The emerging role of noncoding RNA in prostate cancer progression and its implication on diagnosis and treatment

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Abstract

Recent transcriptome studies using next-generation sequencing have detected aberrant changes in the expression of noncoding RNAs (ncRNAs) associated with cancer. For prostate cancer, the expression levels of ncRNAs including microRNAs and long noncoding RNAs are strongly associated with diagnosis, carcinogenesis and tumor growth. Moreover, androgen and its cognate receptor, androgen receptor (AR), regulate various signaling pathways for prostate tumor growth. In addition, progression to lethal castration-resistant prostate cancer (CRPC) is also owing to AR function. Systematic analysis of AR-binding sites and their regulated transcripts revealed that many ncRNAs are widely regulated at the transcriptional level. Thus, recent studies provide new insight into the complicated molecular mechanism of prostate cancer progression. This review focused on the role of various ncRNAs in prostate cancer and the association between their expression and CRPC.

Key words: CRPC; AR; cap analysis of gene expression (CAGE); miRNA; lncRNA; epigenome

The role of noncoding RNAs in prostate cancer biology

Recent advances in transcriptome technology have revealed that >90% of the human genome is actively transcribed [1]. The encyclopedia of DNA elements (ENCODE) project has shown that only 2% of these transcripts are translated into proteins [2]. The noncoding RNAs (ncRNAs), which occupy the majority of transcripts in the nucleus, were initially thought as the ‘dark matter’. Generally, ncRNAs are broadly divided into short (<200 nt) and long (>200 nt) transcripts [3]. In this review, we summarize the function and clinical significance of long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) in prostate cancer and their regulation by the androgen receptor (AR). miRNAs play important roles in cancer by posttranscriptional modification of target mRNA or protein expression. LncRNAs represent most of the transcribed ncRNA in the human genome. GENCODE v19 includes 13870 human lncRNA-related genes, which produce 23898 lncRNAs [4]. These lncRNAs exhibit similar structure and biogenesis as mRNAs. They are polyadenylated and may function in either nuclear or cytoplasmic compartments. Evidence showing that lncRNAs are aberrantly expressed in numerous human diseases including cancer supports their importance [5–7].

The function of AR in castration resistance

AR is a member of the Nuclear Receptor superfamily [8, 9] and is a key molecule for androgen signaling in its target organ, the prostate. Testosterone that is produced in the testes is the most abundant circulating androgen (~90%). Testosterone diffuses into prostate cells and is converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase [10]. DHT directly binds to and activates AR even more tightly than testosterone [11]. Before ligand binding, AR is found in the cytoplasm in a complex that includes molecular chaperones and co-chaperones from the heat shock protein (Hsp) family. After binding to androgen, a conformational change in the complex leads to nuclear translocation of AR. In the nucleus, AR binds as a dimer to specific genomic sequences called androgen-responsive elements (AREs), which are found in the promoter and enhancer.
regions of target genes (Figure 1) [12]. AR regulates its enhancer action by modifying the epigenetic condition of AR-binding sites (ARBBs) [13].

AR also induces prostate cancer development and progression. Despite favorable responses to initial hormone therapy, most patients progress to lethal castration-resistant prostate cancer (CRPC) with elevated AR expression [14], hypersensitivity to androgens [15], intra-tumoral steroidogenesis [16] and development of AR variants [17]. Thus, identification of signaling events downstream of AR is critical for understanding the progression of hormone-responsive prostate cancer to CRPC.

Although AR overexpression is commonly observed in CRPC, the mechanism underlying altered AR expression is not completely understood. However, recent studies showed that both transcriptional and epigenetic changes are important for AR upregulation in prostate cancer. A recent study analyzing an androgen-dependent prostate cancer cell line identified ARBBs in the introns of the AR gene [18]. Androgen treatment causes AR to bind the enhancer and recruit lysine-specific demethylase 1 (LSD1), which represses transcription by inhibiting histone H3K4 methylation, thus demonstrating a negative feedback loop that limits endogenous AR expression. However, if cells are incubated in castration levels of androgens, AR expression increases. Furthermore, low levels of androgens in CRPC are sufficient to activate AR target genes, but are insufficient to suppress AR itself.

