Noncoding RNA for personalized prostate cancer treatment: utilizing the ‘dark matters’ of the genome

Prostate cancer is the most commonly diagnosed cancer in men in western countries, with significant health impact. Clinically, it is complicated with the lack of biomarkers and effective treatments for aggressive disease, particularly castration-resistant prostate cancer. Although we have gained much insight into the biology of prostate cancer through studying protein-coding genes, they represent only a small fraction of our genome. Therefore, it is essential for us to investigate noncoding RNAs, which comprise the majority of our transcriptome, in order to achieve a better understanding of prostate cancer and move toward personalized medicine. In this article, we will address recent advancements in our knowledge of noncoding RNAs, and discuss the clinical potentials and challenges of different types of noncoding RNAs in prostate cancer.

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mediate- and high-risk groups with different treatment options [4].

While patients in low-risk group are often subjected to active surveillance, patients in intermediate- and high-risk groups with localized tumors often undergo surgical resection of the tumor, radiotherapy or chemotherapy. Despite initial response to localized treatment, about a third of patients will eventually relapse with metastatic disease [5]. Androgen-deprivation therapy (ADT) is a first-line therapy for metastatic PCa as prostate cell growth is dependent on androgen pathway signaling [7]. However, ADT is not curative and castration-resistant PCa (CRPC) eventually arises, leading to patient survival benefit of only several months post-treatment [5,6]. In addition, patients in intermediate-risk group often show differential initial responses to treatments due to tumor heterogeneity [8]. Much research efforts, such as the CPC-GENE project, are dedicated to understanding the heterogeneity in intermediate-risk patients and further stratifying them into finer risk groups [9]. Therefore, there is an unmet need to identify novel biomarkers with high sensitivity and specificity for PCa development and progression, and novel therapeutic options for advanced stage PCa patients with metastatic disease [5,6].

With increased understanding of the innate biology of CRPC, novel therapeutic options have emerged in the past several years. CRPC have adaptive mechanisms to maintain AR signaling despite reduced levels of androgen [5,6]. New agents abiraterone acetate and enzalutamide have been approved by the US FDA in 2011 and 2012, respectively to further target the AR signaling pathways. Furthermore, understanding of the genomic profiles of PCa has shifted the field toward personalized medicine and fueled the development of small molecule inhibitors targeting frequently mutated pathways in PCa, such as PI3K, BRCA and AURKA pathways [10–12]. Despite enormous research efforts into protein drivers of PCa, the role of noncoding RNA (ncRNA) in PCa remains largely unexplored.

ncRNA
ncRNA is a species of RNA transcribed by RNA polymerase but lacks the ability to translate into protein [13]. In addition, they also tend to be less evolutionarily conserved than protein coding genes [14,15], and were originally thought to be ‘junk’ RNA resulting from leaky transcription of RNA polymerases [13]. However, research in the past few decades has shed light on the diverse functional roles of ncRNA. At large, ncRNA can be further classified into two groups based on their sizes — short ncRNA and long ncRNA (lncRNA) [16]. Short ncRNA are less than 200 nt in length, and include structural RNAs such as transfer RNA, small nuclear RNA, PIWI-interacting RNA and ribosomal RNA, as well as regulatory RNAs such as miRNA and siRNAs [13]. Structural small ncRNAs have been reported to play an essential role in processes such as protein translation and RNA splicing, while regulatory RNAs regulate the stability of mRNAs [13]. LncRNA is a group of ncRNA over 200 nt in length that can be further subclassified based on their genomic locations, directionality and properties (e.g. intergenic lncRNA, antisense lncRNA and enhancer RNA) [16]. LncRNAs share many similarities with protein coding mRNAs, but are found to be much more cell type and cancer-specific in expression [17]. Importantly, lncRNAs predominantly function as regulators of gene expression, but through various mechanisms of action [16].

