealed the dynamic interplay between lncRNAs and cancer development, which has highlighted the importance of lncRNAs in the etiology of PCa.

Transcriptome analyses have identified hundreds of novel lncRNA transcripts that are dysregulated in PCa. lncRNAs *PCAT1* and *SChLAPI* were first discovered in a cohort of 102 PCa tissues and cell lines by RNA sequencing, and reported to participate in PCa tumorigenesis and progression. *SChLAPI* was recently shown to have significant prognostic value for metastatic progression of PCa [5]. In addition, another lncRNA named *PCAT14* was identified as a novel PCa- and lineage-specific lncRNA by analyzing The Cancer Genome Atlas PCa RNA-seq data, and was reported to be inversely related with disease aggressiveness [10]. However, dysregulation of lncRNAs expression does not necessarily speak for functional importance in PCa pathogenesis. Nevertheless, with rich genome, epigenome as well as transcriptome data of PCa available, it is now feasible to systematically evaluate the function of lncRNAs in PCa.

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While PCa genome-wide association studies (GWAS) have identified multiple common risk-associated SNPs that can cumulatively explain 33% of familial risk [11], very little is known about the effect of risk-associated SNPs on lncRNAs [12]. To systematically evaluate lncRNAs involved in PCa pathogenesis, we performed an integrative analysis of the lncRNA transcriptome with epigenomic and risk SNP data [13]. Interestingly, we found that PCa-risk SNPs are significantly enriched in the regulatory regions marked by DNase I hypersensitive sites (DHSs) but not in the exons of protein-coding genes or lncRNAs. Notably, we observed that PCa-risk SNPs were specifically enriched in the cis-tromes of PCa-related transcription factors, such as *AR* and *HOXB13*. Upon further analysis, these risk-SNP containing DHSs were in close proximity to lncRNAs, suggesting a potential cross talk between risk SNP and lncRNA regulation. Integrative analysis of SNPs in the regulatory regions identified 45 candidate lncRNAs associated with 50% of the PCa risk loci.

The top hit by our prediction was lncRNA *PCAT1*, which is specifically expressed in prostate tissues and PCa, and promotes PCa cell proliferation [14]. Consistent with previous reports, we found that *PCAT1* is significantly upregulated in primary PCa compared with benign prostate tissues, and knockdown of *PCAT1* expression suppressed PCa cell proliferation and tumor growth.

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PCAT1 is located in the 8q24 gene desert region, which contains 10 PCa risk loci. Interestingly, a PCa-specific DHS harboring the risk SNP rs7463708 was predicted to interact with *PCAT1* promoter. By performing chromosome conformation capture (3C), we were able to validate this interaction. Importantly, the *PCAT1* promoter–enhancer interaction was specific to risk ‘T’ allele of rs7463708. The rs7463708 locus with risk allele also showed higher enhancer activity than nonrisk allele, and disruption of rs7463708 locus resulted in the decrease of *PCAT1* expression. In further analyses, we identified that the risk allele ‘T’ of rs7463708 created a stronger *ONECUT2* motif at *PCAT1* enhancer, resulting in allele-specific occupancy of transcription factors *ONECUT2* and *AR*. In addition, chromatin immunoprecipitation experiments revealed that the recruitment of *AR* at the *PCAT1* enhancer was dependent on *ONECUT2* occupancy. In agreement with our binding data, the induction of *PCAT1* by prolonged androgen treatment was dependent on *ONECUT2* expression. Finally, we demon-

strated that *PCAT1* interacts with *AR* and *LSI*, which mediates their occupancy at the enhancers of *GNMT* and *DHCR24*, two androgen late-response genes that participate in PCa development and progression. Altogether, these data establish the capacity of risk SNPs to promote PCa development by modulating lncRNA expression, and provide additional evidence that lncRNAs, such as *PCAT1* are crucial to PCa.

The catalog of lncRNA transcripts in cancer has grown tremendously during the past few years, but the functions of most lncRNAs are still unclear. Our integrative analysis in PCa retrieves the intrinsic connections between risk SNPs and functional lncRNAs from the genome, epigenome and transcriptome data. Since these data are becoming increasingly available for many cancer types, our pipeline will also be useful for the lncRNA study in other cancer types.

Many GWAS SNPs also locate in lncRNA gene bodies [1]. Since most lncRNAs function through interaction with other molecules (such as RNA, DNA and protein), some studies have revealed that those interactions have a sequence-specific pattern [15]. However, it remains to be examined whether these GWAS SNPs located in lncRNAs mediate functional interactions. In addition, with newer approaches being recently established to analyze lncRNA interactomes [16,17], it is time to investigate the implication of SNPs in the lncRNA interactomes.

Recently, two studies reported somatic mutations enriched in regulatory regions that were linked to promoter and nucleotide excision repair activities [18,19]. These somatic mutations in regulatory regions may also modulate lncRNA expression and thus affect tumor growth and progression. Although there are differences in downstream mechanistic analyses of genetic predisposition and acquired variation data, with appropriate modifications, our computational and experimental workflow could be applied for identifying lncRNAs affected by acquired variants in cancer.

Genome editing technologies have been used to validate the function of genetic variations. Transcription activator-like effector nuclease or CRISPR-Cas9 induced homology-directed repair can introduce allele-specific modification to the genome, which has been proved to be a powerful tool to validate the causal SNPs in regulatory regions [20]. In addition, cell lines derived from single-cell cloning with different allelic makeup of a mutation, offer an ideal model to study the allelic dependence of transcription factor binding and subsequent effect on gene expression. To further expand the scale of functional validation of lncRNA-related SNPs, CRISPR-Cas9 based functional screens should be utilized to manipulate the activity of non-coding regions systematically. Thus, our integrative

analysis provides a focused list of lncRNA and SNPs that are likely regulating these lncRNAs. Robust genome editing techniques like CRISPR-Cas9 can immensely contribute to validate the causality by these SNPs and their modus operandi.

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