**Identification of androgen-regulated miRNAs**

miRNAs are a class of naturally occurring, short noncoding RNAs that negatively regulate gene expression by binding to the 3′ untranslated region (UTR) of mRNAs and inhibiting their translation. Recent research demonstrates that dysregulation of miRNA expression profiles contributes to pathogenesis of human malignancies. In prostate cancer, miRNAs regulated by androgen and AR have been identified using short RNA sequence studies or miRNA microarray, and their functions for prostate cancer progression have been investigated.

**miR-21**

miR-21 is an oncogene encoded at a fragile genomic location, 17q23.2, which is amplified in several types of tumors [19]. miR-21 is an androgen-regulated miRNA and is induced by AR recruitment to its promoter in the presence of androgens (Figure 1). Expression analysis studies have shown that miR-21 expression is associated with prostate cancer aggressiveness. Elevated miR-21 expression promotes androgen-dependent cell growth and castration-resistant tumor growth [20]. The target gene of miR-21 is MRCKS (myristoylated alanine rich protein kinase C substrate), which regulates cell motility, mitogenesis and plasma membrane trafficking [21]. Other factors repressed by miR-21 are B-cell translocation gene 2 (BTG2) and phosphatase and tensin homolog (PTEN) [22, 23].

**miR-125b**

This miRNA is induced by androgen and stimulates the growth of prostate cancer cells (Figure 1). Expression of miR-125b is increased in prostate cancer samples compared with normal controls and correlates with Gleason scores, thus demonstrating that miR-125b is an oncogenic miRNA that participates in the development of CRPC [24]. miR-125b targets the 3′ UTR of apoptosis regulators such as p53, p53 downstream modulator of apoptosis (PUMA) and BCL2-antagonist/killer 1 (BAK1). Thus, dysregulation of miR-125b results in an imbalance between pro- and anti-apoptotic processes by targeting these pathways.

**Other androgen-regulated miRNAs (miR-141, -148a and -32)**

We have previously performed short RNA sequence analysis in LNCaP cells and reported several androgen-regulated miRNAs that promote cell proliferation including miR-141, miR-200a and miR-148a [25]. miR-148a is highly expressed in prostate cancer cell line derived from the lymph node (LNCaP) cells and targets cell cycle regulator cullin-associated and neddylation-dissociated 1 to promote cell proliferation (Figure 1). Expression of miR148a is upregulated in prostate cancer samples compared with normal tissues [26].

miR-32 is an androgen-regulated miRNA, which confers a growth advantage as demonstrated by reduction of apoptosis, and this effect is mediated by targeting BCL2 interacting mediator (BIM), a pro-apoptotic member of the BCL2-antagonist/killer 1 (BCL2) family [27, 28]. In addition, miR-32 directly targets the 3′ UTR of BTG2 to induce cell proliferation. Interestingly, both miR-32 and miR-148a are reported to be upregulated in CRPC samples, suggesting roles for both miRNAs in the emergence of castration resistance [27].

**miR-29a/b**

Because the expression of the miR-29 family is reduced in several cancer tissues than in normal tissues, their role in cancer is controversial [29, 30]. While the precise role of the miR-29 family members in cancer is still unclear, they are known to promote metastasis in breast cancer [31, 32]. Uregulated miR-29a enhances hepatoma migration by targeting PTEN [33], suggesting that miR-29 family may exhibit a dual function as a tumor suppressor and a tumor promoter.
suppressing and oncogenic miRNAs depending on cellular context and disease progression.

We found that mir-29a/b is androgen-inducible miRNA targeting ten-eleven translocation 2 (TET2) and is highly expressed in cancers with poor prognoses. In vitro, miR-29 family members promote cell proliferation and migration of hormone refractory prostate cancer cells by activating cell motility and cell cycle-associated gene expression. In vivo, we showed that miR-29 family members enhance tumor growth using several AR antagonists- and castration-resistant prostate cancer models. TET2 repression is associated with decreased 5-hydroxymethylcytosine (5-hmC) in prostate cancer progression. Decreased 5-hmC promotes AR collaborating factor, forkhead box A1 (FOXA1) transcriptional activity and prostate cancer-associated expression of genes such as mTOR (Mammalian target of rapamycin). These results indicate a significant oncogenic role of miR-29 in prostate cancer progression by regulating epigenetic status [34].