It is now well accepted that over 75% of the human genome is actively transcribed, but protein-coding genes only account for about 2% the genome [18]. In addition, many researchers have reported global dysregulation of ncRNAs in various types of cancer, and have begun to explore their functional importance [16,19]. Specifically, the dysregulation of regulatory ncRNAs such as miRNAs and lncRNAs can alter the expression of many downstream target genes involved in signaling pathways. For example, miR-141 is upregulated in PCa and promotes PCa cell proliferation [20]. LncRNA HOTAIR is upregulated in breast and hepatocellular carcinomas and can mediate PRC2- and LSD1-dependent repression of target genes, ultimately leading to cancer progression in vitro and in vivo [21,22]. LncRNA PCAT5 is activated by ERG and regulates CRPC cell proliferation, migration and invasion [23]. Interestingly, these studies sparked great interest in developing therapeutic agents targeting oncogenic miRNAs and lncRNAs. In particular, RNA interference and antisense oligonucleotides (ASO) targeting oncogenic miRNAs and lncRNAs are currently under rigorous preclinical testing [24,25].

Biomarker potential of ncRNAs has also gained increasing attention in the recent years. This is mainly driven by the highly specific expression pattern of ncRNAs in cancer. In particular, the expression of multiple ncRNAs has been reported to be specific to both cancer types and subtypes [19,26]. Furthermore, with the recent advancements in sensitivity of next-generation sequencing technologies, ncRNAs can now be detected more sensitively and noninvasively in blood and/or urine samples [27]. In the era of personalized medicine fueled in part by the advancements in noninvasive liquid biopsy, expression pattern and detectability of ncRNA holds great potential as novel biomarkers for cancer diagnosis and prognosis.
miRNA in personalized medicine

Among all the ncRNAs studied to date, miRNAs are most well characterized, and immense amount of research efforts have focused on applying them clinically for diseases such as cancer [14,28]. miRNA is a class of small ncRNA transcribed by RNA polymerase II and processed into its mature form by two ribonuclease III type endonucleases, DROSHA and DICER [14,29]. It was first discovered in *Caenorhabditis elegans* and later revealed to play essential roles in normal biology [30]. Functionally, miRNAs are loaded onto RNA-induced silencing complex (RISC) and predominantly bind to mRNAs with complementary sequences to mediate post-transcriptional silencing of mRNAs [29]. Remarkably, each miRNA is capable of targeting multiple downstream mRNAs and is thus master regulator of gene expression [31]. Therefore, as expected, dysregulation of miRNAs has been found to contribute to cancer development and progression [19,25]. For example, *miR-21* was reported as an oncogenic miRNA that is upregulated in PCa to promote androgen dependent and independent reported as an oncogenic miRNA that is upregulated in stream mRNAs and is thus master regulator of gene type specific between cancer and benign tissues, and are also cancer The expression patterns of miRNAs are different 

Research efforts have focused on applying them clinically for diseases such as cancer [14,28]. MiRNA is a class of small ncRNA transcribed by RNA polymerase II and processed into its mature form by two ribonuclease III type endonucleases, DROSHA and DICER [14,29]. It was first discovered in *Caenorhabditis elegans* and later revealed to play essential roles in normal biology [30]. Functionally, miRNAs are loaded onto RNA-induced silencing complex (RISC) and predominantly bind to mRNAs with complementary sequences to mediate post-transcriptional silencing of mRNAs [29]. Remarkably, each miRNA is capable of targeting multiple downstream mRNAs and is thus master regulator of gene expression [31]. Therefore, as expected, dysregulation of miRNAs has been found to contribute to cancer development and progression [19,25]. For example, *miR-21* was reported as an oncogenic miRNA that is upregulated in PCa to promote androgen dependent and independent tumor growth [32]. Contrarily, *miR-320a* and *miR-223* were found to be tumor suppressors, downregulated in prostate tumors that normally inhibit cell proliferation, migration and invasion [33,34]. More detailed information of clinical potentials of miRNAs in PCa can be found in a few recent reviews [25,35–36].

