Noncoding RNAs in Prostate Cancer: The Long and the Short of It

Eva M. Bolton1,2, Alexandra V. Tuzova1, Anna L. Walsh1,2, Thomas Lynch2, and Antoinette S. Perry1

Abstract
As the leading culprit in cancer incidence for American men, prostate cancer continues to pose significant diagnostic, prognostic, and therapeutic tribulations for clinicians. The vast spectrum of disease behavior warrants better molecular classification to facilitate the development of more robust biomarkers that can identify the more aggressive and clinically significant tumor subtypes that require treatment. The untranslated portion of the human transcriptome, namely noncoding RNAs (ncRNA), is emerging as a key player in cancer initiation and progression and boasts many attractive features for both biomarker and therapeutic research. Genetic linkage studies show that many ncRNAs are located in cancer-associated genomic regions that are frequently deleted or amplified in prostate cancer, whereas aberrant ncRNA expression patterns have well-established links with prostate tumor cell proliferation and survival. The dysregulation of pathways controlled by ncRNAs results in a cascade of multicellular events leading to carcinogenesis and tumor progression. The characterization of RNA species, their functions, and their clinical applicability is a major area of biologic and clinical importance. This review summarizes the growing body of evidence, supporting a pivotal role for ncRNAs in the pathogenesis of prostate cancer. We highlight the most promising ncRNA biomarkers for detection and risk stratification and present the state-of-play for RNA-based personalized medicine in treating the "untreatable" prostate tumors. Clin Cancer Res; 20(1); 35–43. ©2013 AACR.

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Learning Objective(s)
Upon completion of this article, the reader should have a good understanding of the major small and long noncoding RNAs involved in prostate carcinogenesis, their potential as biomarkers, and the biologic rationale underlying novel therapeutic strategies using noncoding RNAs for castration-resistant prostate cancer.

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Introduction
It was somewhat humbling to learn that the human genome encodes only 20,000 or so protein-coding genes, in the same region as the worm or mouse. Was it really true that >90% of our DNA were so-called ‘junk,’ lying idle? In 2007, it transpired that in fact most of our DNA is transcribed into biologically crucial regulatory molecules, branded noncoding RNAs (ncRNA; refs. 1 and 2). Noncoding because they are not translated into conventionally purposeful protein end-products, but nevertheless, these molecules, both long and short, little and large have emerged as vital parts in our complexity. They are key factors in maintaining normal cellular function and therefore play an enormous role in human disease, including cancer.

So what exactly do ncRNAs do? Broadly speaking, ncRNAs can be divided up into those with “housekeeping” functions such as mRNA processing and protein synthesis and those exhibiting cell-type-specific expression and more “regulatory functions” such as pre- and posttranscriptional...
gene regulation and chromatin assembly. The rapid development of RNA microarrays and next-generation sequencing of transcriptomes (RNA-Seq) has resulted in an unparalleled treasure-trove of data on gene expression, structural rearrangements (i.e., gene fusions, copy number alterations, and alternatively spliced forms), and detection of undiscovered transcripts such as chimeric RNAs (3). Cumulative evidence from these studies supports a significant role for the untranslated, noncoding portion of the human genome in cancer initiation, development, and progression.

Responsible for approximately 30,000 deaths annually in the United States and 258,000 deaths worldwide, prostate cancer is the most common noncutaneous malignancy and third leading cause of cancer-related deaths in men in the Western world (4). This review provides a snapshot of the variety of roles ncRNAs play in prostate carcinogenesis. We also highlight the significant contributions these molecules can make as prostate cancer biomarkers and their potential therapeutic implications.

Small ncRNAs in prostate carcinogenesis

The human genome includes a diverse collection of ncRNAs, which can be broadly grouped according to size and function (Table 1). Noncoding transcripts originate from intergenic sequences, introns of “host” protein-coding genes, or antisense strands. Small ncRNAs (<200 nucleotides) participate in a variety of cellular functions.

**microRNAs**

Without a doubt, microRNAs (miRNA) remain the best-characterized class of small ncRNAs, with 2,578 mature human transcripts listed in miRBase v20 (5). It is estimated that up to 60% of human transcripts are regulated by miRNAs (6). Many excellent reviews have recently summarized the biogenesis of miRNAs and their role in human disease and cancer (7–9). In prostate cancer, several groups have conducted miRNA expression profiling studies using a range of different platforms (10–15). A common finding is that miRNAs tend to be preferentially downregulated during prostate cancer progression and metastatic spread.

