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NON-THEMATIC REVIEW

Deciphering the function of non-coding RNAs in prostate cancer

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Abstract The advent of next-generation sequencing methods is fuelling the discovery of multiple non-coding RNA transcripts with direct implication in cell biology and homeostasis. This new layer of biological regulation seems to be of particular importance in human pathogenesis, including cancer. The aberrant expression of ncRNAs is a feature of prostate cancer, as they promote tumor-suppressive or oncogenic activities, controlling multicellular events leading to carcinogenesis and tumor progression. From the small RNAs involved in the RNAi pathway to the long non-coding RNAs controlling chromatin remodeling, alternative splicing, and DNA repair, the non-coding transcriptome represents the significant majority of transcriptional output. As such, ncRNAs appear as exciting new diagnostic, prognostic, and therapeutic tools.

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However, additional work is required to characterize the RNA species, their functions, and their applicability to clinical practice in oncology. In this review, we summarize the most important features of ncRNA biology, emphasizing its relevance in prostate carcinogenesis and its potential for clinical applications.

Keywords ncRNA · microRNA · lncRNA · Prostate cancer · Transcription

1 Introduction

Following the central dogma of molecular biology, DNA is transcribed into messenger RNAs, which in turn serves as the guide for protein synthesis [1, 2]. Although exceptions to this rule were known to occur, in the form of transfer RNAs and ribosomal RNAs for a long time [3, 4], only over the last few years' evidence emerged that RNA displayed functional roles beyond the messenger between DNA and protein. It is now widely accepted that RNA plays a key role in the regulation of genome organization and gene expression [5, 6]. Multiple studies have demonstrated that the vast majority of the human genome is dynamically and differentially transcribed to produce multiple and complex non-coding RNAs (ncRNAs), *i.e.*, RNA transcripts that do not encode for a protein but rather act as regulatory RNAs, in a phenomenon called pervasive transcription [2, 7–9].

The human genome comprises more than 3.2 billion nucleotides that unfolded correspond to more than 2 m of linear DNA, which is packed into three-dimensional structures in the nucleus of each cell [10]. However, the part of the genome that represents protein-coding genes is approximately only 2–3 %, while a vast and diverse plethora of ncRNAs originate from the remaining nucleotides in the genome [5, 10]. Current

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ENCODE predictions suggest that ~80 % of the genome's DNA is transcribed into RNA and contribute to the overall estimates of 80 % of the genome being biochemically active/ functional [11]. Although these include some functionally well-characterized small and long ncRNAs [11–15], it has been hypothesized that their abundance and sheer complexity alone is reason enough to believe that they must play major regulatory roles in complex organisms. Furthermore, because such complexity is not the result of the amount of synthesized proteins, it should represent the extent and nature of genome regulation [12, 16]. However, caution should be taken when considering those transcribed DNA elements as functional players. Multiple lines of evidence indicate that this genome-wide transcription is a stochastic process rather than a sign of function *per se* [17, 18]. Indeed, any given DNA element can be transcribed when it is associated with specific histone marks, binds to transcription factors, and is located in an open chromatin area [11]. Transcription is certainly a prerequisite for a genetic element to be functional, but it is not synonymous of that condition [18]. Further, if one looks at the term "function" from an evolutionary standpoint and assumes that a putative undetected function of 98 % of the human genome is not a human-specific trait, it might be argued that a given DNA element with an important function would possibly show significant signs of selective pressure (*i.e.*, sequential conservation in related organisms) to maintain this functionality over evolutionary time as demonstrated for a very limited number of long ncRNAs (lncRNAs) [19]. Comparative studies showed that broadly conserved lncRNAs share a short and 5′-biased patches of conserved sequence [20]. Moreover, lncRNA structure is considerably renewed during evolution, in part due to exonization of transposable elements [20].

Nevertheless, an ever-increasing number of novel classes of small and long ncRNAs are being described, regardless of its homology to that of any related organism or demonstrated function. Driven by recent paradigm shifts in the appreciation of genomic architecture, regulation, and transcriptional output, this seems a valid approach to many researchers. Many of these novel ncRNAs are able to interact with DNA, RNA, and proteins. Some take part in diverse structural, functional, and regulatory activities, controlling nuclear organization and transcription, post-transcriptional, and epigenetic regulation [10, 16]. This expanding inventory of ncRNAs is implicated in a broad spectrum of processes including organ homeostasis and pathogenesis. This growing index of ncRNAs is fuelled by the discoveries of large-scale consortia, aiming to dissect the functional genomic elements such as ENCODE and FANTOM [5]. These projects exposed the complexity and plasticity of the genome: It encompasses not only protein-coding genes with multiple transcription start sites, alternative promoter and enhancer elements, splicing initiation, and donor sites, as well as variable 3 -untranslated regions (UTRs), but also an unpredictably large number of ncRNAs [5]. These

display numerous regulatory functions and similarly serve as substrates for transcriptional and post-transcriptional diversification [12, 16]. The advent of sequencing technologies revealed that the vast majority of the genome is transcribed either in sense or antisense, and it is also expressed in a highly cell type-, subcellular compartment-, and developmental stage-specific manner [21]. The current view of RNA transcription is that each nucleotide can contribute to context-dependent transcription, mediated by specific RNA polymerases, ultimately giving rise to numerous and overlapping transcripts [21].

Several reports shed light on the global deregulation of non-coding transcriptome that occurs in cancer cells. One of the best examples is the overexpression of lncRNA *HOTAIR* in breast cancer. *HOTAIR* reprograms breast cells' epigenome in a Polycomb repressive complex 2 (PRC2)-dependent manner, contributing to increased invasiveness and metastasization [22]. Recently, it was shown that intronic RNA may serve as molecular scaffold for epigenetic regulation through recruitment of PRC2 proteins to specific gene loci [23]. This misregulation of RNA–protein interactions ultimately leads to tumor formation [23]. Interestingly, R-loop-formation and head-to-head antisense transcription are known to be involved in transcriptional activation in cancer [24]. However, one of the first hints on ncRNAs involvement in cancer was the deletion and concomitant downregulation of miR-15 and miR-16 in chronic lymphocytic leukemia [25]. The de-regulation of some PIWI-interacting RNAs may also contribute to breast cancer-specific biology, possibly by remodeling the cancer epigenome [26]. Taken together, this body of evidence sets ncRNA as critical components of cancer biology: ncRNAs are cancer-related genes due to their potential tumor suppressive and/or oncogenic functions [27].

2 The diversity of non-coding RNAs in humans

According to its size, ncRNAs are classified in two main families: lncRNAs, corresponding to transcripts with over 200 nt that does not appear to contain a protein coding sequence, and small ncRNAs (sncRNAs), when the RNA sequence contains less than 200 nt [12]. The ncRNAs localize both to the nucleus and cytoplasm and may be found in exosomes and other microvesicles present in bodily fluids such as urine, blood, and seminal fluid, although the abundance and activity of ncRNAs in exosomes remains unclear [28]. Exosomes are released from tumor cells and may transfer proteins and RNA across cells. Thus, it is tempting to speculate whether in the PCa microenvironment, miRNAs (the most commonly studied ncRNA in exosomes) may be transferred among stromal cells and cancer stem cells, although it is not clear how miRNAs reassemble into a functional miRISC upon import into other cells.

Moreover, circulating ncRNAs in serum, plasma or urine, although at low levels, may provide new opportunities for biomarker development [29].

2.1 Small non-coding RNAs

Small ncRNAs differ from lncRNAs by its length and are typically classified according to different biogenesis pathways and genomic origins (Table 1). Classically, sncRNAs include all transfer RNAs (tRNAs), some ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs and its derivatives, microRNAs (miRNA), short interfering RNAs, and piwi-interacting RNAs [30]. More recently, several other small RNAs associated with protein-coding gene transcription and splicing regulation, such as transcription initiation RNAs (tiRNAs), promoter-associated short RNAs, termini-associated short RNAs, 3′-untranslated region-derived RNAs, and antisense termini-associated short RNAs, have been added to this class [30, 31].

2.1.1 microRNAs

Presently, sncRNAs involved in post-transcriptional regulation of target RNAs *via* the RNAi pathway, such as miRNA, siRNA, and piRNA, are considered the most biologically relevant [32]. Owing to its involvement in human diseases such as cancer and its potential as disease biomarkers, miRNAs are undoubtedly the best-studied sncRNA class. Mature miRNAs are typically ~22 nucleotide single-stranded RNAs (ssRNA), canonically derived from longer primary transcripts (pri-miRNAs) which are processed to intermediate precursor-miRNAs (pre-miRNAs) by DROSHA-DGCR8 microprocessor complex [33]. These hairpin precursors are exported from the nucleus to the cytoplasm, *via* exportin 5 (XPO5), where the terminal loop region of the hairpin is removed by DICER/TRBP2 [34], resulting in a double-stranded mature/star RNA molecule (dsRNA) (Fig. 1) [35]. Both Drosha and Dicer are RNase-Type III proteins and leave characteristic 2 nt offsets on their substrate that can be used for bioinformatics