miRNA as PCa biomarkers

The expression patterns of miRNAs are different between cancer and benign tissues, and are also cancer type specific [19]. Therefore, miRNAs are potential biomarkers and therapeutic targets for personalized medicine. Aside from their specific expression patterns in cancers, miRNAs have also been reported to be stable in body fluids such as blood, which further highlighted their clinical potential as cancer biomarkers [37]. Specifically, miRNAs in body fluids are found either as a complex with the Argonout protein or within microvesicles, and are thus protected from extracellular nucleic degradation [37–39]. Although there are controversies as to which form of extracellular miRNA is most predominant, they can both be isolated and detected experimentally through centrifugation and quantitative analyses [38,39]. However, the amount of extracellular miRNAs in circulation is extremely low compared with intracellular miRNAs, and the amount of tumorspecific miRNAs in circulation is further diluted by the greater presence of nontumor miRNAs [37]. Therefore, extremely sensitive assays are needed to detect these tumor miRNAs for clinical purposes. RT-qPCR is currently widely used for circulating miRNA detection with high sensitivity and specificity, but in a low throughput manner. In addition, the recent advancements in next-generation sequencing technologies allowed for higher sensitivities capable of detecting circulating miRNAs [40]. These improvements in technologies have fueled advancements in evaluating circulating miRNAs as biomarkers of cancer. Various researchers have reported differential expression of miRNAs in blood of patients with low- and high-risk PCa [41,42]. Furthermore, circulating *miR-20a*, *miR-21*, *miR-145* and *miR-221* are predictive of PCa aggressiveness and can be used to differentiate PCa patient in different risk groups [43]. Recently, several circulating miRNAs have also been found predictive of chemotherapy response [44], biochemical recurrence [45], and progression to CRPC [46]. Their strong potential as biomarkers has led to several ongoing clinical studies testing miRNA as predictive biomarker for ADT and enzalutamide treatment response (NCT02366494, NCT02471469).

Therapeutic potentials of miRNA

miRNAs have also been widely explored as potential therapeutic targets owing to their functional roles in cancer [25,31]. Targeting these master regulators poses as an effective way to regulate multiple genes involved in cancer [25,31]. Currently, multiple therapeutic approaches have been developed and tested experimentally. These include miRNA sponges [47], small molecule inhibitors [48] and chemically modified ASO [49], which affect miRNA target binding or expression. Among these, the ASO approach is most popular due to its high specificity and effectiveness [25]. ASO works by binding complementarily to miRNAs, thereby blocking their abilities to bind to target mRNAs [25]. *In vitro* ASO has been shown to effectively inactivate the oncogenic functions of miRNAs [50]. However, despite its effectiveness, several challenges remain for the clinical utility of ASO-based miRNA targeted therapy. Two major challenges are the issue of ASO stability due to nuclease degradation and ASO specificity [25]. Various chemical modifications have been tested in ASO in the past few years to improve ASO stability and specificity for *in vivo* applications. One common modification is the use of locked nucleic acids to enhance these properties. Many researchers have found that locked nucleic acids modified ASO have sufficient specificity and stability for *in vivo* targeting of miRNAs [51,52]. An additional challenge for ASO-based therapy is its effective delivery *in vivo* [25]. Unlike *in vitro* systems, accessibilities of different tissues vary greatly, and different means of delivery are thus needed to target different locations and to minimize off targeting to other organ sites. Recent Phase I study of a modified ASO targeting AR in CRPC patients resulted in minimal effect, which in part may be due to inefficient delivery [53].
There are currently two new strategies in delivering ASOs – nanoparticle delivery and conjugate delivery. Nanoparticle method encapsulates ASOs in lipid or polymer based particles of 100–200 nm in size. These particles are often coated with polymers with various chemical properties to reduce nonspecific uptake and enhance target tissue uptake. Nanoparticle delivery method is popular for delivery of ASOs to organs with fenestrated endothelium, such as liver and spleen, due to its relatively large size [54]. The second approach involves conjugating ASOs to different receptors or antibodies for specific delivery to targeted cells. Small sizes of these conjugates allow for rapid diffusion across various endothelial barriers and thus can target most organs in vivo. In addition, these conjugates have enhanced target specificity due to receptor or antibody recognition [54]. Although delivery of ASOs using these methods has not yet been tested clinically, siRNA-based therapies using these delivery methods are in various clinical trials [55]. In summary, therapeutic targeting of miRNAs using ASOs offers great clinical potential for personalized medicine in PCa in the future, but additional improvements on stability and delivery of these agents in vivo are needed.