**Table 1. Functional classification of major human genomic ncRNAs**

<table>
<thead>
<tr>
<th>RNA type</th>
<th>Symbol</th>
<th>Length (nt)</th>
<th>Function</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translation and protein synthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribosomal RNA</td>
<td>rRNA</td>
<td>121–507</td>
<td>Facilitates passage of tRNAs along the mRNA during translation</td>
<td>4 genes present in hundreds of copies</td>
</tr>
<tr>
<td>Transfer RNA</td>
<td>tRNA</td>
<td>73–94</td>
<td>RNA adaptor molecule that physically links the mRNA nucleic acid sequence with the peptide amino acid sequence at the ribosome</td>
<td>~500</td>
</tr>
<tr>
<td>Ribonuclease P</td>
<td>RPPH1</td>
<td>341</td>
<td>RNA component of ribonuclease P, involved in tRNA maturation and RNA polymerase III transcription</td>
<td>1</td>
</tr>
<tr>
<td>Chromosome structure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telomerase RNA</td>
<td>TERC</td>
<td>451</td>
<td>RNA component of telomerase that provides the template for de novo synthesis of telomeric DNA</td>
<td>1</td>
</tr>
<tr>
<td>Regulatory RNAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miRNA</td>
<td>miR</td>
<td>~22</td>
<td>Negatively regulate gene expression posttranscriptionally through base pairing to the 3’-UTR of target mRNAs and inhibiting protein translation and/or mRNA degradation</td>
<td>~2,578</td>
</tr>
<tr>
<td>Piwi-interacting RNA</td>
<td>piRNA</td>
<td>25–33</td>
<td>Silence transposons during spermatogenesis</td>
<td>~23,000</td>
</tr>
<tr>
<td>Long noncoding RNA or LincRNA</td>
<td></td>
<td>&gt;200</td>
<td>Various</td>
<td>Unknown, estimated at ~20,000</td>
</tr>
<tr>
<td>Long intergenic noncoding RNA</td>
<td>LincRNA</td>
<td>~150</td>
<td>Assemble around newly transcribed pre-mRNA in the spliceosome to remove introns during mRNA processing</td>
<td>~9</td>
</tr>
<tr>
<td>Small nuclear RNA</td>
<td>snRNA</td>
<td>~150</td>
<td>Guide chemical modifications (methylation and pseudouridylation) of other ncRNAs (tRNA, tRNA, snRNA); alternative splicing; in cis and trans gene regulation; may also function as miRNA</td>
<td>~200, some present in several copies</td>
</tr>
<tr>
<td>Small nucleolar RNA</td>
<td>snoRNA</td>
<td>70–200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
However, this finding could be unduly influenced by differences in sample tissue composition and a reduction in the stromal contribution in advanced and metastatic prostate cancer, rather than true biologic meaning. Most recently, a profiling study by Liu and colleagues examined miRNA expression across 6 prostate cancer stem/progenitor cell populations and proposed that a distinct set of miRNAs (downregulation of miR-34a, let-7b, miR-106a, and miR-141 and upregulation of miR-301 and miR-452) coordinately regulate prostate cancer stem cells (16). Of primary interest in this review are those miRNAs experimentally proven to be directly involved in prostate cancer development and progression.

Prostate epithelial cells require androgens and androgen receptor signaling for their proliferation and survival and as such hormone deprivation by chemical castration is the first-line therapeutic modality for patients with advanced disease. Favorable responses are short-lived and progression to lethal castration-resistant prostate cancer (CRPC) is inevitable. The androgen receptor is expressed throughout prostate cancer progression and its overexpression at both gene and protein level is a consistent feature of CRPC, where its activity as a transcriptional activator has been shown to induce a distinct set of mitotic cell cycle genes resulting in androgen-independent growth (17). A gain-of-function analysis of 1,129 miRNAs combined with androgen receptor protein quantification by reverse-phase protein lysate microarray and 3'-UTR luciferase reporter assays in a panel of prostate cancer cell lines identified 13 miRNAs that target AR mRNA (miR-135b, miR-185, miR-297, miR-299-3p, miR-34a, miR-34c, miR-371-3p, miR-421, miR-449a, miR-449b, miR-634, miR-654-5p, and miR-9); several of these also inhibited androgen-induced proliferation. Analysis in clinical specimens confirmed a negative correlation with miR-34a and miR-34c expression and androgen receptor levels (18). The members of the miR-34 family are regulated by transcription factor P53 and have been suggested to be potent mediators of tumor suppression by P53, implicated in the negative control of the cell cycle, senescence, and apoptosis (19, 20). miR-34c was previously found to be significantly downregulated in prostate tumors and linked with disease aggressiveness (21). In addition to the AR, MYC, and cell adhesion and stem cell marker CD44 have been identified and validated as direct and functional targets of miR-34a (22, 23). miR-34a is underexpressed in CD44+ prostate cancer cells from both xenografts and primary tumors. Enforced expression of miR-34a inhibited clonogenic expansion, tumor regeneration, and metastasis, whereas delivery of miR-34a antagonirs in CD44+ prostate cancer cells promoted tumor development and metastasis. This would suggest that miR-34a negatively regulates the tumor initiating capacity of prostate cancer stem cells (23).