description of miRNAs [36]. While the canonical microRNA biogenesis and action model are being constantly refined, Drosha [37] and Dicer [38] independent biogenesis mechanism have been described, but they represent rare exceptions. After the canonical, multistep processing, typically only one of the strands (mature miRNA) is loaded by Argonaute proteins and coupled with diverse components of the RNA-induced silencing complex (RISC). Constrained by the structure of the Argonaute protein, only 7 nucleotides (position 2–8) of the mature miRNA are exposed [39]. This so-called "seed region" defines the range of potential target RNAs by usually perfect complementarity of these few nucleotides. In the vast majority of cases, target interaction of miRNAs occurs at the 3′UTR of protein coding genes [40]. miRNA:mRNA interactions that include the RISC complex then lead to repression or degradation of those transcripts and, ultimately, to a moderate downregulation of the corresponding proteins (Fig. 2) [35]. While this seemed a straightforward model when miRNA function was first discovered, complexity was quickly added when researchers realized that multiple copies of nearly identical and evolutionarily related miRNAs might be found in the genome that share seed sequences and, consequently, the range of targets (miRNA families). Moreover, single miRNAs have not only one but hundreds of target RNAs and single protein coding genes are targeted by multiple miRNAs (Fig. 3). Consequently, there is redundancy in microRNA targeting. Indeed, one miRNA may have different MRE in the same target RNA. It is, thus, likely that most miRNA act as rheostats, fine tuning the expression of hundreds of genes, in intricate gene networks [41]. miRNAs may, in fact, establish thresholds and increase the coherence of the expression of its targets, as well as reduce the cell-to-cell variability in target gene expression [42].
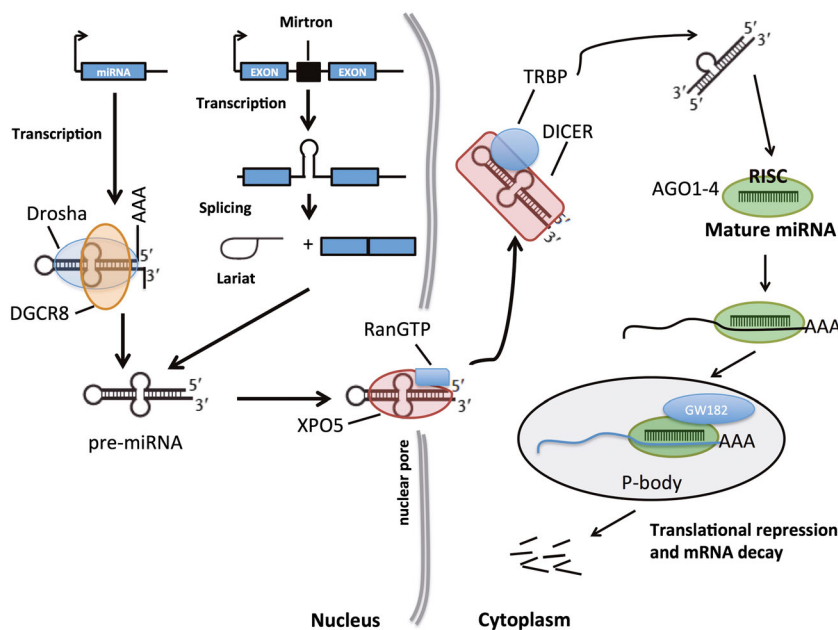
miRNAs themselves are also subject to modifications, including post-transcriptional RNA editing (methylation, uridylation, and adenylation) [27, 43] and miRNA tailing [35]. Deep sequencing data revealed that the majority of miRNAs show length and sequence isoforms (isomiRs) with largely unknown functions in cancer biology [43], although it

Table 1 mRNA/lncRNA convergent and divergent features

mRNA	lncRNA
Tissue-specific expression	
Form secondary structure	
Undergo post-transcriptional processing (*e.g.* 5′CAP, polyadenylation, splicing)	
Important roles in disease	
Protein-coding transcripts	Non-protein coding, regulatory functions
Well conserved across species	Poorly conserved
Present in both nucleus and cytoplasm	Predominately in the nucleus
Around 20–24,000 mRNAs	Predicted 3–100 fold of mRNA in number
Expression level: low to high	Expression level: very low to moderate

has been shown that they can selectively associate with specific RNA-binding proteins (*e.g.*, Argonaute) or exosomes [44] implying context-dependent functional roles. These alterations may, thus, affect miRNA maturation and de-regulate target-genes:miRNA interactions stoichiometry.

Despite growing knowledge on miRNAs biology and the ever-increasing amount of sncRNA sequencing data, defining what is and what is not a miRNA has become a challenge. This uncertainty led to descriptions of several hundreds to several thousands miRNAs in the human genome [45, 46]. Remarkably, when structural criteria for annotation and nomenclature of human miRNA genes were recently updated, only a small proportion of the previously reported human miRNAs was recognized, constituting about one third of the 1881 putative miRNA entries in the widely used online repository miRBase (last updated June 2014) were considered *bona fide* miRNAs [36]. Consequently, the 523 currently accepted human miRNAs represent a solid foundation for future studies, but a reassessment of all published (but not yet in miRBase listed) miRNAs is desirable to further expand our understanding of the human microRNAome.

## Box 1: miRNA structural features

- miRNAs are between 20-26 nt long
- They are genome-encode and derive from hairpin precursor that shows imperfect complimentarity (~16 nts)
- Mature products of both hairpin arms are expressed (mature, co-mature or star sequence)
- Show a 5′ read homogeneity in 90 % of the reads
- Display a 2 nt offset on both ends which is a consequence of Drosha/ Dicer processing
- Mature miRNA sequences usually start with A or U.
- Flanking region upstream shows UG motif at Position 14, loop shows UGU motif at the 3′ end of the 5′ arm, and flanking region downstream shows CNNC motif at Position 17–18.
- At least some miRNAs of any higher animal taxon are representatives of phylogenetically conserved miRNA families and show very high sequence similarities.

2.1.2 siRNAs

Endo-siRNAs are double-stranded, 21–26 nt, RNAs (dsRNA) that are cleaved from longer dsRNA intermediates precursors

Fig. 1 MicroRNA biogenesis in human cells. In the canonical miRNA biogenesis pathway, primary transcripts are processed by Drosha in the nucleus and by Dicer in the cytoplasm. Both Drosha and Dicer are RNAse III enzymes and produce a hairpin precursor with 2-nt offsets at the 5p and 3p arm. The lncRNA pri-miRNA displays a 7-methylguanosine cap (m7Gppp), ends with a 3 poly(A) tail, and is transcribed by RNA polymerase II (Pol II). The pri-miRNA contains a stem–loop structure that is cleaved in the nucleus by the endonuclease Drosha together with its double-stranded RNA (dsRNA)-binding protein parner DGCR8, forming a complex called Microprocessor. The output of this trimming is a precursor miRNA (pre-miRNA). It is then exported to the cytoplasm by exportin 5 and further cleaved by the endonuclease Dicer, together with its dsRNA-binding partner TRBP, to produce a miRNA-miRNA* duplex. Further maturation steps reject miRNA* and

incorporate the mature miRNA strand into the miRNA-induced silencing complex (miRISC). Alternative biogenesis pathways are also acknowledged. Mirtrons are short introns with hairpin potential that are spliced and disbranched into pre-miRNAs and bypass the Drosha cleavage of the canonical miRNA pathway. It lacks a lower stem and basal single-stranded segments, which are structural features of pri-miRNA and mediate recognition/cleavage by the DGCR8/Drosha complex. In this pathway, pri-miRNA is generated from a branched mirtron structure that undergoes lariat debranching. Another alternative biogenesis pathway involves pre-miRNA escaping to Dicer processing after nuclear export that is directly loaded into AGO2 protein. AGO2 is responsible for processing the pre-miRNA into a single-stranded miRNA (hsa-miR-451)
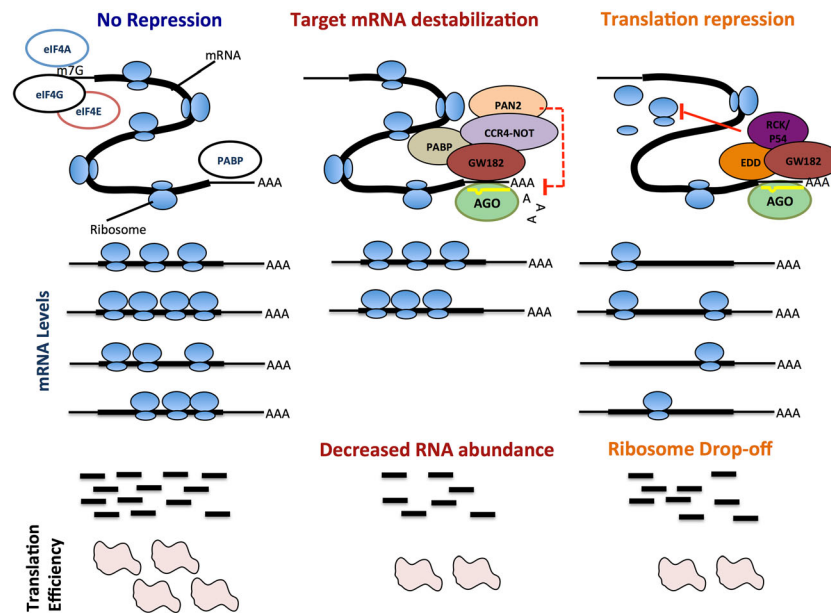
Fig. 2 miRNA-mediated gene regulation. Most plant and a few animal miRNAs direct endonucleolytic cleavage of their mRNA targets by perfect complementarity. However, highly complementary sites in animals' transcriptomes are infrequent. Accordingly, miRNA-directed translational repression is indistinguishable from mRNA destruction *via* decapping and 5-to-3 decay. Thus, it was suggested that miRNAs mainly direct target mRNAs for decay. Nonetheless, the predominant mode of miRNA-mediated repression may be context-dependent. The core RISC complex is formed by Argonaute proteins (1–4 in mamals) and GW182. If mRNA decay and subsequent target mRNA destabilization is observed, it suggests that miRISC interacts with the CCR4–NOT and PAN2 deadenylase complexes to facilitate deadenylation of the poly(A) tail. Following deadenylation, the 5-terminal cap (m7G) is removed by decapping the DCP1–DCP2

complex, and mRNA decay is affected by an exonuclease. The miRISC inhibits translation initiation by interfering with eIF4E-cap recognition and 40S small ribosomal subunit recruitment or by antagonizing 60S subunit joining and preventing 80S ribosomal complex formation. Furthermore, miRISC might inhibit translation at post-initiation steps by inhibiting ribosome elongation. MicroRNA-target interactions might be additionally mapped by Ribosome profiling, providing a "snapshot" of all the ribosomes active in a cell at a specific time point. MicroRNA manipulation would allow systematic monitoring of cellular translation processes and prediction of protein abundance. Coupled with bioinformatic target predictions, this would help determine which mRNA is being translated and which region of the mRNA is being targeted by the miRNA

derived from repetitive sequences, sense–antisense pairs (derived from transposons), or long stem-loop structures [47, 48]. Endo-siRNAs biogenic pathway in humans is DICER-dependent (although Drosha-independent) and involves Argonaute proteins (AGO2) [47, 49]. Endo-siRNAs originate from diverse genomic locations and have been implicated in post-transcriptional (mRNA cleavage) and epigenetic silencing of protein-coding genes and transposon-derived ncRNAs, respectively, as well as other unclear functions [49]. Contrasting with miRNAs, endo-siRNAs bind only RNA molecules containing perfectly complementary sequences [32].