**IncRNA in personalized medicine**

Compared with miRNAs, IncRNAs are less well understood but have gained increasing interest from researchers due to their functional importance [16]. Although the genomic loci of IncRNAs are less conserved than protein-coding mRNAs, their promoters are well conserved and the distribution of histone marks at the loci are remarkably similar to mRNAs [17]. In addition, most IncRNAs and mRNAs are transcribed by RNA polymerase II and often undergo similar post-transcriptional modifications [16]. The major criteria distinguishing IncRNAs from mRNAs are their lack of functional open reading frame and thus low protein-coding potential [56]. Although recent studies have argued that IncRNAs can contain cryptic open reading frames coding for micropeptides [57], and that these peptides may be functional [58]. Nonetheless, they predominantly function at the RNA level as master regulators of gene expression. As nucleic acids with secondary structure, IncRNAs can interact with DNA, RNA and proteins. Therefore, they often function by tethering protein complexes such as histone modifiers, transcriptional modulators and spliceosomes to specific genomic and transcriptomic regions [16,59]. Similar to miRNAs, IncRNAs are also master regulators capable of modulating the expression of multiple downstream targets. Hence they are attractive therapeutic targets that can modulate entire functional programs or signaling networks, such as the essential AR signaling pathway in PCa [16]. While the clinical applications of targeting IncRNAs are still being actively evaluated, numerous data have already presented IncRNAs as attractive diagnostic and prognostic biomarkers of cancer.

**IncRNA: novel biomarkers in PCa**

As mentioned earlier, IncRNAs are highly specific in their expression, which can be utilized to identify molecular subtypes and stages of cancer [26,60]. Therefore, IncRNAs have been rigorously evaluated as diagnostic and prognostic biomarkers of cancer. Although their global expression patterns are generally lower than mRNAs, advancements in microarray and sequencing technologies have allowed for their detection even in small amounts of clinical samples. Nonetheless, it is still important to select IncRNA with high expression as candidates of biomarker studies, and various publically available IncRNA databases may be helpful in this aspect [61,62]. Promisingly, IncRNA PCA3 that is highly expressed in PCa cells, was approved by the FDA in 2012 in the form of a urine test to aid PCa diagnosis [63]. While it currently has issues that may limit its clinical usefulness, such as specificity issue in a subset of cases and unclear correlation with aggressive PCa, it is nonetheless available as a biomarker to determine the need for re-biopsy to confirm PCa diagnosis [63]. Recently, Prensner et al. have found IncRNA SChLAPI to be prognostic for metastatic PCa in a large cohort of PCa patients and highlighted its potential as a prognostic biomarker [64]. With hundreds of uncharacterized IncRNAs significantly and differentially expressed in PCa, we have only just begun to explore the world of IncRNAs and the clinical potentials they hold [64].