miR-205 is possibly the best-characterized tumor suppressor miRNA in prostate cancer. Hypermethylation of the MIR-205 locus is associated with a decrease in miR-205 expression in prostate cancer cell line LNCaP (40-fold induction upon 5-Aza-CdR treatment) and localized prostate cancer compared with matched histologically benign prostate tissue (24). MIR-205 hypermethylation was also shown to be a significant predictor of biochemical recurrence (24). Argonaute-2 co-immunoprecipitation experiments revealed that miR-205 targets mRNAs involved in mitogenesis-activated protein kinase and androgen receptor signaling pathways, including the AR itself (25, 26). miR-205 also plays an important role in counteracting epithelial-to-mesenchymal transition (EMT) and reducing cell migration and invasion by inactivating EMT regulators ZEB1 and ZEB2, which downregulate epithelial marker e-cadherin and upregulate mesenchymal marker vimentin (27, 28). It was recently shown that metastasis suppressor P63 mediates its repressive effects on cell migration and EMT marker ZEB1 through transcriptional activation of MIR-205 in the PC3 cell line (29, 30).

Allelic loss of the MIR-15A-MIR-16-1 cluster on chromosome 13 is correlated with progression of prostate cancer from early stage to metastatic disease. Antagonirs designed to specifically sequester and inhibit miR-15a and miR-16 activity resulted in increased proliferation, migration, and survival in nontumorigenic prostate cells in vitro and in vivo. Furthermore, restoration of miR-15a and miR-16 expression in LNCaP prostate cancer cells resulted in growth arrest and apoptosis. Luciferase assays showed a direct interaction between both miRs and CCND1, WNT3A, and BCL2 transcripts, indicating that this miRNA cluster contributes to prostate carcinogenesis by targeting multiple oncogenic pathways, namely cell-cycle progression, Wnt signaling, and apoptotic resistance (31).

A number of studies have focused on miRNAs involved in the transformation of hormone-sensitive prostate cancer to the lethal castration-resistant phenotype. miRNA expression profiling in androgen-dependent LNCaP cell line and LNCaP-derived androgen-independent LNCaP-Abl cell line identified miR-221 and miR-222 as the most strongly upregulated miRNAs in LNCaP-Abl (10.8- and 6.5-fold, respectively, P < 0.001; ref. 32). Analysis in clinical specimens revealed that overexpression of miR-221/222 in bone metastatic CRPC relative to normal prostate tissue was highly significant (P < 0.001; ref. 33). Functional investigations using LNCaP cells showed that overexpression of miR-221/222 reduced dihydrotestosterone-induced growth and expression of certain androgen receptor–responsive genes [including prostate-specific antigen (PSA)] and resulted in androgen-independent growth (32, 34). miR-221 and miR-222 are upregulated in several cancer types and many different targets have been proposed. However, these transcripts were found to be irrelevant in CRPC and two new potential targets of miR-221 were identified: HECTD2 and RAB1A, although the mechanisms by which they mediate the miR-221/222–induced CRPC phenotype remain to be deciphered (34).

Other small ncRNAs

Studies addressing the expression and functional role of other small ncRNAs (Table 1) in prostate cancer are surprisingly lacking. Deep sequencing of the entire small transcriptome in organ-confined and metastatic lymphomas

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node prostate cancer revealed that the total count and diversity of tRNAs and snoRNAs increased by $>20\%$ during tumor metastasis (15). The authors hypothesized that this was indicative of the high metabolic activity and elevated protein synthesis of advanced tumors. The snoRNA gene $U50$ has been proposed as a tumor suppressor; located within 6q14-15 (commonly deleted in multiple human cancers), it displays a homozygous 2-bp deletion detected in approximately 10% of prostate tumors, which was significantly associated with clinically significant disease (35).

