Non-coding RNAs in Prostate Cancer: the Long and the Short of it

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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 non-coding RNAs (ncRNAs), 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, whilst 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 multi-cellular events leading to carcinogenesis and tumor progression. The characterization of RNA species, their functions and their clinical applicability is a major area of biological 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.
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 was so called “junk”, lying idle? In 2007, it transpired that in fact most of our DNA is transcribed into biologically crucial regulatory molecules, branded non-coding RNAs (1, 2). Non-coding 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 post-transcriptional 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, non-coding 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 (PCa) is the most common non-cutaneous 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 PCa 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). Non-coding 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 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 microRNAs (6). Many excellent reviews have recently summarized the biogenesis of microRNAs and their role in human disease and cancer (7-9). In PCa, several groups have conducted microRNA expression profiling studies using a range of different platforms (10-15). A common finding is that microRNAs tend to be preferentially down-regulated during PCa progression and metastatic spread. However, this finding could be unduly influenced by differences in sample tissue composition and a reduction in the stromal contribution in advanced and metastatic PCa, rather than true biological meaning. Most recently, a profiling study by Liu et al examined miRNA expression across six PCa stem/progenitor cell populations and proposed that a distinct set of microRNAs (down-
regulation of miR-34a, let-7b, miR-106a and miR-141 and up-regulation of miR-301 and miR-452) coordinately regulate PCa stem cells (16). Of primary interest in this review are those microRNAs experimentally proven to be directly involved in PCa development and progression.

Prostate epithelial cells require androgens and androgen receptor (AR) 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 AR is expressed throughout PCa progression and its over-expression 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 microRNAs combined with AR protein quantification by reverse-phase protein lysate microarray and 3’UTR luciferase reporter assays in a panel of PCa cell lines identified 13 microRNAs 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 AR levels (18). 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 down-regulated 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-34-a is under-expressed in CD44^+ PCa cells from both xenografts and primary tumors. Enforced expression of miR-34a inhibited clonogenic expansion, tumor regeneration and metastasis, whilst delivery of miR-34a antagonirs in CD44^- PCa cells promoted tumor development and metastasis. This would suggest that miR-34a negatively regulates the tumor initiating capacity of PCa stem cells (23).

miR-205 is possibly the best characterized tumor suppressor microRNA in PCa. Hypermethylation of the _MIR-205_ locus is associated with a decrease in miR-205 expression in PCa cell line LNCaP (40-fold induction upon 5-Aza-CdR treatment) and localized PCa compared to 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 genes involved in mitogen-activated protein kinase (MAPK) and AR 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 -2, which down-regulate epithelial marker e-cadherin and up-regulate 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 PCa from early stage to metastatic disease. Antagomirs 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 PCa 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 PCa 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 -222 as the most strongly up-regulated miRNAs in LNCaP-Abl (10.8-fold and 6.5-fold, respectively, P<0.001) (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) (33). Functional investigations using LNCaP cells showed that overexpression of miR-221/222 reduced dihydrotestosterone-induced growth and expression of certain AR-responsive genes (including *PSA*) and resulted in androgen-independent growth (32, 34). miR-221/222 are up-regulated 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 PCa are surprisingly lacking. Deep sequencing of the entire small transcriptome in organ-confined and metastatic lymph node PCa 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 (IncRNAs, >200 nucleotides) are structurally similar to protein-coding genes. They contain proximal promoter sequences, and consist of exons & intervening introns but possess no open reading frames. Their biogenesis is also similar to mRNAs; transcribed by RNA polymerase II and then spliced, polyadenylated and 5’-capped. Yet, they exhibit both nuclear and cytoplasmic localization (Figure 1). Although IncRNAs constitute the majority of the transcriptome, we certainly understand less of their biological functions than those of their small counterparts (Table 1) (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 PCa, was PCGEM1 (prostate cancer gene expression marker 1), 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 PCa incidence (41).

In the most comprehensive analysis of lncRNAs in prostate carcinogenesis to date, Prensner et al. analyzed the transcriptomes of 102 prostate tumors and cell lines by RNA-Seq (43). The authors reported 121 long intergenic ncRNAs (lincRNAs) whose expression patterns distinguished benign, localized and metastatic cancers. They described PCAT1 (prostate cancer associated-transcript 1), a novel PCa lincRNA on 8q24, in the locality of well-characterized PCa risk-related SNPs and the c-MYC oncogene (44). PCAT1 was found to be up-regulated 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 knock-down or inhibition of EZH2 caused re-expression of PCAT1 and down-regulation of its target genes.

That same year, Chung et al. reported PCa susceptibility SNPs within a 13 kb intron-less lincRNA also on 8q24, which they termed PRNCR1 (prostate cancer non-coding RNA 1) (45). PRNCR1 was found to be up-regulated in a small sample set of precursor prostatic intraepithelial neoplasia and prostate tumors. siRNA mediated knockdown of PRNCR1 reduced cell viability and transactivation by the AR, although the precise mechanisms behind these observations were not elucidated. Fascinatingly, a search on the UCSC Human Genome Browser (Feb 2009 assembly) reveals that PCAT1 and PRNCR1 are adjoining
neighbors on 8q24, separated by only 60kb of DNA desert (46). Other SNP analysis with respect to PCa risk-related loci have revealed an enrichment in IncRNA sequences and also identified new risk-related loci, such as 19p13 (47).