As highlighted by PCA3, IncRNAs are also detectable in body fluids such as urine and blood, which hold great clinical relevance in PCa as noninvasive biomarkers. Recently, there has been a shift of attention toward studying IncRNAs in exosomes, as it offers great stability for IncRNAs and can be isolated experimentally through ultracentrifugation. Many studies are already underway to evaluate the biomarker potential of exosome in cancer [65,66]. Given their superior stability in body fluids and predictive potential, IncRNAs in exosomes will likely be a new generation of noninvasive cancer biomarkers for liquid biopsy. Interestingly, recent work by Ahadi et al. suggests that the presence of IncRNAs in exosomes is not random but rather selective for IncRNAs with miRNA binding regions [67]. By identifying IncRNAs enriched in exosomes, we can potentially narrow the pool of candidate IncRNA biomarkers for clinical evaluation. In summary, IncRNAs hold great potential as cancer biomarkers due to their specific expression patterns and detectability in body fluids. While currently only PCA3 have been FDA
approved, several other lncRNAs are under intensive review, and we may see additional FDA-approved lncRNA-based biomarkers in the near future.

**lncRNA as therapeutic targets in PCa**

With advancements in sequencing technologies and processing software, a large number of lncRNAs has been identified in the past few years. Currently, lncRNAs represent the largest group of transcript in the human genome, with one study citing twice more number of lncRNAs than mRNAs in our transcriptome [61]. Despite efforts in understanding the functional roles of these lncRNAs, relatively few lncRNAs have been well characterized to date. Nonetheless, these lncRNAs are often dysregulated in cancer and have been reported to play an essential role in tumorigenesis. For example, lncRNAs **PRNCR1** and **PCGEM1** were found to interact with AR to drive the expression of proliferative genes [68]. In addition, lncRNA **SChLAP1** was reported to promote progression of PCa through antagonizing the tumor suppressive SWI/SNF complex [69].

Recently, we have identified AR- and LSD1-dependent oncogenic functions of lncRNA **PCAT1** in PCa development and progression through the upregulation of AR late response genes [70]. Furthermore, knockdown of these lncRNAs resulted in reduced proliferative and invasive potential of PCa both in vitro and in vivo. Interestingly, several lncRNAs and enhancer RNAs were found recently to confer resistance to conventional cancer therapies [71–73]. Therefore, targeting lncRNAs holds great promise for the treatment of PCa.

Two main approaches in suppressing oncogenic lncRNAs are RNAi silencing and ASOs. Due to their diverse functional mechanisms, lncRNAs can exhibit nuclear or cytoplasmic localization, or both. While RNAi silencing (e.g. siRNAs and shRNAs) is effective in targeting lncRNAs in the cytoplasm, its effectiveness in targeting nuclear localized lncRNAs is less apparent. By comparing both techniques in suppressing a panel of lncRNAs with different cellular localization, Lennox and Behlke have reported superior silencing of nuclear lncRNAs by ASOs [74]. Therefore, ASO is currently the most popular way to target oncogenic lncRNAs in a clinical setting. Similar to miRNA-targeting ASOs, challenges and improvements associated with oligonucleotide stability, specificity and mode of delivery mentioned earlier also apply to lncRNA-targeting ASOs. Presently, several clinical trials have tested ASO treatments in cancer patients (NCT00466583, NCT00120288, NCT00159028). Although ASOs in these studies were targeting protein-coding genes, ASOs targeting lncRNAs are being tested rigorously in the preclinical setting and may eventually make their way into clinical trials [75].