### Long ncRNAs in prostate carcinogenesis

Human long ncRNAs (lncRNAs, >200 nucleotides) are structurally similar to protein-coding genes. They contain proximal promoter sequences and consist of exons and intervening introns but possess no open reading frames. Their biogenesis is also similar to that of mRNAs; they are transcribed by RNA polymerase II and then spliced, polyadenylated, and 5’-capped. Yet, they exhibit both nuclear and cytoplasmic localization (Fig. 1). Although IncRNAs constitute the majority of the transcriptome, we certainly understand less of their biologic functions than those of their small counterparts (Table 1; ref. 36). They are attributed with an ever-increasing number of functional activities including genomic imprinting and both cis- and trans-acting transcriptional regulation. This is achieved via a variety of mechanisms such as natural antisense inhibition of contiguous genes, transcriptional interference, recruitment of chromatin remodeling complexes to specific gene loci, and promoter inactivation by binding to basal transcription factors (36–39).

One of the earliest lncRNAs described in prostate cancer was prostate cancer gene expression marker 1 ($PCGEM1$), a prostate-specific transcript encoded on 2q32 (40). It promotes cell proliferation and inhibits apoptosis in vitro, although the molecular mechanisms behind this remain to be elucidated (41, 42). $PCGEM1$ has also been hypothesized to contribute to ethnic variation in prostate cancer incidence (41).

In the most comprehensive analysis of lncRNAs in prostate carcinogenesis to date, Prensner and colleagues analyzed the transcriptomes of 102 prostate tumors and cell lines by RNA-Seq (43). The authors reported 121 long intergenic ncRNAs (lincRNA), whose expression patterns distinguished benign, localized, and metastatic cancers. They described prostate cancer–associated transcript 1 ($PCAT1$), a novel prostate cancer lincRNA on 8q24, in the locality of well-characterized prostate cancer risk-related single-nucleotide polymorphisms (SNP) and the $c$-MYC oncogene (44). $PCAT1$ was found to be upregulated in a subset of metastatic and high-grade localized tumors and to promote cell proliferation in vitro through transcriptional regulation of target genes. $PCAT1$ and $EZH2$ expression were shown to be mutually exclusive and knockdown or inhibition of $EZH2$ caused reexpression of $PCAT1$ and downregulation of its target genes (43).

That same year, Chung and colleagues reported prostate cancer susceptibility SNPs in a 13-kb intron-less lincRNA also on 8q24, which they termed prostate cancer susceptibility SNPs within a 13-kb intron-less lincRNA ($PRNCR1$; ref. 45). $PRNCR1$ was found to be upregulated in a small sample set of precursor prostatic intraepithelial neoplasia and prostate tumors. siRNA-
mediated knockdown of\textit{PRNCR1} reduced cell viability and
transactivation by the androgen receptor, although the
precise mechanisms behind these observations were not
elucidated. Fascinatingly, a search on the UCSC Human
Genome Browser (Feb 2009 assembly) reveals that \textit{PCAT1}
and \textit{PRNCR1} are adjoining neighbors on 8q24, separated
by only 60 kb of DNA desert (46). Other SNP analyses with
respect to prostate cancer risk-related loci have revealed an
enrichment in lncRNA sequences and also identified new
risk-related loci, such as \textit{19p13} (47).

A handful of other lncRNAs have also been associated
with prostate cancer. A small RNA-Seq study on 14 Chinese
prostate tumors and adjacent benign tissues identified 137
lncRNAs that were significantly altered (48). The \textit{CDKN2A–
CDKN2B} tumor suppressor locus is subject to frequent
deletion and hypermethylation in cancers, including pros-
tate cancer. \textit{ANRIL} is an antisense lncRNA elevated in
prostate cancer that overlaps this locus, interacting directly
with polycomb repressive complex 1 and histone H3K27
methylation to repress \textit{CDKN2A–CDKN2B} expression (49).