A handful of other IncRNAs have also been associated with PCa. A small RNA-Seq study on 14 Chinese prostate tumors and adjacent benign tissues identified 137 IncRNAs that were significantly altered (48). The *CDKN2A-CDKN2B* tumor suppressor locus is subject to frequent deletion and hypermethylation in cancers including PCa. *ANRIL* is an antisense IncRNA elevated in PCa that overlaps this locus, interacting directly with polycomb repressive complex 1 and histone H3K27 methylation to repress *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. Telomerase activation and subsequent maintenance of telomeres are required for tumor cell survival and proliferation. The telomerase core enzyme consists of an RNA component (*TERC*) that provides the template for *de novo* synthesis of telomeric DNA, and a protein catalytic subunit (*TERT*) with reverse transcriptase activity. *TERC* is expressed in all human tissues regardless of telomerase activity, whereas TERT is highly expressed in >90% of tumor cells. Antisense oligonucleotide mediated knock-down of *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 *TERC* gene has been reported in 5% of hormone-naive prostate tumors and in approximately 16% of CRPC (52). In support of this, *in situ* hybridization for *TERC* showed up-regulation in luminal epithelial cells during
malignant transformation of the prostate, although a high degree of heterogeneity was observed in neoplastic cells (53).

**ncRNAs as diagnostic and prognostic biomarkers for PCa**

Development of diagnostic and prognostic PCa biomarkers has the potential to dramatically improve disease management, reduce over-treatment 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 PCa biomarker is in fact a IncRNA. *PCA3* (Prostate cancer antigen 3) is a unique, polyadenylated, atypical alternatively-spliced IncRNA specifically over-expressed in >95% of primary prostate tumors (56). Urinary detection of *PCA3* has been developed as a PCa-detection test with superior tumor-specificity to PSA (57); The Progensa *PCA3* test is approved by the US FDA and commercially available to aid in the decision of repeat biopsies. Correlations between *PCA3* and prognostic factors (histologic Gleason grade and tumor stage) are conflicting, although most studies report the *PCA3* test is negative in men with indolent cancer (58, 59). Efforts to improve the prognostic value of *PCA3* are focusing on teaming it with *TMPRSS2-ERG*, a highly PCa-specific family of gene fusion transcripts (60). Two independent prospective, multi-center evaluations of the combined quantification of *PCA3* and *TMPRSS2-ERG* revealed that the superior PCa-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-stratification in an active surveillance setting (62).

Circulating small ncRNAs have *bone fide* appeal as blood/urine-based biomarkers, demonstrating resistance to variations in temperature and pH as well as endogenous RNase
activity (63). Serum samples from men with low-risk, localized PCa and metastatic CRPC have been shown to exhibit distinct circulating miRNA signatures (Figure 2) (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 miR-21, miR-141 and miR-375 in the plasma/sera of men with advanced disease and the association of these microRNAs with poor prognosis.

ncRNAs as PCa therapeutics

Our ever-expanding appreciation of molecular tumor heterogeneity, coupled with transcriptomic profiling and mechanistic studies (that reveal widespread dysregulation of ncRNAs in PCa), suggest bespoke treatments, which could be tailored to distinct molecular phenotypes. microRNAs 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 mRNAs are targeted by a single microRNA, off-target effects are likely to be substantial. There are a number of other major obstacles 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, conjugation to cholesterol moieties and encasement in nanoparticles, 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 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).