**Circular RNA in personalized medicine**

Circular RNA (circRNA) is a novel class of ncRNA predominantly generated through backsplicing events involving the covalent linkage between 3’ donor site of a downstream exon with 5’ acceptor site of an upstream exon. CircRNAs were first identified decades ago but were originally thought to be byproducts of aberrant RNA splicing with no apparent function. However, advancements in sequencing technologies have led to the identification of a large number of circRNAs in our transcriptome [69]. Interestingly, these circRNAs were also found to be conserved, functional and display tissue-specific expression patterns [77]. CircRNA can be generated from both mRNAs and lncRNA loci, and they often exhibit different but nonetheless important functions as compared with their linear counterparts. For example, Zheng et al. reported oncogenic function of **circHIPK3** but not HIPK3 protein in cancer cell growth through inhibition of **miR-124** [78]. Therefore, targeting oncogenic circRNAs holds potential as a novel cancer therapy. Furthermore, owing to their circular structures and lack of 5’ cap or polyA tail, circRNAs are resistant to exonuclease-mediated degradations and thus have higher stability than linear RNAs. This property sparked great interest in investigating the biomarker potential of circRNAs. Unsurprisingly, the field of circRNA research has exploded in the recent years. Recent finding on the dysregulation of circRNAs in PCa tumor provided strong rationale to further investigate the functional role and clinical potential of circRNAs in PCa [78].

**circRNA: the rising star of cancer biomarkers**

As mentioned before, circRNAs are highly stable and display specific expression patterns. These properties have made circRNAs an ideal candidate for noninvasive biomarker used in liquid biopsy. Therefore, the stability and detectability of circRNAs are actively tested in patient body fluids. Memczak et al. recently revealed that circRNAs can be robustly detected in patient blood samples [79]. In particular, they also reported higher expression of circRNA in blood than their corresponding linear mRNAs. This may be due to higher stability of circRNAs or biased secretion of circRNAs from cells. Nonetheless, robust detectability of circRNAs in body fluids demonstrates their strong biomarker potential. Furthermore, a recent study has identified several cancer-related fusion-circRNAs (f-circRNAs) resulted from oncogenic gene fusions such as **MLL–AF9** in acute myeloid leukemia. These f-circRNAs were found to contribute to cell proliferation and even drug resistance [80]. Importantly, PCR assays are sensitive enough to detect these f-circRNAs, further supporting their robust expression. The iden-
Noncoding RNA biomarkers from patient fluids (e.g. urine and blood) can assist the diagnosis and prognosis of prostate cancer. Noncoding RNA-directed therapies may be alternative treatments for prostate cancer patients.


Figure 1. Potential clinical integration of prostate cancer-specific noncoding RNAs in personalized medicine. Noncoding RNA biomarkers from patient fluids (e.g. urine and blood) can assist the diagnosis and prognosis of prostate cancer. Noncoding RNA-directed therapies may be alternative treatments for prostate cancer patients.

Crosstalk between ncRNA: a complicated regulatory network
Cancer is not the result of a single genetic aberration but rather a collection of aberrations affecting both protein coding genes and noncoding transcripts. In this perspective, ncRNAs mainly act as epigenetic regulators that normally tightly control the expression of multiple oncogenic or tumor suppressive protein coding genes. Therefore, dysregulation of ncRNAs can either lay down predisposition for cancer or further augment the abnormal genomes of cancerous cells. Expression of ncRNA themselves is also tightly regulated, in part by a network of crosstalk between different classes of ncRNAs. For example, while miRNAs can regulate the stability of mRNAs, both IncRNAs [85] and circRNAs [82] can act as miRNA sponges to inhibit the activity of miRNAs. On the contrary, some miRNAs are also capable of targeting functional IncRNAs such as MALAT1 [86]. Furthermore, IncRNAs are also capable of producing miRNAs [87] and circRNAs [88]. Therefore, to understand the role of ncRNA in cancer, it is important to consider not only their downstream targets but also the upstream regulatory network.
Noncoding RNA for personalized prostate cancer treatment: utilizing the ‘dark matters’ of the genome

**Conclusion & future perspective**

There is currently an unmet need to identify novel biomarkers for PCa risk stratification and prediction of aggressive disease. Equally important is the identification of novel therapies for the treatment of CRPC, of which no cure is currently available. Since ncRNAs are highly specific in their expression patterns and are functional as epigenetic regulators of the genome, they are attractive candidates for such biomarker and therapeutic research in PCa. A myriad of preclinical research efforts, together with the technological advancements in sensitivity of next generation sequencing platforms in the recent years, have found ncRNAs to be highly promising biomarkers for PCa diagnosis and prognosis (Table 1). For example, IncRNA PCA3 expression is specific to PCa and it is currently FDA approved for PCa diagnosis, miR-21 and miR-145, as well as IncRNA SchLAP1 were reported to be prognostic for aggressive PCa and are capable of stratifying PCa patients into different risk groups [32,68,70].