2.1.3 PIWI-interacting RNAs

Another important family of RNAi comprises piRNAs [50], 24–30 nucleotide ssRNAs, derived from single-stranded RNA precursors transcribed from intergenic repetitive elements, transposons, or large piRNA clusters [47]. The biogenesis is Drosha-/DICER-independent and requires Piwi proteins of the Argonaute/Piwi family [50, 51]. During piRNAs biogenesis, piRNA precursors undergo nuclear processing and export, primary or cyclic secondary processing (the ping-pong cycle,

catalyzed by PIWI proteins MILI and MIWI2), and PIWI ribonucleoprotein complex (piRNP) assembly [21, 52]. The ping-pong amplification cycle generates antisense piRNAs capable of suppressing the transcript of origin [52]. The assembly of the piRNP is essential to establish post-transcriptional regulation and transposon modulation. The piRNAs functions are connected to its origin: If derived from transposons, piRNAs are implicated in regulating cognate transposon activity, whereas piRNAs resulting from piRNA clusters are involved in gene expression control [52, 53]. piRNAs were primarily found in germ cells, but recent studies have recognized that piRNAs are expressed in somatic cells, including non-tumorous and tumourous tissues from 11 organs [54].

Current views suggest that both endo-siRNAs and piRNAs are defensive mechanisms against nucleic acid-based parasites, acting as genome's guardian. However, both endo-siRNAs and piRNAs are not considered cancer-related genes, and consequently, additional data is need to ascertain the true relevance of these ncRNA families in tumorigenesis [54, 55]. Remarkably, these three major families of sncRNAs associate with different AGO protein subclades to perform sequence-specific gene silencing [35].
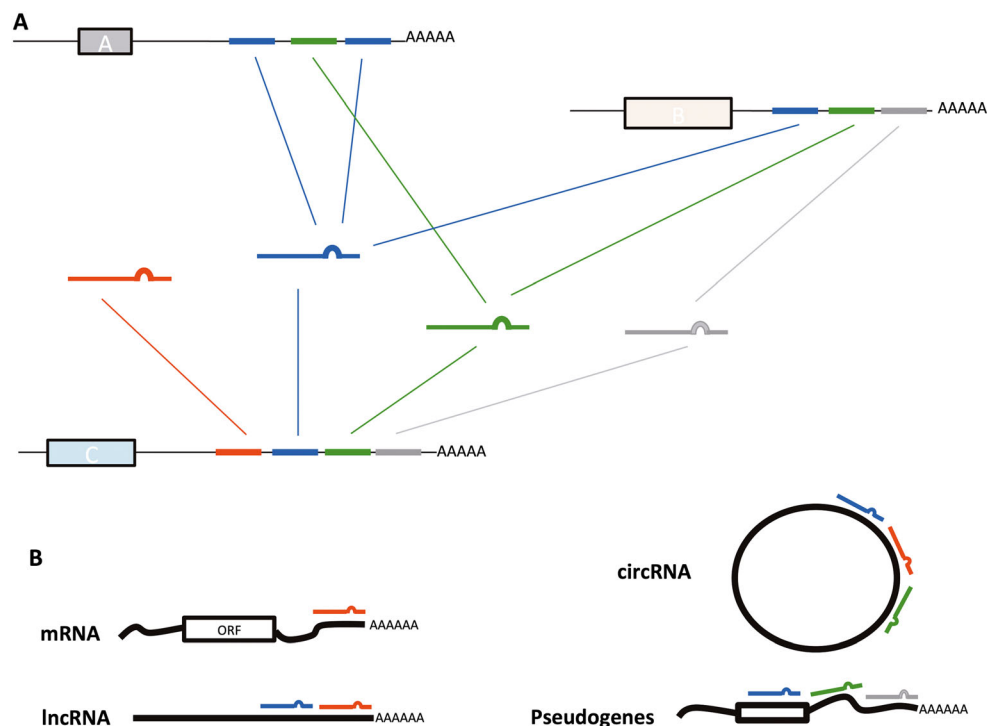
Fig. 3 Magnitude of microRNA-mediated gene regulation. **a** Redundancy of microRNA targeting. Most miRNA act as rheostats, fine tuning the expression of hundreds of genes, in intricated gene networks. A single miRNA can target multiple transcripts and one specific mRNA is able to be targeted by several miRNAs. Indeed, one microRNA may have different MRE in the same target mRNA (miRNA binds to the specific MRE in the same color). **b** Competing endogenous RNAs: natural occurring miRNA decoys. Linear or circular lncRNAs may function as miRNA decoys to sequester miRNAs from their target mRNAs of functionally relevant lncRNAs. Base pairing is also the mode of action of ceRNAs. Those lnc-ceRNAs can mask miRNA-binding sites on a target mRNA to block miRNA-induced silencing through the RNA-induced silencing complex (RISC). The ceRNA comprises both circular RNA (circRNA), lncRNAs, and pseudogenes competing for the complementarity with miRNAs. The ultimate impact of these interactions is that protein-coding RNAs and non-coding RNAs may crosstalk by competing for miRNA binding through their miRNA recognition motifs impairing cell homeostasis. (ORF, open reading frame)

2.1.4 snoRNAs

Small non-coding RNAs exert far more biological regulation rather than just RNAi-silencing. One of the firstly described classes of small ncRNAs was snoRNAs. SnoRNAs are 60–300 nt long, mainly localized in the nucleolus, which are encoded by introns of coding and non-coding genes [56, 57]. Their function is to guide RNA for post-transcriptional modification of ribosomal RNAs and some spliceosomal RNAs, with a few others involved in nucleolytic processing of the original rRNA transcript [57, 58]. Two subdivisions of snoRNAs are known to exist and are involved in two different types of RNA post-transcriptional modification. The C/D box snoRNAs define the target sites for 2′-O-ribose methylation and H/ACA box snoRNAs demarcate the target sites for pseudouridylation [57, 59]. C/D box and H/ACA box snoRNAs are structurally distinct, and those differences make the connection with the binding of specific proteins to form the small nucleolar ribonucleoprotein (snoRNP) complexes that identify and modify the cognate targets [60]. During processing of rRNA, snoRNA guide sequences hybridize to the target rRNA and lead the snoRNP to direct the modification of ribose 2′-hydroxyl groups or the isomerization of uredines to pseudouridines within pre-rRNAs [58, 61]. Dyskerin is the enzyme recruited by H/ACA box snoRNAs to catalyze pseudouridylation at specific ribonucleotides, whereas C/D box snoRNAs activity requires the methyltransferase fibrillarin, to mediate the 2′-O-methylation [59]. The reactions occur generally at conserved sites in nascent rRNAs [58, 61]. In addition to catalyze nucleotide modification, snoRNP association with pre-rRNAs may also serve to chaperone correct RNA folding for rRNA processing and ribosome assembly [28]

Additionally, snoRNAs have other functions (*e.g.*, Small Cajal body RNAs [62]) and it has recently been found that snoRNA loci may also produce miRNA-like small RNAs [63, 64], uncovering a putative complex crosstalk between snoRNA-guided RNA processing and RNAi pathways. Strikingly, novel evidences implicate snoRNA as controllers of cell homeostasis and snoRNA dysregulation may thus contribute to carcinogenesis [57, 65].

2.1.5 Small RNAs incertae sedis

Although the previously described sncRNA families are relatively well understood from a biological standpoint, others are still poorly characterized. These include sncRNAs resulting from gene regulatory regions and gene boundaries [subclasses of promoter-associated small RNAs, such as transcription initiation RNAs (tiRNAs)], termini-associated short RNAs, antisense termini-associated short RNAs, and splice-site RNA (spliRNA) [66]. Others are structural components of chromosomes—the centromere-associated RNAs and telomere small RNAs. Additionally, some small RNAs are cleavage sub-products of other ncRNAs [e.g., transfer RNA-derived RNA fragments (tRFs)] or are derived from different sources (mitochondrial ncRNAs and miRNA-offset RNAs) [12, 66]. tRFs are one of the most abundant sncRNAs, thought to be present in most organisms and generated by ribonucleolytic processing of tRNAs by Dicer and RNAse Z [67]. The definition of the multiple tRFs classes is made according to the position of the tRNA cleavage site that gives rise to tRFs. Among the known classes, the most prominent includes 5′- and 3′-tRNA halves (cleaved in the anti-codon loop), 5′- and 3′-tRFs (also known as 3′CCA tRF), and 3′U tRFs [68]. The stress induction of tRFs results in stress granule assembly and inhibition of protein synthesis, linking tRFS to cell homeostasis through control of cell proliferation and mediating RNA inactivation through Argonaute engagement [68].