Providing a link between ncRNAs and chromosome
structure is telomerase, a ribonucleoprotein polymerase
responsible for the synthesis of the tandem hexameric
repeat sequence (TTAGGG) at chromosome termini. Telo-
merase activation and subsequent maintenance of telo-
meres are required for tumor cell survival and proliferation.
The telomerase core enzyme consists of an RNA component
(\textit{TERC}) that provides the template for \textit{de novo} synthesis
of telomeric DNA, and a protein catalytic subunit (\textit{TERT})
with reverse transcriptase activity. \textit{TERC} is expressed in all human
tissues regardless of telomerase activity, whereas \textit{TERT}
is overexpressed in >90% of tumor cells. Antisense oligo-
nucleotide–mediated knockdown of \textit{TERC} significantly
reduced cell viability in PC3 and DU145 cell lines and
reduced tumor growth in nude mice via induction of
apoptosis (50), although this effect was not seen by others
(51). Amplification of the \textit{TERC} gene has been reported in
5% of hormone-naïve prostate tumors and in approximate-
ly 16% of CRPC (52). In support of this, \textit{in situ} hybridiza-
tion for \textit{TERC} showed upregulation in luminal epithelial
cells during malignant transformation of the prostate,
although a high degree of heterogeneity was observed in
neoplastic cells (53).

\textbf{ncRNAs as diagnostic and prognostic biomarkers for
prostate cancer}

Development of diagnostic and prognostic prostate can-
cer biomarkers has the potential to dramatically improve
disease management, reduce overtreatment, and eradicate
death from this disease. PSA is currently the only serum
marker in widespread clinical use, although its limitations
are well established (54, 55). Perhaps surprisingly, the most
clinically advanced prostate cancer biomarker is in fact an
lncRNA. Prostate cancer antigen 3 (\textit{PCA3}) is a unique,
polyadenylated, atypical alternatively spliced lncRNA spe-
cifically overexpressed in >95% of primary prostate tumors
(56). Urinary detection of \textit{PCA3} has been developed as a
prostate cancer detection test with superior tumor specificity
to PSA (57). The Progensa \textit{PCA3} test is approved by the U.S.
Food and Drug Administration and commercially available
to aid in the decision of repeat biopsies. Correlations
between \textit{PCA3} and prognostic factors (histologic Gleason
grade and tumor stage) are conflicting, although most
studies report the \textit{PCA3} test is negative in men with indolent
cancer (58, 59). Efforts to improve the prognostic value of
\textit{PCA3} are focusing on teaming it with \textit{TMPRSS2-ERG},
a highly prostate cancer–specific family of gene fusion tran-
scripts (60). Two independent prospective, multicenter
evaluations of the combined quantification of \textit{PCA3} and
\textit{TMPRSS2-ERG} revealed that the superior prostate cancer
specificity of this urinary biomarker panel over serum PSA
could reduce a substantial number of unnecessary prostate
biopsies (61) and could also have utility for risk stratifica-
tion in an active surveillance setting (62).

Circulating small ncRNAs have \textit{bone fide} appeal as blood/
urine-based biomarkers, demonstrating resistance to varia-
tions in temperature and pH as well as endogenous RNase
activity (63). Serum samples from men with low-risk,
localized prostate cancer and metastatic CRPC have been
shown to exhibit distinct circulating miRNA signatures (Fig.
2; refs. 64–66). Similarly, plasma miRNA panels have been
shown to differentiate patients by tumor aggressiveness (67,
68). A common feature to these studies is the detection of
\textit{miR}-21, \textit{miR}-141, and \textit{miR}-375 in the plasma/sera of men
with advanced disease and the association of these miRNAs
with poor prognosis.

\textbf{ncRNAs as prostate cancer therapeutics}

Our ever-expanding appreciation of molecular tumor
heterogeneity, coupled with transcriptomic profiling and
mechanistic studies (that reveal widespread dysregulation
of ncRNAs in prostate cancer), suggest bespoke treat-
ments, which could be tailored to distinct molecular
genotypes. miRNAs constitute one of the most abundant
classes of gene-regulatory molecules (6). On one hand,
this makes these micromolecules highly attractive for
therapeutic manipulation. Conversely, because many
miRNAs are targeted by a single miRNA, off-target effects
are likely to be substantial. A number of other major
obstacles are impeding development of (nc)RNA-based
therapeutics, such as the inherent low stability of RNA
molecules and tumor-specific delivery and retention.
Some solutions exist: Locked nucleic acid design, conju-
gation to cholesterol moieties, and encapsement in nano-
particles have all been shown to improve stability.
Targeted delivery to specific tissues can be achieved by
linking tumor-specific ligands to nanoparticle surfaces.
Prostate tumor cells could be selectively targeted through
the cell-surface receptor \textit{PSMA} (69). Nanoparticles can be
further specified to target tissues by engineering their size
so that they can only pass through the larger pores present
in tumor–blood vessels, allowing them to accumulate
inside tumor cells (70).