Currently, increasing number of ncRNAs is being evaluated in large clinical cohorts for their diagnostic, prognostic and predictive potentials. Although miRNAs are mostly well reported to date, both IncRNAs and circRNAs are gaining great popularity due to their specific expression patterns and/or superior stability in body fluids. In particular, the ‘new player’ in ncRNA biomarkers, circRNA, is actively being tested noninvasively in patients [66]. However, the field still faces several preanalytical and analytical challenges that need to be addressed, including the standardization of sample handling and processing, as well as consensus on data normalization [89]. Nonetheless, ncRNAs, especially circRNAs, hold enormous potential as noninvasive biomarkers for cancer and there will likely be more PCa-specific ncRNAs approved for clinical use in the future.

Undoubtedly, ncRNAs are also essential regulators of normal biology, and their dysregulation directly promotes tumor development and progression. This is supported by countless in vitro studies with direct manipulation of ncRNA expression. For example, knockdown of miR-21 and IncRNAs PCGEM1 and PCAT1 inhibited the growth of PCa cells [32,68,70]. More importantly, knockdown of IncRNA SchLAP1 can reduce PCa metastasis in vitro and in vivo [69], and knockdown of circRNA f-circM9 decreases drug resistance of acute myeloid leukemia cells [80]. Mechanistically, most ncRNAs function as master regulators of gene expression, and can thus impact numerous genes in one or more signaling pathways. One such essential pathway in PCa tumorigenesis is the AR pathway, in which recent studies reported upregulation of AR signaling pathway in CRPC cells despite the absence of androgen [97]. Therefore, therapeutic targeting of oncogenic ncRNAs presents attractive alternative treatments for PCa, particularly CRPC, in which silencing of a single ncRNA can simultaneously affect numerous oncogenic drivers. However, not all ncRNAs are equal in therapeutic potential, and it is essential to identify functional ncRNAs to focus our attentions on. As exemplified by Paralkar et al., not all ncRNAs identified from functional deletion screens are truly functional [98]. We therefore have to apply stringent criteria in our search for functional ncRNAs.

One integrative approach by Guo et al. utilized post-genome wide association study data and gene expression data in the identification of 45 functional IncRNAs [70]. In the future, we will likely see more studies applying integrative approaches from multiple datasets to identify ncRNAs for clinical evaluation. Currently the introduction of ASOs is the most popular way to silence oncogenic ncRNAs therapeutically. While they hold great promises, several challenges involving nucleotide stability, target specificity and effective in vivo delivery need to be addressed. Numerous studies have already begun addressing these chal-