To further ascertain the specific biological roles of these enigmatic small RNAs, functional studies are needed. For instance, when deleting tiRNAs associated with binding sites for RNAPOLII CTCF binding factor, there is a dramatic alteration in CTCF binding and nucleosome density at genomic loci proximal to sites of tiRNA biogenesis [69]. Further research is thus required to dissect the evolution, biogenesis, and functions of these small ncRNAs classes and explore its potential connections with cancer.
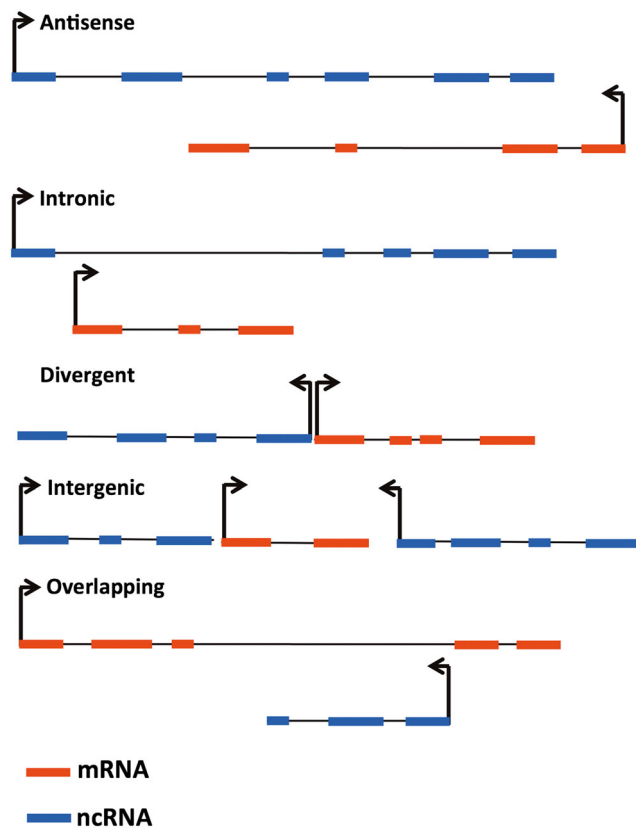
2.2 Long non-coding RNAs

According to GENCODE annotation v7, there are 20,687 protein-coding genes and in total, GENCODE-annotated exons of protein coding genes cover 2.94 % of the genome or 1.22 % of protein-coding exons [5]. These data clearly point out that the vast majority of the human genome is transcribed not into a biochemically active RNA but rather into a structural, nc RNA. Transcripts lacking the capacity to code for a protein, are uniformly abundant in all organisms, from yeast to humans [16]. There is growing evidence that ncRNA have biologic functions and operate through defined mechanisms. However, this compelling abundance of ncRNAs triggered the discussion whether ncRNA transcription is the output of transcription or ordinary by-products of the transcriptional system or simply a methodological artifact [12, 70].

Thanks to global efforts, it has been possible to assign specific features to define lncRNAs as distinct transcripts: The vast majority of lncRNAs is generated by the same transcriptional machinery, similar to other mRNAs, as emphasized by RNA polymerase II occupancy and histone modifications associated with transcription initiation (promoter, H3K4me3) and elongation (H3K36me3 in the gene body) [16, 30]. lncRNAs possess a 5′ terminal methylguanosine cap, are often spliced *via* canonical genomic splice site motifs, and some of them are polyadenylated whereas other are not. Alternative pathways also contribute to the generation of lncRNAs such as non-polyadenylated lncRNAs, likely expressed from RNA polymerase III promoters [16]. Not only lncRNA regulation is made by well-established transcription factors, but also lncRNA are frequently expressed in a tissue-specific manner (Table 1) [30].

Generally, lncRNAs are expressed in lower amounts compared to their protein-coding counterparts, making it difficult to robustly detect in clinical samples [12, 16]. Consistent with the many regulatory functions assigned to lncRNAs, the low expression may restrict these lncRNAs to subtle or redundant roles, or reflect incomplete repression in nonspecific cells [16, 66]. By comparison to protein-coding genes, lncRNA expression has higher cell specificity than proteins, consistent with their proposed role in architectural regulation in which each cell displays a unique transcriptome [16]. The organization of lncRNA loci in the genome revealed transcriptional complexity as lncRNA genes often display large numbers of isoforms. Moreover, lncRNAs are often organized in association with protein-coding genes and half of the protein coding genes have complementary non-coding antisense transcription, further expanding the complexity of genome transcriptional dynamics. lncRNAs may be transcribed from intergenic regions [large intergenic ncRNAs (lincRNAs)]; in antisense, overlapping, intronic, and bidirectional orientations relative to protein-coding genes (Fig. 4); from gene regulatory regions—UTR, promoters, and enhancers; from specific chromosomal domains (telomere) or derived from the mitochondrial genome [12, 16, 66].

lncRNAs act by a multitude of regulatory mechanisms according to its specific location in the cell. lncRNA play a role as organizing factors in the dynamic nuclear organization that shapes the cell nucleus through nucleosome remodeling [71, 72]. Nuclear lncRNAs might be involved in gene-to-gene interactions either locally or in the context of cross chromosome interactions, *i.e.*, cis- and *trans*-mediated regulatory roles, respectively [6, 10]. In most cases, nuclear lncRNAs function by recruiting chromatin-remodeling complexes to particular DNA loci [22], as it have been shown to form ribonucleoprotein (RNP) complexes by recruiting DNA methyltransferases, the Polycomb repressive complex (PRC) 2 (promotes H3K27 trimethylation) [22], and H3K9 methyltransferases, resulting in the formation of repressive heterochromatin and

Fig. 4 Descriptive structure of a long non-coding RNA loci. Normally, lncRNAs are defined by their location accordingly to protein-coding genes in the vicinity. Antisense lncRNAs transcription initiate inside or 3′ of a protein-coding gene. They are transcribed in the opposite direction of protein-coding genes, overlapping any portion of a mRNA. Intronic lncRNAs initiate inside an intron of a protein-coding gene in either direction and terminate without overlapping exons. Bidirectional lncRNAs are transcripts that initiate in a divergent fashion from the promoter of a protein-coding gene; the precise distance cutoff that constitutes bidirectionality is not defined but is generally within ~100 base pairs. Intergenic lncRNAs (also termed large intervening non-coding RNAs or lincRNAs) are lncRNAs with separate transcriptional units from protein-coding genes. A key structural feature is that lincRNAs need to be 5 kb away from protein-coding genes. LincRNA genes are preferentially found within 10 kb of protein coding genes. These are defined as lncRNA transcripts that encompass a protein-coding gene within the "intron" of a lncRNA or as lncRNAs that overlap the intron of a protein coding gene

transcriptional inhibition. However, lncRNAs are also associated with transcriptional activation through engaging of chromatin-modifying complexes, including H3K4 methyltransferases, specific transcription factors, and recruiting POLII [73–75]. Nuclear lncRNAs may also bind and sequester transcription factors away from their target chromosomal regions, thus indirectly impairing gene expression [76].

Nonetheless, a significant number of lncRNAs are transferred to and lodged in the cytoplasm. Functions of cytoplasmic lncRNAs include protein localization, mRNA translation, and stability. By recognition of the target by base pairing, they can modulate mRNA at different levels: (a) base pairing between *BACE1* and *BACE1-AS* induces stabilization of target

mRNA and increases the BACE1 protein expression [77], (b) repression of translation (*e.g.*, lincRNA-p21 suppresses target mRNA translation) [78], and (c) competition with endogenous RNAs (ceRNAs) for miRNA binding [79]. This regulatory system in which multiple RNAs (both coding genes, pseudogenes, and lncRNA) may crosstalk and compete for shared miRNA binding are thought to be relevant for many processes, including cancer [79]. Moreover, circular RNAs (circRNA) also function as miRNA "sponges" [80], and given that linear ceRNAs have a short half-life, it provides superior stability and its turnover can be controlled by the occurrence of a perfect miRNA binding site (Fig. 3b) [81, 82].

Another function of cytoplasmic lncRNA is related to protein localization: lncRNA contains distinct domains that interact with specific protein complexes and, through a combination of domains, bring specific regulatory components into proximity, resulting in the formation of a specific functional complex to coordinate gene expression [83]. Additionally, lncRNAs not only act as decoys for sncRNAs but they may also function as precursors of sncRNAs, including small nucleolar RNAs (snoRNAs) and miRNAs [84].

2.2.1 Natural antisense transcripts

Natural antisense transcripts (NATs) are endogenous RNAs that partially or totally overlap transcripts (either coding or non-coding) originating from the opposite DNA strand [85]. NATs can be originated from independent promoters, shared bidirectional promoters or cryptic promoters that are situated within genes [85]. Depending on the orientation of the sense transcript, overlapping pairs are classified as: head-to-head (5′-regions overlap, HTH), tail-to-tail (3′-regions overlap, TTT), embedded (one transcript is fully contained within the other) (EMB), or intronic (INT) pairs [85, 86]. NATs function locally (in the nucleus, preferentially) or distally (in the cytoplasm) [85], and are usually not abundant (around 10-fold lower abundance than associated mRNA) [85].

Although NATs clearly typify *cis* regulation, affecting alleles on the DNA strand from which they are produced, in a local fashion, they also act in *trans* because they can interact with other loci taking advantage of 3-D organization of chromatin [86]. *Cis*-regulation is due to antisense transcription in a given locus, whereas *trans*-regulation is mediated by the antisense transcript corresponding to the RNA being transcribed [85]. *Cis*-acting NATs function either locally (*e.g.*, in promoter–gene interactions) or distally (*e.g.*, in enhancer–gene interactions). Local *cis*-regulation comprises epigenetic alterations proximal to a target gene (*e.g.*, regulation of transcription initiation by affecting DNA methylation), whereas distal *cis*-regulation requires RNA-RNA interactions amid transcripts originated from the same locus [85]. Moreover, when NATs remain at the loci of origin, they can mediate *cis* effects due to formation of R-loops, triple helices, or stalled polymerases.

The functional output of *cis*-regulation by NATs leads to activation or silencing of the corresponding sense mRNA, *via* transcriptional activation or silencing, mRNA stabilization, alternative splicing or post-translational regulation [86]. Because antisense and sense transcripts are transcribed from the same locus, it is suggested that antisense transcripts function recurrently in *cis* whereas other ncRNAs commonly function in *trans*, although there is evidence for *trans*-acting antisense transcripts. Antisense transcription might be far more extensive than previously anticipated, with around 50 % of sense transcripts having antisense partners [86]. Interestingly, NATs' genomic distribution suggests that they might act as self-regulatory loops that control its own expression.