miRNAs seem particularly appealing from a therapeutic
standpoint and can be manipulated in two ways: miRNA
replacement and miRNA reduction (using antisense
oligonucleotides, antagomiRs; ref. 71). The aim of miRNA replacement is to reintroduce tumor-suppressor miRNAs depleted in the tumor (by use of a miRNA mimic), thus reactivating specific pathways to drive a therapeutic response (70). Given that evidence supports downregulation as the more widespread mode of miRNA dysregulation in prostate carcinogenesis (as opposed to oncoMir activation), this is an active area of research for novel prostate cancer therapeutics. Systemic delivery of atelocollagen-conjugated miR-16 in a mouse xenograft model of prostate cancer inhibited bone metastases (72). In an independent study, reintroduction of miR-15-16 induced tumor regression and enhanced docetaxel sensitivity in LNCaP cell lines and primary tumor cells (31). TP53 mutations are frequent in prostate cancer, and thus miR-34 (downstream effector of P53) replacement therapy could be of great therapeutic benefit. Systemic delivery of miR-34a was found to inhibit prostate cancer metastasis and improve survival in tumor-bearing mice (23).

Another approach utilizes a small RNA molecule as both a targeting (cell-type specific) and silencing moiety (via RNA interference) by generating an aptamer-siRNA chimera. The miRNA-processing enzyme Dicer acts upon the chimeric RNA, thus directing it into the RNA interference pathway, where it silences its target mRNAs. Aptamer-siRNA chimeras were designed to target prostate cancer cells specifically through interaction with PSMA at the cell surface, and effectively silenced two antiapoptotic genes (PLK1 and

**Table 1. ncRNAs in the molecular pathogenesis of prostate cancer (PCa).**

<table>
<thead>
<tr>
<th>Stem cell progenitor model</th>
<th>Dietary genistein consumption (prevention of ncRNA hypermethylation, i.e., miR-205)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(downregulation of miR-34 family)</td>
<td><strong>HGPIN</strong></td>
</tr>
<tr>
<td>Epigenetic dysregulation</td>
<td><strong>Cell proliferation</strong> (overexpression of PCGEM1 and PCAT1; downregulation of miR-23b and miR-124)</td>
</tr>
<tr>
<td>(promoter hypermethylation–associated silencing of miR-23b, miR-34b, and miR-205)</td>
<td><strong>Cell-cycle progression</strong> (downregulation of miR-15, miR-16 and miR-23b)</td>
</tr>
<tr>
<td><strong>Apoptotic resistance</strong></td>
<td><strong>AR dysregulation</strong> (downregulation of miR-34 family, miR-124 and miR-205; overexpression of miR-221/222 and PRNCR1)</td>
</tr>
<tr>
<td>(allelic loss of the miR-15a/miR-16-1 cluster; overexpression of PCGEM1)</td>
<td><strong>EMT</strong> (downregulation of miR-23b, miR-34b, miR-200 family, and miR-205; overexpression of miR-21)</td>
</tr>
<tr>
<td><strong>Cell proliferation</strong> (overexpression of PCGEM1 and PCAT1; downregulation of miR-23b and miR-124)</td>
<td><strong>Localize prostate cancer</strong></td>
</tr>
<tr>
<td><strong>Biomarker and therapeutic potential</strong></td>
<td><strong>Urinary detection of clinically significant PCa</strong> (overexpression of PCA3)</td>
</tr>
<tr>
<td></td>
<td><strong>ncRNA-replacement therapy</strong> (miR-16, miR-34, and miR-205)</td>
</tr>
<tr>
<td></td>
<td><strong>Systemic delivery of microRNAs targeting the AR</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Plasma/serum detection of metastatic PCa</strong> (overexpression of miR-21, miR-141, and miR-375)</td>
</tr>
</tbody>
</table>

Figure 2. ncRNAs in the molecular pathogenesis of prostate cancer (PCa). Prostate cancer arises in the glandular epithelial cells. High-grade prostatic intraepithelial neoplasia (HGPIN) is the earliest accepted stage in prostate carcinogenesis, characterized by architecturally benign prostatic ducts, with changes to the spatial arrangements of the glandular luminal cells and to their nuclear size and shape and focal disruption of the basal cell layer. In carcinoma, there is an increased nuclear:cytoplasmic ratio of the luminal cells, a disappearance of the basal cellular layer, and an infiltrative growth pattern. Somatic genetic and epigenetic aberrations to ncRNAs accumulate during the pathogenesis of prostate cancer and have far-reaching consequences for the cell. ncRNAs also have potential in the management of prostate cancer as diagnostic and prognostic biomarkers and as vector-based therapies.