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**Table 1. Promising noncoding RNAs in prostate cancer clinical research.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Class</th>
<th>Expression</th>
<th>Annotation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>miR-21</strong></td>
<td>miRNA</td>
<td>Upregulated</td>
<td>Diagnostic/prognostic/therapeutic</td>
<td>[32,35,41,43]</td>
</tr>
<tr>
<td><strong>miR-221</strong></td>
<td>miRNA</td>
<td>Upregulated</td>
<td>Diagnostic/prognostic/therapeutic</td>
<td>[41,43,90]</td>
</tr>
<tr>
<td><strong>miR-145</strong></td>
<td>miRNA</td>
<td>Downregulated (tumor)/upregulated (circulating)</td>
<td>Prognostic</td>
<td>[30,43,45]</td>
</tr>
<tr>
<td><strong>miR-20a</strong></td>
<td>miRNA</td>
<td>Upregulated</td>
<td>Prognostic</td>
<td>[63]</td>
</tr>
<tr>
<td><strong>PCA3</strong></td>
<td>IncRNA</td>
<td>Upregulated</td>
<td>Prognostic</td>
<td>[64]</td>
</tr>
<tr>
<td><strong>SchLAP1</strong></td>
<td>IncRNA</td>
<td>Upregulated</td>
<td>Prognostic/therapeutic?</td>
<td>[94,95]</td>
</tr>
<tr>
<td><strong>PCAT18</strong></td>
<td>IncRNA</td>
<td>Upregulated</td>
<td>Diagnostic/prognostic</td>
<td>[93]</td>
</tr>
<tr>
<td><strong>MALAT1</strong></td>
<td>IncRNA</td>
<td>Upregulated</td>
<td>Diagnostic/therapeutic?</td>
<td>[94,95]</td>
</tr>
<tr>
<td><strong>PCAT1</strong></td>
<td>IncRNA</td>
<td>Upregulated</td>
<td>Therapeutic?</td>
<td>[70,96]</td>
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</table>
Lenges, but it will likely take some time before therapeutic targeting of ncRNAs make their way to the clinical market. Nonetheless, it is an exciting time for ncRNA research as they hold enormous clinical potential and may soon appear as clinically approved biomarkers and therapeutic targets in personalized PCa treatment.

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Executive summary

**Prostate cancer**
- There is an urgent need to identify diagnostic and prognostic biomarkers with high sensitivity and specificity in prostate cancer (PCa).
- There are currently no effective treatments for patients with aggressive castration-resistant PCa.

**Noncoding RNA**
- Noncoding RNAs (ncRNAs) are the most abundant RNA transcripts in the human genome.
- Many ncRNAs play important role in cancer development and progression, and can be exploited as therapeutic targets.
- Expression of ncRNAs is highly specific and may be utilized as novel biomarkers.

**miRNA**
- miRNAs are key regulators of gene expression, and their dysregulation contributes to PCa.
- miRNAs can be targeted therapeutically by antisense oligonucleotides, but improvements in stability, specificity and effective in vivo delivery are needed.
- miRNAs are detectable in blood and are potential noninvasive biomarkers for PCa.

**Long ncRNA**
- Long ncRNAs (lncRNAs) are master regulators of genes and pathways, and their dysregulation in PCa is well documented.
- lncRNA can be targeted therapeutically through chemically modified RNAi agents and antisense oligonucleotides.
- Some lncRNAs exert highly PCa-specific expression and can be exploited as biomarkers.

**Circular RNA**
- Circular RNAs (circRNAs) are highly stable in body fluids and detectable as noninvasive biomarker.
- circRNAs can have different oncogenic or tumor suppressive functions than their corresponding linear RNAs.
- Targeting circRNAs at their backsplice junction site is possible.

**Noncoding RNA crosstalk**
- There is a complex relationship and layer of regulation between different types of ncRNA and mRNAs.

**Future perspective**
- ncRNAs are excellent noninvasive biomarker candidates for PCa diagnosis and prognosis.
- Many ncRNA biomarkers are under clinical testing and may appear in clinical settings in the near future.
- Therapeutic targeting of oncogenic ncRNAs holds great potential in PCa, but is still in its early stages.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest
Reports that dysregulation of miRNAs is cancer type-specific.


**Addresses therapeutic approaches to target miRNAs, and the current clinical challenges.**


**One of the first key studies in the field to propose noninvasive detection of miRNA as a biomarker.**

- Arroyo JD, Chevillet JR, Kroh EM et al. Argonaute2 complexes carry a population of circulating microRNAs.


**Highlights the importance of cellular localization in determining therapeutic agents to target IncRNAs.**


**First study on the properties and functional roles of circRNAs.**


**Shows the existence of a unique class of circRNA involving gene fusions, and their functional roles in cancer.**