2.2.2 Enhancer elements and RNAs

Enhancers are non-coding genomic regions that activate transcription of target genes at long distances. Mammalian genomes contain hundreds of thousands of putative enhancer elements, located upstream and downstream of coding target gene promoters, which are critical for cell-specific gene expression programs [87, 88]. Enhancers are also considered transcription units, giving rise to transcription of a class of lncRNAs, the eRNAs [88]. Histone modification signatures characterize enhancer-like regions, including enrichment of H3K4me1 and H3K4me2 and reduced levels of H3K4me3, compared to promoters [87, 88]. eRNAs may be either polyadenylated or non-polyadenilated and are subdivided into unidirectional and bi-directional transcripts. eRNAs exhibit a 5′cap, usually are not spliced or polyadenylated, and can be produced as unidirectional or bi-directional transcripts. A growing body of evidence suggests that eRNAs are functionally important *per se* and contribute to enhancer-mediated transcriptional activation of target genes. Primed enhancers are marked with H3K4me1 and H3K4me2 and lack histone acetylation. The repressive histone modification H3K27me3 marks enhancers that are considered to be poised. In contrast, active enhancer regions are enriched for H3K27ac and are bound by actively transcribing RNA Polymerase II (PolII) [88]. eRNA expression is a hallmark of active enhancers, and it has been used as a signature to identify those regions through transcriptomic profiling. The mechanisms by which eRNAs regulate gene expression are not completely clear, but it has been hypothesized that it may stabilize enhancer-promoter looping and facilitate PolII recruitment and its transition into productive elongation [87]. As such, eRNAs are likely to have important functions in many regulated programs of gene transcription including those mediated by androgen receptor [89], p53 and ERα (ESR1) [90].

2.2.3 Long intervening non-coding RNAs

lincRNAs (long intervening non-coding), also called long intergenic non-coding RNAs, are lncRNAs that do not overlap exons of either protein-coding or other non-lincRNA types of genes [19]. They are transcribed from multiple loci in the human genome and are located in nucleus, although they are more frequently reported in cytoplasm. lincRNAs lack defining sequence or structure characteristics as they combine multiple classes of non-coding RNAs (such as intronic and intergenic genes) (Fig. 4). However, a few common features are observed, including being composed of few exons (normally 2–3) which makes them shorter than mRNAs [19]. The average length of lincRNAs exons is no larger than its counterparts in PCG. Although the transcriptional regulation, chromatin modifications, and splicing signals are similar to PCG, lincRNAs seem to be less efficiently spliced. Interestingly, lincRNAs significantly overlap repetitive elements, probably due to the fact that lincRNA functions are more tolerant to retrotransposon insertions. Repetitive elements were reported to play important mechanistic roles in lincRNAs roles, enabling base pairing with other RNAs containing repeats from the same family. Finally, the median lincRNA levels are only about a tenth of that of mRNAs, as lincRNA expression is typically more variable among tissues and enriched in testis and brain. LincRNAs' functions include co-transcriptional regulation, regulation of gene expression (both in *cis* and *trans*) by bridging proteins and chromatin, scaffolding nuclear and cytoplasmic complexes, and RNA-RNA interactions. Consequently, lincRNAs are believed to play a widespread role in gene regulation and maintenance of cell's homeostasis [19].

2.2.4 Pseudogenes

Pseudogenes are ancestral copies of protein-coding genes that arose from genomic duplication or retrotransposition of mRNA sequences into the genome, followed by accumulation of deleterious mutations due to loss of selection pressure [91]. A pseudogene shares an evolutionary history with a functional protein-coding gene, but it has been mutated through evolution to contain frameshift and/or stop codon(s) that disrupt the open reading frame [92]. Pseudogenes pervade the genome in close sequence similarity with their cognate genes. There are three main types of pseudogenes: (a) unitary pseudogenes—species-specific unprocessed pseudogene without a parent gene in the same species but with an active orthologue in another species; (b) processed pseudogenes—which appear to have been produced by integration of a reverse transcribed mRNA into the genome; and (c) unprocessed pseudogene—those that may contain introns as it resulted from gene duplication [92]. Pseudogenes are of capital importance owing to its competing endogenous RNAs (ceRNAs) action as natural miRNA "sponges" [79]. Moreover, they may also regulate the

expression of their parent gene by decreasing mRNA stability of the functional gene through its own over-expression [93]. Interestingly, pseudogenes are also a source of small interfering RNAs, impacting on gene expression by means of RNAi pathway [94] and by generating antisense transcripts [95].

3 Non-coding RNAs in prostate cancer

The constantly expanding inventory of ncRNAs has been implicated in a broad spectrum of processes including prostate homeostasis and pathogenesis. The emergence of ncRNAs is of crucial importance for prostate biology because prostate cells are transcriptionally active, and numerous reports documented the deregulation of ncRNAs in prostate cancer (PCa) [27, 30]. This is the most commonly diagnosed cancer among men worldwide and a major cause of morbidity and mortality [96]. Although radical prostatectomy reduces mortality among men with localized prostate cancer, up to 40 % of patients experience disease progression and recurrence [97]. Numerous studies using mRNA-based techniques contributed to a better understanding of the molecular pathways involved in prostate carcinogenesis [98]. It is likely then that unraveling the biological functions of ncRNAs in PCa will provide new insights into their functions, mechanisms of action, and potential usefulness as tools for PCa management [27]. Despite the myriad of ncRNA families described thus far, only a small proportion is known to be involved in PCa. The best examples include small RNA families, mainly miRNAs and some lncRNAs, including eRNAs and antisense RNAs. Other classes are under active investigation, including pseudogenes, lincRNAs, and tRNAs. These ncRNAs not only control functional pathways of cell biology, but it may also constitute novel therapeutic targets or diagnostics biomarkers. In the following sections, we review the rapidly growing knowledge on ncRNAs as key players in prostate tumorigenesis and highlight their translational potential into the clinics.

3.1 Small non-coding RNAs

To date, the most extensively studied sncRNAs in PCa are miRNAs. These are classified as oncomirs (when miRNA expression favors tumor development) or tumor suppressor miRNA (*i.e.*, when its expression normally counteracts tumor initiation and/or development) and play a critical role in PCa [58]. Dysregulation of miRNAs in cancer may occur through epigenetic changes (commonly, promoter CpG island hypermethylation) or genetic alterations, as well as miRNA biogenesis machinery dysfunction, which subsequently affects transcription of primary miRNA, its processing to mature miRNAs, and/or interactions with mRNA targets [59].

3.1.1 Dysregulation of microRNAs in PCa

NGS-based profiling, which enables high-throughput analysis of the miRNAome with single-base resolution [99], revealed common downregulation of miR-205, miR-143, and miR-145 and upregulation of miR-375 and miR-148, among others (Table 2). However, due to the inherent heterogeneity of PCa, sample selection, and technological platforms used, some discrepancies in results are apparent. Downregulation of miR-15a-miR-16-1 cluster (putative tumor suppressors through targeting of BCL2, CCDN1, and WNT3A) in PCa due to 13q14 deletion is commonly acknowledged [135]. Deletion of this cluster fuels survival, proliferation, and invasion of PCa cells, whereas *in vivo* overexpression results in growth arrest, apoptosis, and marked regression of PCa xenografts [135]. Strikingly, *in vitro* blockade of miR-15a and miR-16-1 promotes survival, proliferation, and invasiveness of previously untransformed prostate cells, which become tumorigenic in immunodeficient NOD-SCID mice [135]. On the other hand, loss of miR-101 contributes to overexpression of EZH2, linking PCa progression and altered of epigenetic reprogramming [136]. Indeed, one or both genomic loci encoding for miR-101 [located in chromosome 1 (MIR101-1) and in chromosome 9 (MIR101-2)] are lost in a sizeable proportion of primary PCa and up to 2/3 of metastatic PCa [136]. In DU145 cells, forced expression of miR-101 impairs cell invasion and reduces tumor growth in a mouse xenograft, whereas miR-101 re-expression globally decreases H3K27me3 histone mark levels at PRC2 target genes' promoters, demonstrating that manipulation of miR-101 expression may be of therapeutic usefulness [136].

Mir-34a, a p53 target [137], is downregulated due to promoter methylation [138] and underexpressed in CD44+ PCa cells purified from xenograft and primary tumors [139]. Its overexpression in cell pool or purified CD44+ PCa cells inhibits clonogenic expansion, tumor regeneration, and metastasis, whereas delivery miR-34a antogomirs to CD44− PCa cells has the opposite effects [139]. These effects are mediated by CD44, a target of miR-34a, and CD44 silencing phenocopied miR-34a overexpression [139]. Additionally, miR-34 cooperates with p53 to counteract cancer progression and jointly regulates prostate stem/progenitor cell activity [139]. This action is also mediated by MET, a mutual p53/miR-34 downstream target and a critical regulator of stem cell compartment [139]. This suggests a therapeutic potential for miR-34a against PCa by directly acting on cancer stem cells [139].

One of the main focuses on miRNA research in PCa is AR-signaling pathway. Not only AR is targeted by multiple miRNAs, but it also modulates miRNA expression, mediated by androgen-responsive elements within the promoter region [140]. miR-21 is overexpressed in primary PCa and DU145 and PC-3 PCa cell lines [141] and AR binding to its promoter enhances transcriptional activity, promoting hormone-dependent and hormone-independent PCa growth [142].