AR-regulated miRNAs such as miR-21-125b-141 and -148a regulates apoptosis, cell cycle, and cell proliferation by repressing various factors for tumor progression as AR downstream. In addition, our research highlights the epigenetic regulation by androgen-mediated miRNAs for activating FOXA1 and AR downstream. In vivo, delivery of miR-15 and -16 suppressed tumor formation from nontumorigenic cells in severe combined immunodeficiency (SCID) mice [42]. These miRNAs control cell survival, proliferation and invasion via deregulation of oncogenes such as BCL2, cyclinD1 and WNT3A (wingless type MMTV integration site family).

Thus, in addition to AR-mediated pathways, these tumor-suppressive miRNAs possess other critical roles for progression to metastatic or advanced prostate cancer.

**Integrative analysis to discover androgen-regulated lncRNAs**

Next-generation sequencing has been used to analyze the transcriptome of prostate cancer cells. Combined analyses of genome-wide ARBSs obtained by chromatin immunoprecipitation (ChIP)-based analyses such as ChIP-chip [43–48] or ChIP-seq [49–51] and the androgen-regulated transcriptome have recently been reported. A new technique, global nuclear run-on sequencing (GRO-seq), has been used to analyze sequential gene expression on androgen treatment [52]. For nuclear run-on reactions, cell nuclei are isolated after treatment with androgens for a specific amount of time. In the run-on step, RNA polymerases are allowed to run on about 100 bases in the presence of a ribonucleotide analog [5-bromouridine 5'-triphosphate (BrUTP)]. BrU-containing RNA is selected by purification with an antibody specific for the nucleotide analog. A cDNA library is then prepared for next-generation sequencing. Using this method, the authors discovered the production of enhancer-templated noncoding RNAs (enhancer RNA, eRNAs).

Cap analysis of gene expression (CAGE) [53] is a high-throughput method for analysis of gene expression and to profile transcription start sites (TSS), including those for promoter usage. CAGE is based on sequencing of concatamers of DNA tags derived from the initial 20 nucleotides at the 5' ends of mRNAs. The frequency of CAGE tags is consistent with results from other analyses, such as microarrays. This analysis is high-throughput, and enables understanding of gene networks via correlation between promoter usage and expression of gene transcription factors. We performed CAGE to determine androgen-regulated TSSs and ChIP-chip analysis to identify genome-wide ARBSs and histone H3 acetylated sites in the complete human genome [54]. Using CAGE, we identified 13,110 distinct, androgen-regulated TSSs. Cross-referencing with the gene expression database for prostate cancer (Oncomine), the majority of androgen-upregulated genes containing adjacent ARBSs and CAGE tag clusters in our study were previously confirmed as upregulated genes in prostate cancer. The integrated high-throughput genome analyses of CAGE and ChIP-chip provide useful information for elucidating the AR-mediated transcriptional network that contributes to the development and progression of prostate cancer. ncRNAs, including miRNAs, were also identified as androgen target transcripts in this study. We found many androgen-dependent TSSs widely distributed throughout the genome, including in the antisense (AS)
direction of RefSeq genes. Several pairs of sense/AS promoters were newly identified within single RefSeq gene regions, suggesting the involvement of AS ncRNA in transcriptional regulation.

Functional analysis of AS ncRNA in prostate cancer

Global transcriptome analysis revealed that the majority of the mammalian genome can generate transcripts from both strands of the DNA double helix, and identified paired sense/AS transcripts, indicating that AS transcription is important for gene regulation [55–57]. One example of AS transcripts is the cyclin-dependent kinase (CDKN) 2B antisense RNA1 (CDKN2B-AS1) [58], which is a natural AS transcript located within the CDKN2A/CDKN2B tumor suppressor locus on 9q21.3. Cycline dependent kinase (CDK) inhibitors, p14, p15 (CDKN2B) and p16 (CDKN2A) are produced from this locus and regulate cell cycle progression at G1/S. CDKN2B is specifically silenced by hormone deprivation. In addition, CDK inhibitors interact with a second histone modification complex, the LSD1/corepressor for Rest (CoREST)/RE1 silencing transcription factor (REST) complex, which coordinates targeting of polycomb repressive complex2 (PRC2) and LSD1 to chromatin for coupled histone H3K27 methylation and K4 demethylation [62].