**Biologic implications**

**Stem cell progenitor model**

- **Benign prostate**
  - (downregulation of miR-34 family)

**Epigenetic dysregulation**

- **HGPIN**
  - (promoter hypermethylation–associated silencing of miR-23b, miR-34b, and miR-205)

**Cell proliferation**

- **Localized prostate cancer**
  - (overexpression of PCGEM1 and PCAT1; downregulation of miR-23b and miR-124)

**Cell-cycle progression**

- **Urinary detection of clinically significant PCa**
  - (overexpression of PCA3)

**Apoptotic resistance**

- **ncRNA-replacement therapy**
  - (miR-16, miR-34, and miR-205)

**AR dysregulation**

- **Systemic delivery of microRNAs targeting the AR**

**EMT**

- **Plasma/serum detection of metastatic PCa**
  - (overexpression of miR-21, miR-141, and miR-375)
ncRNAs in Prostate Cancer

BCL2) inducing tumor-regression in a mouse xenograft model of prostate cancer (69).

Far less is known on the potential of lncRNAs as therapeutic modalities for prostate cancer. It has been argued that truly effective treatment regimens must specifically target the subpopulation of prostate cancer stem cells. This avenue has recently been explored by targeting the telomerase IncRNA TERC, which was shown to be enriched in $\alpha_2\beta_1^{\text{high}}$ CD44$^+$ putative prostate cancer stem cells. A two-pronged "telomerase-interference" approach consisting of ectopic expression of a TERC (with a mutated template region) and an siRNA (against wild-type endogenous TERC) effectively reprogrammed telomerase, eliciting a DNA damage response and apoptosis (73). This novel approach was also shown to abrogate the tumorigenicity of DU145 $\alpha_2\beta_1^{\text{high}}$ CD44$^+$ prostate cancer cells in SCID mice (74).

Conclusions

The field of ncRNA biology and its contribution to human disease is experiencing a well-deserved upsurge in research activity. Differential expression of ncRNAs is now a recognized trait of prostate tumorigenesis; however, the functional role of many of these molecules unearthed during profiling studies remains undetermined. We are only just beginning to understand how these noncoding molecules are involved in prostate cancer. Teasing apart their diverse range of target molecules and modes of action offers an unparalleled opportunity to open the drapes and shed light onto the altered biology of the so-called "dark matter" inside the prostate tumor cell. It was recently shown that prostate cancer cells are enriched in chimeric miRNAs, compared with their benign counterparts (75). One could logically extrapolate from these findings that chimeric ncRNAs might also be a prominent feature in prostate cancer. The decreasing cost of whole transcriptome RNA-Seq and its reciprocal increased accessibility to more laboratories will be instrumental in validating studies to date and addressing other questions too: What of the expression or role of piRNAs or even mitochondrial ncRNAs in prostate cancer? Is the elevated expression of snoRNAs in advanced prostate cancer simply a net result of elevated protein synthesis or are they playing a more sinister role? Relatively speaking, biomarker studies into prostate cancer ncRNAs are in their infancy. Further work is needed to establish the importance of distinguishing between free-circulating ncRNAs, those bound to Argonaute proteins and circulating microvesicle-encapsulated ncRNAs. The therapeutic applications of ncRNAs in prostate cancer are still in a formative stage and require extensive investigation in vitro and in animal models before their true potential can be realized.

Authors’ Contributions

Conception and design: E.M. Bolton, A.S. Perry

Development of methodology: E.M. Bolton

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.M. Bolton, T. Lynch

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.S. Perry

Writing, review, and/or revision of the manuscript: E.M. Bolton, A.L. Walsh, T. Lynch, A.S. Perry

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.M. Bolton, T. Lynch, A.S. Perry

Study supervision: T. Lynch

Other (prepared figures): A. Tuzova

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