Table 2 Representative microRNA in PCa biology

miRNA	Function(s)	Disruption	PCa hallmarks	Target(s)	Ref
miR-1	TS-miRNA	Epigenetic silencing	Cell cycle control, mitosis, DNA replication/repair and actin dynamics. Suppresses xenograft growth of PCa cells	*LASP1, PTMA, XPO6, NOTCH3*	[100]
miR-31	TS-miRNA	Epigenetic silencing	Suppresses androgen receptor and inhibits prostate cancer growth *in vivo*	AR, *CDK1, E2F2, EXO1, FOXM1, MCM2*	[101]
miR-34b/c	TS-miRNA	Epigenetic silencing	Suppresses cell proliferation, colony formation, migration/invasion, cell-cycle arrest and apoptos is due to demethylation, active chromatin modifications, and AKT pathways Decreases tumor growth in nude mice	*cMYC, DNMT1, HDAC1, HDAC2, HDAC4, AKT2*	[102, 103]
miR-145	TS-miRNA	Epigenetic ilencing and p53 mutations	Regulates androgen-dependent cell growth *in vitro*. Promotes cell cycle arrest. Suppresses PCa tumor growth *in vivo*	*ERG*, AR, *SENP1*	[104–107]
miR-193b	TS-miRNA	Epigenetic silencing	Regulates anchorage independent growth and controls cell cycle	*CCND1, uPA*	[108–110]
miR-203	TS-miRNA	Epigenetic silencing	Controls proliferation, migration and invasive potential. Suppresses PCa metastasis *in vivo*. Loss of miR-203 promotes tyrosine kinase inhibitors resistance	*LASP1, Rap1A, GOLM1, AREG, EREG, TGFA*	[111–115]
miR-205	TS-miRNA	Epigenetic silencing	Cell viability impairment, Neutralizes epithelial-to-mesenchymal transition and reducing cell migration/invasion. Inhibits metastasis *in vivo*	*MED1, PKC1,* AR, *ZEB1*	[116–119]
miR-224	TS-miRNA		Suppresses PCa cell proliferation, apoptosis, invasion, and migration	*TRIB1, TPD52*	[120, 121]
miR-21	Onco-miR	AR-regulated	Promotes hormone-dependent and hormone-independent prostate cancer growth. Induces EMT and luminal markers expression. Enhances PCa tumor growth *in vivo*	*BTG2, FBXO11*	[122–124]
miR-32	Onco-miR	AR-regulated	Regulates autophagy and induces radioresistance. Conferees growth advantage and blocks apoptosis	*BTG2, PIK3IP1, DAB2IP*	[125, 126]
miR-96	Onco-miR		Regulates autophagy under hypoxia, enhances growth and cellular proliferation, promotes prostate bone metastasis *in vivo*.	*MTOR, ATG7, FOXO1, AKT1S1*	[127–129]
miR-183	Onco-miR		Involved in zinc homeostasis, regulates cell growth and motility, positively controls synthesis and serum levels of PSA	*hZIP1, Dkk-3, SMAD4, PSA*	[130–132]
miR-375	Onco-miR		Stimulates cell growth, invasion ability, and impairs apoptosis in a cell-specific context	*SEC23A, CCND2*	[133, 134]

miR-21 inhibition using antisense oligonucleotides does not affect proliferation, although it increases sensitivity to apoptosis and inhibits cell motility and invasion by targeting MARCKS, a gene with a role in cell motility [143], whereas miR-21 overexpression represses BTG2, which induces expression of luminal markers and promotes epithelial-mesenchymal transition (EMT) [144]. Increased miR-21 expression is associated with shorter biochemical recurrence-free survival and predicts biochemical recurrence in PCa patients submitted to radical prostatectomy [145]. The list of AR-regulated miRNA also includes miR-27a, miR-141, miR-101, and miR-125b [146]. Conversely, miR-135b, miR-185, miR-297, miR-34a, miR-34c, miR-421, miR-634, miR-654-5p, and miR-9 influence androgen signaling by targeting AR [140]. Thus, miRNAs are involved in hormone-dependent and hormone-independent PCa growth, constituting putative therapeutic strategies to inhibit AR function and androgen-dependent cell growth in PCa.

PTEN is a tumor suppressor that antagonizes PI3K/AKT signalling and its expression is frequently abrogated in PCa. Decreased PTEN abundance due to upregulation of miR-106b~25 cluster (due to genomic amplification) and miR-22 in PCa is critical for malignant transformation of prostate cells [147]. In DU145 cells, stable over-expression of pri-miR-22 markedly increases colony formation and caused increased proliferation and tumor growth, as well as over-stimulation of AKT pathway in xenografts [147]. The same effects are apparent when miR-106b~25 cluster is stably expressed in PCa cells, leading to decreased PTEN abundance and activity [147]. Strikingly, that miRNA locus also collaborates with its host gene, MCM7, to promote malignant transformation. In nude mice, larger tumors were formed, compared to control cells, when miR-106b~25 cluster was overexpressed [147]. Moreover, miR-22 and miR-106b~25 clusters cooperate with c-MYC, further emphasizing its proto-oncogenic properties. Indeed, MCM7, and, consequently, miR-106b~25 cluster

transcription is enhanced by c-MYC, suggesting that its oncogenic activity may also involve transcriptional activation of PTEN-targeting miRNAs [147].

MicroRNAs might be also involved in development of PCa bone metastasis, as loss of miR-15 and miR-16 and increased miR-21 expression stimulate dissemination and bone marrow colonization, through aberrant TGF-β and Hedgehog signalling [141].

In exosomes derived from PCa bulk and cancer stem cells (CSC), miR-100-5p and miR-21-5p were the most abundant miRNAs in both cell types, among 1839 miRNAs isolated [148]. Strikingly, biological processes controlled by the differentially expressed miRNAs in bulk exosomes were related to fibroblast growth, epithelial proliferation, and EMT through MMPs activation, whereas those from CSCs exosomes controlled proliferation, epithelial differentiation, and angiogenesis [148]. Overexpression of miR-100-5p, miR-21-5p, and miR-139-5p in a normal prostate fibroblast cell line (WPMY-1) resulted in increased expression of MMPs [148], with a predominant effect of miR-21-5p on MMP9 and of miR-100-5p on MMP2 and MMP13, whereas miR-139 induced expression of all MMPs. Ultimately, transfection of those miRNAs significantly increased RANKL expression, which induces cell proliferation, emphasizing that miRNAs contained in exosomes may play a significant role in cancer invasion and metastasis [148].

It was recently demonstrated that PCa-derived adipose stem cells (pASCs) stimulated with conditioned media or exosomes (isolated from PC-3 and C4-2B cells) induced prostate-like neoplastic lesion *in vivo* [99]. The oncogenic stimulation of pASCs might be a consequence of the RNA transfer by PCa-derived exosomes and activation of oncomiRNAs (*e.g.*, miR-125b, miR-130b, and miR-155), along with oncogenic factors (*e.g.*, *H-RAS* and *K-RAS*) [99]. In fact, expression of miR-125b and miR-130b promoted downregulation of tumor suppressors *Lats2* and *PDCD4* in pASCs exposed to PCa-derived exosomes. Functionally, pASC tumors acquire cytogenetic aberrations and mesenchymal-to-epithelial transition features and expressed neoplastic markers reminiscent of molecular features of PCa xenografts [99]. Due to its plasticity and cargo potential, PCa-derived exosomes might play a critical role in clonal expansion of tumors through neoplastic reprogramming of tumor-ASCs in cancer patients. This also emphasizes that deregulated expression of oncomiRs causes oncogenic transformation of pASCs due to disruption of transcriptional networks of tumor suppressor genes [99]. Further research on other ncRNA families and different prostate cells (*e.g.*, basal, luminal, and fibroblasts) might help understand how exosomes are involved in crosstalk between tumor and stromal cells to synergistically promote tumor progression and drug resistance.

3.1.2 Small nuclear and nucleolar RNAs in PCa

The role of other small ncRNAs in prostate tumorigenesis has been also investigated. snoRNA U50 is mutated and downregulated in PCa, and a homozygous 2-bp (TT) deletion was identified both in PCa cell lines and primary tissues. Ectopic expression of snoRNA U50 abrogates colony formation, a feature associated with tumor suppression [135].

The nucleolar protein dyskerin (DKC1) catalyzes pseudouridylation of rRNA, and it is also required for the formation of hTR, the RNA component of telomerase. Compared to benign tissues, *DKC1* mRNA levels were higher in PCa samples, especially in lymph node metastases [136]. SiRNA-mediated depletion of *DKC1* decreased cell proliferation of prostate cells [136], suggesting that deregulation of snoRNA machinery is important for prostate carcinogenesis.

Using a deep sequencing approach to characterized small non-coding RNA transcriptome, an increase in both global snoRNAs and tRNA expression in PCa metastatic to lymph node compared to that of primary PCa was shown, suggesting a possible oncogenic role for snoRNAs, particularly in more advanced tumors [138]. In addition, there is a strong differential expression of snoRNAs and tRNAs, comparing PCa and normal prostate samples [138]. Additionally, snoRNA-derived RNAs (sdRNAs) display higher differential expression than miRNAs and they are greatly upregulated in PCa. Using qPCR, SNORD44, SNORD78, SNORD74, and SNORD81, sdRNAs were shown to be upregulated in PCa. The higher expression levels of SNORD78 and its sdRNA—sd78-3′—were associated with metastatic PCa [138].

The ribosome biogenesis begins in the nucleolus [137]. Here, the ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomal subunits [139]. It hosts a transcriptional unit encoding a 45S ribosomal RNA precursor that is processed into the mature 18S, 5.8S, and 28S RNA species [140]. 45S percursor rRNA and mature rRNAs 28S, 18S, and 5.8S are overexpressed in PCa samples compared to morphologically normal prostate tissues [139]. The mechanism leading to the aberrant expression is not well characterized, but apparently, overexpression is not associated with rDNA promoter hypomethylation [139]. In fact, 45S, 18S, and 5.8S rRNA expression levels altered nucleolar structure and function and are more closely associated with *MYC* mRNA levels [139], suggesting that *MYC* might be involved in rRNA biogenesis. In a different report, *MYC* was found to be required for rRNA transcription and processing [142]. In PCa cells, MYC binds to the 5′ upstream region of *Fibrillarin* (*FBL*), a gene required for rRNA production and processing [142]. *FBL* is overexpressed in PCa samples and siRNA-mediated depletion of *FBL* suppressed cell proliferation and clonogenic

survival. Moreover, *FBL* knockdown decreased the levels of 5.8s, 18s, and 28s rRNAs, whereas only a modest reduction in 45S pre-rRNA was observed [142]. Conversely, *MYC* knockdown associated with decreased levels of pre-rRNA as well as of processed rRNAs, indicating that *MYC* is required for rRNA transcription and processing [142]. Genome-wide analysis of *MYC* depletion revealed down-regulation of 133 nucleolus-associated genes and of 64 genes associated with rRNA processing [142]. Those comprised *fibrillarin*, *nucleolin*, *UBF*, and *nucleophosmin*. In addition, overall nucleolar size was reduced after *MYC* depletion *in vitro* [142]. Considering these findings, *MYC* overexpression in PCa cells can drive enhanced *de novo* nucleolar and ribosomal gene expression, thus fostering the malignant phenotype.

rRNA is crucial for both androgen-dependent and androgen-independent growth of PCa cells. The androgen-AR signaling leads to the accumulation of rRNA in androgen-dependent prostate cells, and angiogenin (ANG) is upregulated in PCa cells, mediating androgen-stimulated rRNA transcription [141]. In androgen-dependent cells, androgen stimulation promotes ANG nuclear translocation, where it binds to rDNA promoter, stimulating rRNA transcription [141]. Blocking ANG leads to inhibition of androgen-induced rRNA transcription. Moreover, ANG signalling is not only critical for androgen-dependent growth but also for the castration-resistant phenotype. In an androgen-independent context, ANG stimulation leads to constitutive nuclear translocation in androgen-insensitive cells, ensuing a continuous rRNA overproduction and thereby stimulating cell proliferation [141].