HOTAIR expression is associated with hormone-dependent cancer such as breast and prostate cancer. HOTAIR expression level was higher in breast tumors than in normal breast epithelia, and high HOTAIR expression is associated with poor prognosis of patients. This clinical finding also reflects the HOTAIR-mediated regulation of hormone action. Although the regulation of HOTAIR by estrogen is controversial [63, 64], HOTAIR enhanced estrogen receptor (ER) protein level and enhanced downstream signaling [64]. Moreover, HOTAIR is repressed by androgen and upregulated by hormone deprivation. In addition, HOTAIR binds to AR and blocked E3-ubiquitine ligase murine double minute 2 (MDM2) to stabilize AR and enhance the AR-mediated transcriptional activity and drives CRPC development [65].

We investigated the functional role of the novel androgen-responsive AS RNA, CTBP1-AS [66]. We focused on the mechanism of androgen-mediated cancer proliferation by this IncRNA and the clinical significance in prostate cancer progression. We showed that C-terminal binding protein 1 (CTBP1) [67] is a transcriptional corepressor of AR and negatively regulates androgen signaling. CTBP1-AS is directly upregulated by AR and interacts with RNA binding protein, PSF [68, 69], for transcriptional repression using HDACs. Downstream signals of CTBP1/PSF (polypyrimidine tract binding protein associated splicing factor) complex include cell cycle regulators such as p53 or SMAD3 in addition to CTBP1. Castration-resistant tumor growth was promoted by androgen-mediated repression of these cell cycle regulators and activation of AR signaling.

Activation of AR transcription activity by IncRNAs

In addition to CTBP1-AS and HOTAIR, several IncRNAs have been reported to regulate AR activity for prostate cancer progression.

Steroid receptor RNA activator

Steroid receptor RNA activator (SRA) interacts with nuclear receptors, including AR, ER, progesterone receptor, glucocorticoid receptor (GR) and thyroid hormone receptor, and regulates their transcriptional activities. As a mechanism for activation, SRA interacts with a nuclear receptor coactivator SRC-1 (steroid receptor coactivator). Mutation analysis revealed that six stem-loop motifs are important for coactivation by SRA [70, 71]. Ablation SRA transcription was observed in various tumors. It is upregulated in tumors such as breast cancer, ovarian cancer and prostate cancer, compared with normal tissues. In prostate cancer, SRA and its alternative splicing form, steroid receptor RNA activator protein (SRAP), were shown to be required for expression of AR target genes in the presence of androgens [72, 73].
Enhancer RNA

In AR signals, AR-activated enhancers marked by increased eRNA are required for activation of genes in their vicinity. eRNA knockdown disrupts ligand-induced promoter-enhancer interaction. As a mechanism of enhancer-promoter interaction, eRNA interacts with a cohesion complex and this interaction is required for its recruitment to enhancers [74].

Prostate cancer gene expression marker 1 and Prostate cancer noncoding RNA 1

Prostate cancer gene expression marker 1 (PCGEM1) was originally identified as a prostate tissue-specific long ncRNA [75]. It is involved in inhibition of apoptosis by delaying p53 and p21 induction in an androgen-dependent manner [76]. PCGEM1 is overexpressed in at least half of all prostate tumors. LncRNA prostate cancer noncoding RNA 1 (PRNCR1) was found by a single nucleotide polymorphism (SNP) associated with prostate cancer susceptibility. Two SNPs, rs1456315 and rs7463708 in the chromosome 8q24, which is a gene desert region, were shown to be most significantly associated with prostate cancer susceptibility. PCGEM1 and PRNCR1 are both involved in AR-mediated gene transcription [77]. Knockdown of these IncRNAs inhibits AR target gene regulation in a DHT-dependent manner and is associated with tumor growth in CRPC xenograft model. Interactions of PCGEM1 and PRNCR1 with AR were confirmed by RNA pull down and RNA immunoprecipitation assay assays. For the mechanism of AR activation, PCGEM1 was shown to interact with pygopus homolog2 (Pygo2), and PRNCR1 interacts with DOT1-like histone H3 methyltransferase for AR methylation. By 3C-ChIP experiments, these two IncRNAs were found to be required for AR-bound enhancer promoter loop formation. However, this mechanism is still controversial because another study failed to reproduce the interaction of AR with these two IncRNAs [78].