MicroRNAs appear particularly appealing from a therapeutic standpoint and can be manipulated in two ways: microRNA replacement and microRNA reduction (using antisense oligonucleotides, antagomiRs) (71). The aim of microRNA replacement is to reintroduce tumor-suppressor microRNAs depleted in the tumor (by use of a microRNA mimic), thus reactivating specific pathways to drive a therapeutic response (70). Given that evidence supports down-regulation as the more widespread mode of microRNA dysregulation in prostate carcinogenesis (as opposed to oncoMir activation), this is an active area of research for novel PCa therapeutics. Systemic delivery of atelocollagen-conjugated miR-16 in a mouse xenograft model of PCa 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 PCa 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 PCa 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 microRNA-processing enzyme Dicer acts upon the chimeric RNA, thus directing it into the RNAi pathway, where it silences its target mRNAs. Aptamer-siRNA chimeras were designed to target PCa cells specifically through interaction with PSMA at the cell surface, and effectively silenced two anti-apoptotic genes (PLK1 and BCL2) inducing tumor-regression in a mouse xenograft model of PCa (69).
Far less is known on the potential of IncRNAs as therapeutic modalities for PCa. It has been argued that truly effective treatment regimens must specifically target the sub-population of PCa stem cells. This avenue has recently been explored by targeting the telomerase IncRNA \( \text{TERC} \), which was shown to be enriched in \( \alpha2\beta1^{\text{high}} \) CD44\(^{+}\) putative PCa stem cells. A two-pronged “telomerase-interference” approach consisting of ectopic expression of a \( \text{TERC} \) (with a mutated template region) and an siRNA (against wild-type endogenous \( \text{TERC} \)) effectively reprogrammed telomerase, eliciting a DNA damage response and apoptosis (73). This novel approach was also shown to abrogate the tumorigenicity of DU145 \( \alpha2\beta1^{\text{high}} \) CD44\(^{+}\) PCa 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 non-coding molecules are involved in PCa. 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 PCa cells are enriched in chimeric mRNAs, compared to their benign counterparts (75). One could logically extrapolate from these findings that chimeric ncRNAs might also be a prominent feature in PCa. 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 PCa? Is the elevated expression of snoRNAs in advanced PCa simply a net result of elevated protein synthesis or are they playing a more sinister role? Relatively speaking, biomarker studies into PCa 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 PCa are still in a formative stage and require extensive investigation \textit{in vitro} and in animal models before their true potential can be realized.
# TABLES

## 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><strong>Translation &amp; protein synthesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ribosomal RNA</td>
<td>rRNA</td>
<td>121-5070</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><strong>Chromosome structure</strong></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><strong>Regulatory RNAs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>microRNA</td>
<td>miR</td>
<td>~ 22</td>
<td>Negatively regulate gene expression post-transcriptionally 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 non-coding RNA or Long intergenic non-coding RNA</td>
<td></td>
<td>&gt;200</td>
<td>Various</td>
<td>Unknown, estimated at ~20,000</td>
</tr>
<tr>
<td>Small nuclear RNA</td>
<td>snRNA</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 nucleolar RNA</td>
<td>snoRNA</td>
<td>70-200</td>
<td>Guide chemical modifications (methylation and pseudouridylation) of other ncRNAs (rRNA, 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>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Functions of non-coding RNAs in the eukaryotic cell

ncRNAs play a diverse range of functions in the normal eukaryotic cell. Small ncRNAs have both nuclear and cytoplasmic functions: microRNAs regulate post-transcriptional expression of specific target genes through mRNA cleavage and/or translational inhibition by recruiting the RNA-induced silencing complex. Other small ncRNAs function in house-keeping roles such as mRNA translation and protein synthesis. They also play more regulatory roles, such as alternative splicing and transposon silencing. LncRNAs can act as guides and tethers for chromatin-modifying complexes; can act as natural antisense transcripts inhibiting gene expression, and can bind directly to other RNA species, modifying their stability.

Abbreviations: CMVs: circulating microvesicles, such as exosomes.

Figure 2. ncRNAs in the molecular pathogenesis of prostate cancer.

PCa 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 PCa, and have far-reaching consequences for the cell. ncRNAs also have potential in the management of PCa as diagnostic and prognostic biomarkers and as vector-based therapies.
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Figure 1:

- Long ncRNA
- Small ncRNA
- Transposon silencing
- piRNA
- Chromatin assembly
- Antisense inhibition
- pre-mRNA
- mRNA
- Posttranscriptional modifications of rRNA and tRNA
- mRNA stability
- mRNA degradation/translation inhibition
- CMV-mediated transfer of miRNA
- intron splicing
- TERC
- Telomere replication
- snRNA, snoRNA
- Posttranscriptional RNA and TRNA
- Small rRNA
- Small tRNA
- Small ncRNA
- Small rRNA
Figure 2:

### Biologic implications

#### Stem cell progenitor model
- (Downregulation of miR-34 family)

#### Epigenetic dysregulation
- (Promoter hypermethylation–associated silencing of MIR-23B, MIR-34B and MIR-205)

#### Cell proliferation
- (Overexpression of PCGEM1 and PCAT1; downregulation of miR-23b and miR-124)

#### Cell cycle progression
- (Downregulation of miR-15, miR-16 and miR-23b)
- **Apoptotic resistance**
  - (Allelic loss of the miR-15a-miR-16-1 cluster; overexpression of PCGEM1)
- **AR dysregulation**
  - (Downregulation of miR-34 family, miR-124 and miR-205; overexpression of miR-221/222 and PRNCR1)

#### EMT
- (Downregulation of miR-23b, miR-34b, miR-200 family and miR-205; overexpression of miR-21)

### Biomarker and therapeutic potential

#### Benign prostate

#### HGPIN

#### Localized PCa

#### Metastatic PCa

### Biomarker and therapeutic potential

- **Dietary genistein consumption**
  - (Prevention of ncRNA hypermethylation, i.e., MIR-205)

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

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

- **Plasma/serum detection of metastatic PCa**
  - (Overexpression of miR-21, miR-141 and miR-375)

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

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