3.1.3 tRNA-derived RNA fragments

Global expression profile of prostate cell lines revealed that the second most abundant group of sncRNA was that of fragments derived from tRNA, the tRNA-derived RNA fragments (tRFs) [143]. Deep sequencing characterization of LNCaP and C4-2 cell lines disclosed 17 tRNA-related small RNAs, including the most abundant: tRF-1, tRF-3, and tRF-5. For downstream validation, tRF-1001, a member of tRF-1 series, was selected. tRF-1001 is derived from the 3′ end of a Ser-TGA tRNA precursor transcript, which is not retained in the mature tRNA [143].

The tRF-1001 is expressed more abundantly in cell lines than in tissues, but its expression decreases either upon starvation or high cell density in DU145 and LNCaP cells. Reduction of cellular metabolism also decreased expression of tRF-1001 precursor, but the corresponding mature tRNA levels were unaffected. The tRF-1 series of small RNAs are 3′ sequences from pre-tRNA, released through a cleavage by tRNA endonuclease ELAC2 during the 3′-end maturation of tRNA. Knockdown of ELAC2 decreased tRF-1001 expression, leading to accumulation of the pre-tRNA [143]. tRF-1001 and its precursor tRNA

are exclusively localized in the cytoplasm, providing evidence that biogenesis occurs in the cytoplasm, rather than in the nucleus as it happens for tRNAs. These data sustain a functional role for tRFs, putting aside the idea of a mere by-product of tRNA biochemical processing [143].

Recently, RNA sequencing was used to profile tRFs in fresh frozen tissue samples derived from normal adjacent prostate and PCa at different stages [144]. A total of 598 unique tRFs were identified, and several are deregulated in PCa. Strikingly, 5′-tRFs constitute approximately 75 % of all tRFs detected in prostate tissues. Notably, most of the identified tRFs are derived from 5′- and 3′- of mature cytosolic tRNAs. Nonetheless, tRFs derived from different segments of tRNAs, including pre-tRNA trailers and leaders, as well as tRFs from mitochondrial tRNAs were catalogued. Globally, 110 tRFS were found deregulated (72 upregulated, 24 downregulated, and 13 upregulated in one group but downregulated in the other group [144]). Most of the upregulated tRFs were 5′-tRFs and most of downregulated were 3′-tRFs. Downstream qPCR validation of 6 different tRFS revealed that 4 tRFs (three 5′-tRFs and one D-tRF) were upregulated (Fig. 3c–f), and 2 tRFs (3′-tRF class) were downregulated in PCa. tRF-544 (isotype Phe, anticodon GAA - tRNA^{PheGAA}) is thought to be associated with aggressive forms or advanced stages of PCa. Interestingly, high expression level ratio tRF-315/tRF-544 significantly associated with poorer progression-free survival and shorter time to disease relapse.

Sex hormone-dependent tRNA-derived RNAs (SHOT-RNAs) are commonly expressed in AR-positive PCa cancer cell lines [145]. In LNCaP-FGC cells, both 5′- and 3′-tRNA halves from SHOT-RNA^{AspGUC} and SHOT-RNA ^{HisGUG} are detected by northern blot, but not in DU145 or PC3 cells, and AR knockdown reduced tRNAs expression levels. One of must abundant SHOT-RNAs detected by Honda *et al.*—5′- SHOT-RNA RNA^{LysCUU}—was knocked down using siRNAs in LNCaP-FGC, and cell growth rate was decreased compared to control siRNA [145]. Because levels of mature tRNA were not changed by siRNA transfection, reduced proliferation seems to be solely attributable to the change in SHOT-RNA RNA levels. This strategy was also applied to SHOT-RNA^{AspGUC} and SHOT-RNA^{HisGUG} and depletion of each SHOT-RNA impaired cell growth as well. Nevertheless, 3′-SHOT-RNA^{AspGUC} depletion failed to impair cell growth [145]. Overall, these data support SHOT-RNAs as functional RNA molecules and different species of 5′-SHOT-RNA are involved in cell proliferation [145]. To determine whether 3′-SHOT-RNA holds functional relevance or not, additional studies are required.

The current understanding of tRFs, however, suggests that they are not merely byproducts of random cleavage of tRNAs, but might act as mediators of translational and/or gene regulation. Although some isolated functions have been indicated, the vast majority of tRFs appear to operate *via* uncharacterized

mechanisms. It has been proposed that 5′- but not 3′-derived tRFs play a role in stress granule assembly or inhibition of protein synthesis *in vitro* [146]. However, 3′-derived tRFs are able to repress their mRNA targets in a miRNA-like fashion and may exert tumor-suppressive functions [147].

3.1.4 Other short RNAs

Although no direct involvement of piRNA in PCa has been reported, some genes implicated in piRNA biogenesis are deregulated in PCa. Defects in Tudor-domain proteins significantly impair piRNA pathway, especially its ping-pong components, although not abolishing it. Because multiple Tudor-domain-containing proteins exists, one may argue that it exhibits overlapping or redundant roles in the piRNA pathway, explaining the somewhat minor phenotypes of the individual mutants [148].

Tudor domain-containing protein 1 (TDRD1) is a direct target gene of ERG, strongly correlating gene with ERG overexpression [149]. Mechanistically, ERG is able to disrupt tissue-specific DNA methylation pattern at the *TDRD1* promoter, resulting in *TDRD1* transcriptional activation [149]. Piwil2 has been recently described as an oncogene able to modulating invasion and metastasis, as well as EMT [150]. Of note, global piRNA levels were not assessed to quantify the deregulation caused by TDRD1 and Piwil2 aberrations.

3.2 Long non-coding ncRNA

Although ncRNAs research, and specially lncRNAs, is still in its infancy, significant roles have been ascribed to some lncRNAs in PCa and these are summarized in Table 3.

Table 3 lncRNA manipulation and consequential phenotypes in PCa

| lncRNA	Biotype	Function	Phenotype	Ref
MEG3	lincRNA	Tumor suppressor	Promotes apoptosis by blocking BCL2, enhancing BAX and activating CASP3. Induces G0/G1 arrest by inhibiting CCND1	[151]
PCAT-29	lincRNA	Tumor suppressor	Androgen-regulated lincRNA. Suppresses PCa growth and metastases in chick chorioallantoic membrane assays. Identifies a subset of patients at higher risk for disease recurrence	[152]
NANOGP8	Pseudogene	Tumor suppressor	Decreases clonogenic and migratory potential of PCa cell lines ultimately impairs tumor development *in vivo*. *NANOGP8* knockout increases sensitivity to docetaxel	[153]
DRAIC	lincRNA	Tumor suppressor	Activated by FOXA1 and NKX3-1, and repressed by AR, promoting downregulation of *DRAIC* during prostate cancer progression. Impairs cell invasion and migration, blocking the acquisition of fibroblast-like morphology in PCa cells	[154]
PCAT29		Tumor suppressor	Repressed by AR and induced by FOXA1. Migration and metastasis suppressor	[154]
GAS5	lncRNA/ Retained intron	Tumor suppressor	Involved in cellular growth arrest and apoptosis probably due to GAS5 encoded transcript mimics glucocorticoid response element (GRE). Hosts multiple snoRNA, containing multiple C/D box snoRNA genes in its introns	[138, 155]
PCGEM1	lincRNA	Oncogene	Transcriptional regulator of key metabolic pathways in PCa cells acting as a coactivator for both c-Myc and AR, providing growth advantage. Also recruits PYGO2 and enhances selective looping of AR-bound enhancers to target gene promoters in PCa cells	[156, 157]
PlncRNA-1 *(CBR3-AS1)*	Antisense	Oncogene	Regulates cell proliferation and apoptosis, by targeting AR	[158]
HOTAIR	Antisense	Oncogene	Androgen-repressed ncRNA upregulated following androgen deprivation therapies and in CRPC. Binds to the AR protein to block its interaction with MDM2, thereby preventing AR ubiquitination and protein degradation.Involved in cell growth and invasion	[159]
PRNCR1	lincRNA	Oncogene	Binds to the carboxyterminally acetylated AR on enhancers, and binds DOT1L. Promotes transcriptional activation by AR and ultimately cell proliferation	[157]
PCAT5		Oncogene	Implicated in cell growth, migration/invasion, colony-forming, and apoptosis. PCAT5 is a regulatory target of ERG	[160]
PCAT18		Oncogene	Involved in cell proliferation and apoptosis, migration and invasion. Activated by AR	
TRPM2-AS	Antisense	Oncogene	Associated with poor clinical outcome. Depletion of *TRPM2-AS* induces apoptosis both *in vitro* and *in vivo* in androgen-independent PCa cells. Critical to maintain the cell cycle progression	[161]

3.2.1 Antisense regulatory lncRNAs in PCa

The role of dysregulated antisense transcript expression is under investigation in PCa. The polyadenilated antisense transcript *ANRIL* (encoded by CDKN2B-AS1) is expressed from the tumor-suppressor locus INK4b-ARF-INK4a (9q21.3). *ANRIL* and *CBX7* (member of Polycomb Repressor Group 1) are both upregulated in PCa samples [162]. Furthermore, CBX7 is responsible for maintaining silenced chromatin states through recognition of H3K27me3 [162]. CBX7 binds to H3K27me3 and interacts with *ANRIL* at the INK4b-ARF-INK4a locus. CBX7 employs different regions within its chromodomain for binding to H3K27me3 and *ANRIL* RNA, suggesting that both interactions are important for the sustained *cis*-repression of the locus [162]. Thus, RNA–protein interaction underlies the ability of PRC1 to repress the INK4b-ARF-INK4a cluster, and its disruption contributes to PCa development by reducing senescence [162]. Interestingly, these data might indicate that the frequent promoter hypermethylation observed at this locus occurs as a secondary event after cell differentiation.