Growth arrest-specific 5

Growth arrest-specific 5 (GAS5) interacts with GR and suppresses GR-mediated transcriptional activity. The interaction of GAS5 and GR reduces GR binding to GR-responsive elements (GREs). The binding element of GAS5 with GR contains GRE-like sequences, suggesting a competing role of GAS5 with GRE DNA for GR binding. GAS5 also binds to other nuclear receptors such as AR. GAS5 was found to be downregulated in breast cancer and prostate cancer and then inhibit cell proliferation [79]. However, GAS5 inhibition of ERα transcriptional activity has not been found. Noncoding multiple small nuclear RNAs (snRNAs) encoded in introns of GAS5, particularly U50, showed tumor suppressor characteristics in breast cancer [80].

Functional IncRNA extends the insight of regulatory mechanism of AR. The major mechanism is the direct interaction with AR and subsequent modulation of cofactors (SRA), stability of AR protein itself (HOTAIR), inhibition of genomic binding (GASS) and enhancer promoter loop forming (eRNA, PCGEM1 and PRNCR1). Regulation of co-repressor or co-activator by epigenetic action of androgen-regulated lncRNA (CTBP1-AS) would be another mechanism to enhance AR activity in prostate cancer. However, the number of IncRNAs described in detail is small. We have to investigate the tissue-, spatial-specific function of IncRNAs or the secondary structure or high-level structure to determine the interaction and recruitment to specific genomic regions.

Molecular mechanism of IncRNAs in prostate cancer

Some IncRNAs are not related to the function of AR, but play an important role in prostate cancer progression. These IncRNAs could serve as cancer markers for clinical diagnosis.

Metastasis-associated lung adenocarcinoma transcript 1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is involved in pre-mRNA processing and regulates the alternative splicing of pre-mRNA by modulating levels of serine/arginine splicing factors. Although the mechanism of MALAT1 upregulation in cancer cells is still unclear, multiple copy-number gains at the locus and chromosomal translocations have been reported [81]. In prostate cancer, MALAT1 overexpression is correlated with poor prognosis and cancer progression [82]. The regulation of MALAT1 by androgen has not been reported, and MALAT1 is upregulated in primary breast cancer and inhibited by estrogen treatment. MALAT1 knockdown inhibits breast cancer cell proliferation, suggesting the tumor-promoting effect of MALAT1 in breast cancer [83, 84]. Thus, MALAT1 may play a critical role in hormone-dependent cancer development.

Prostate cancer-associated ncRNA transcript-1

Prostate cancer-associated ncRNA transcript-1 (PCAT-1) is a relatively small intergenic ncRNA located in the 8q24 gene desert, and it was identified through global transcriptomic sequencing of prostate tumors [85]. PCAT-1 expression is inversely correlated with expression of the EZH2, a histone methyltransferase that encodes components of PRC2 and a marker for prostate cancer progression; thus, PRC2 represses PCAT-1 expression. PCAT-1 induces cell proliferation in vitro and has a predominantly repressive effect on gene expression, most notably on the expression of the tumor suppressor gene breast cancer 2 (BRCA2) [86]. PCAT-1 also interacts with the SUZ12 component of PRC2.

Second chromosome locus associated with prostate 1

Second chromosome locus associated with prostate 1 (SChLAP1) is an lncRNA transcribed from within an intergenic gene desert on 2q31.1. It was originally identified in an analysis of intergenic IncRNAs that are selectively upregulated in aggressive prostate cancer samples. SChLAP1 is highly expressed in 25% of prostate tumors and shows increased expression in metastatic cancer cells. SChLAP1 is involved in the regulation of the switch/sucrose nonfermenting (SWI/SNF) complex (Figure 2). This complex canonically controls transcription by using ATP hydrolysis to remodel chromatin and physically mobilize nucleosomes, particularly at the gene promoters. SChLAP1 co-immunoprecipitates with SNF5 and prevents its genomic binding, thus antagonizing tumor-suppressive SWI/SNF-mediated gene regulation [87]. Using another cohort of 1008 patients, SChLAP1 was identified and validated as the highest-ranked overexpressed gene in cancer with metastatic progression [88]. These findings demonstrated the usefulness of SChLAP1 as a biomarker of prostate cancer progression. Thus, both PCAT1-1 and SChLAP1 can be useful biomarkers for clinically detecting advanced prostate cancer.
Prostate cancer gene 3

Prostate cancer gene 3 (PC3A) is an lncRNA associated with prostate cancer and is a potentially useful biomarker [89, 90]. It was originally discovered in 1999 by a differential display analysis of prostate tissues and cell lines. Its expression in 95% of the prostate tumors is up to 100-fold higher than in adjacent non-neoplastic tissues. PC3A regulates the expression of its overlapping gene PRUNE2, a tumor suppressor gene in prostate cancer, by posttranscriptionally regulating the stability of mRNA [91]. Urinary measurement of PC3A RNA levels can be helpful to detect prostate cancer with superior tumor specificity to prostate-specific antigen [92, 93].

H19

This lncRNA is transcribed from the H19/Igf2 gene cluster on chromosome 11p15.5. Some evidence suggests H19 plays a critical role in tumor progression by exhibiting oncogenic activity in some types of tumors, such as bladder cancer. However, H19 was shown to have a tumor-suppressive role in metastatic prostate cancer by repression of TGFβ1 signals, which is involved in metastasis. H19 and miR-675, derived from H19, were downregulated in metastatic cancers. miR-675 inhibits tumor growth factor beta 1 (TGFβ1) signals by directly binding to the 3’ UTR of TGFβ1. Overexpression of miR-675 and H19 repressed prostate cancer cell migration [94]. In contrast, miR-675 enhances tumorigenesis and metastasis of breast cancer cells [95]. H19 mediates estrogen-dependent cell proliferation in breast cancer cell, suggesting the oncogenic role of H19 in estrogen-dependent breast cancer [96].

These lncRNAs are likely to function through interaction with general epigenetic and chromatin remodeling factor (PCAT-1, SCHLAPI) to impact the transcription for cancer progression. Modulation of miRNAs (H19) or snoRNAs (GASS) would be important regulatory mechanism of lncRNAs in cancer. Regulation of stability or splicing by lncRNAs (PC3A and MALAT1) broadens the insight of transcriptional regulation in cell biology.

Future plans for clinical applications of ncRNA

Long ncRNAs or miRNAs can be used as diagnostic and prognostic markers, and as novel specific therapeutic targets for CRPC. Current prostate cancer genomic data can be fully exploited if noncoding regions are studied in detail. In addition, ncRNAs are interesting targets in cancer therapy because their cancer- and tissue-specific expression can be a major advantage over other therapeutic options. Several other studies have also demonstrated the clinical significance of ncRNAs. However, before we can make use of these new therapeutic options, we have demonstrated the functional and structural studies will be required. The exponentially growing number of studies reporting new ncRNAs or androgen signaling molecules will contribute to progress in this field.

Key Points

- Oncogenic miRNAs (miR-21, 29a/b, 148a and 125b) are androgen-regulated and regulate tumor growth by targeting epigenetic status, apoptosis and cell cycle regulators.
- High-throughput analysis using RNA-seq, GRO-seq and CAGE has identified abundant androgen-regulated and prostate cancer-associated lncRNAs.
- RNA-seq analyses of tumor samples have identified lncRNAs such as PCAT-1 and SCHLAPI that could be biomarkers for prostate cancer.
- AR interacts with some lncRNAs such as HOTAIR, eRNAs, SRA, PCGEM and PNCRI to regulate AR transcripational activity and promote prostate cancer.

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