Another NAT with critical impact in PCa cells is *CTBP1-AS*, an androgen-responsive lncRNA that promotes PCa growth through sense-antisense repression of the transcriptional co-regulator *CTBP1* and global epigenetic regulation of tumor suppressor genes [163]. The upregulation of *CTBP1-AS* is inversely correlated with *CTBP1* in primary and metastatic PCa, associating with high AR expression status. Depletion of *CTBP1-AS* mRNA abolished the androgen-dependent reduction of *CTBP1*, indicating that *CTBP1-AS* directly regulates *CTBP1* at RNA level [163]. Silencing *CTBP1-AS* reduced LNCaP cell proliferation, and *in vivo* tumor growth was also reduced, concomitantly with an increased CTBP1 expression. Microarray analysis showed that transcriptional activation of androgen-induced genes was diminished by siCTBP1-AS [163]. Interestingly, *CTBP1-AS* overexpression stimulated cell growth and promoted resistance to growth inhibition by bicalutamide, ultimately rendering *in vivo* tumor growth after castration. Mechanistically, *CTBP1-AS* coordinates *cis*-repression of *CTBP1* promoter, reducing H3Ac and H4K4me levels but not altering repressive marks [163]. *CTBP1-AS* binds to HDAC-Sin3A complex and coordinates HDAC-mediated repression by chromatin deacetylation within *CTBP1* promoter in the AR-dependent system. Moreover, *CTBP1-AS* also interacts with PSF, which binds at *CTBP1* promoter to induce histone deacetylation by HDACs to promote transcriptional repression of CTBP1. Additionally, *CTBP1-AS* may also act as trans-acting regulator of androgen-regulated genes by recruiting the HDAC/Sin3A repressor complex *via* PSF, prompting cell cycle progression by repressing cell cycle regulators and modulating global androgen signaling (*e.g.*, p53, SMAD3) [163].

Another example of antisense gene regulation is the transcriptional control of tumor-suppressor gene *RASSF1A* by *RASSF1A*-antisense RNA 1 [164]. *RASSF1A-AS1* is upregulated is PCa cell lines, inversely correlating with *RASSF1A* expression [164]. *RASSF1A* and RASSF1A-AS1 form a RNA-DNA hybrid at the *RASSF1A* promoter and recruits the polycomb repressor complex PRC2. PRC2 contributes to chromatin compaction by catalyzing the methylation of histone H3 at lysine 27, which is enriched at *RASSF1A* promoter, and specifically blocks *RASSF1A* expression [164].

3.2.2 Enhancers and enhancer RNAs in PCa

Cancer cells display altered expression patterns and enhancer usage in comparison with their normal counterparts [165]. In PCa, enhancer RNAs (eRNAs) have been implicated in assisting AR-mediated signaling, as mediators of enhancer-promoter looping and in altering transcription factor binding (Fig. 5).
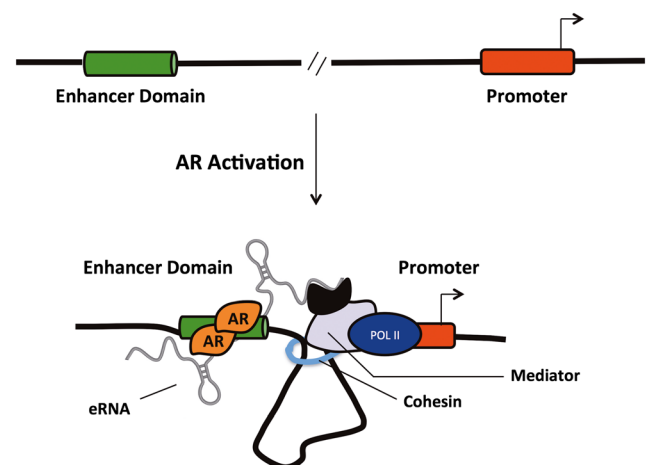
Fig. 5 Transcripton derived from enhancer is important for long-range transcriptional control. eRNAs are lncRNA derived from short regions of DNA that enhance the expression of genes at varying distances. Effects can be mediated by transcription factor binding to these sites, such as androgen receptor (AR). AR controls PCa cell-specific gene expression programs through interactions with diverse co-activators and the transcription machinery. Gene activation may involve DNA loop formation between enhancer-bound AR and the transcription machinery at the core promoter. This interaction seems to be mediated by mediator complex and cohesin, as they have been reported to interact physically and functionally connect the enhancers and core promoters of active genes. The eRNAs produced from AR-bindind DNA segments, facilitate the spatial interaction between enhancer and promoter, ultimately enhancing long-distance transcriptional regulation. Moreover, specific eRNA might encompass androgen response elements (ARE), supporting AR and mediator interactions. This mechanism is critical for PCa cells, as androgen-induced eRNAs scaffolds the AR-associated protein complex that modulate chromosomal architecture and selectively enhance AR-dependent gene expression involved in PCa initiation and progression

FoxA1 has been reported to contribute to the enhancer code in PCa cells, as FoxA1 regulates AR genomic targeting by simultaneously anchoring AR to cognate loci and restricting AR from other ARE-containing loci in the human genome [72]. In addition, knockdown of *FoxA1* markedly elevated di-hydro-testosterone (DHT) response and caused AR binding to a distinct cohort of enhancers. Global nuclear run-on sequencing (GRO-seq) was applied to understand how differential AR binding is translated into hormonal gene response [72]. After DHT treatment, GRO-seq detected ncRNA expression from a subset of H3K4me1-positive and H3K4me3-negative regions. These differentially expressed eRNAs are largely symmetrical and bidirectional (as depicted for the KLK3 enhancer). Moreover, these AR-activated enhancers marked by increased eRNA expression are responsible for activation of nearby coding transcription units [72]. Chromosome conformation capture (3C) suggested that eRNA induction *per se* is the most precise mark of the functional looping between an activated enhancer and its regulated gene promoter, rather than p300 or MED12 binding [72]. Moreover, both DHT and FoxA1 knockdown demonstrated a strong H3K4me2-marked central nucleosome, suggesting that nucleosome remodeling is not required to induce specific enhancer-promoter looping and subsequent target gene activation [72].

Furthermore, it has been reported that PolII binds to a large number of intergenic AR-bound enhancers, marked by H3K4me1 and H3K27ac, which produce eRNAs that may regulate neighbor or distantly located genes [89]. This evidence suggests than eRNAs may contribute to AR-driven looping complex that enhances spatial communication of distal enhancers and target promoters, leading to transcriptional activation of specific genes [89]. The KLK3 enhancer is marked by AR binding, H3K27ac and H3K4me1, and produces a bidirectional eRNA named KLK3e [89]. Both KLK3 and KLK3e expression is induced by DHT treatment and blocked by bicalutamide, indicating a high correlation of activity-dependent induction between eRNAs and adjacent protein coding genes. KLK3e sense strand gives rise to a >2 kb polyadenylated transcript that is substantially more expressed than the antisense transcript. KLK3e facilitates the spatial interaction of the KLK3 enhancer and the KLK2 promoter, enhancing long-distance KLK2 transcriptional activation [89]. KLK3e contains the core enhancer element derived from the androgen response element III (AREIII) required for the interaction of AR and Mediator 1 (MED1). Suppression of either KLK3e or MED1 reduced the interaction of KLK3/2 loci, supporting a role for MED1 as a mediator of the long-range chromatin looping and cooperating with KLK3e in the enhancer target-promoter interaction. Globally, these data suggests that KLK3e forms a functional complex with AR and MED1 that facilitates the association of AR-bound enhancers with promoters, resulting in transcriptional activation of target genes [89]. Supporting this hypothesis, KLK3e expression is

significantly correlated with KLK3 and KLK2 ($R^2 = 0.62$; 0.59, respectively). Further understanding of how AR-induced eRNAs act as a scaffold for AR-associated protein complex that selectively modulate chromosomal architecture and gene expression may translate into new RNA-based therapy to improve response to androgen deprivation therapy [89].

Recently, a new role for single-strand nicks was identified, mediated by DNA topoisomerase 1 (TOP1), in relaxing supercoiled DNA at gene enhancers to promote enhancer-dependent transcription [166]. In LNCaP cells, TOP1 was recruited to AR-regulated enhancers in response to androgen treatment. Using ChIP-seq, of the 6545 putative AR-bound enhancers, 96 % were occupied by TOP1. Of these, 60 % revealed an androgen-stimulated increase in TOP1 binding as well as in RNAPOL II occupancy, indicative of active transcriptional activity [166]. GRO-seq analysis of serum-starved LNCaP cells treated with DHT identified 644 putative enhancers (74 % of them showed increased TOP1 occupancy) with significantly upregulated eRNA expression. Knockdown of endogenous *TOP1* resulted in decreased eRNA expression of 79 % of AR-regulated enhancer, accompanied by lower expression levels of 368 protein-coding mRNAs (including *KLK3*, *KLK2*, *TMPRSS2*, and *NDRG1*) [166]. Having proved that TOP1 reduces both eRNA and mRNA production of most AR-regulated target genes, the authors found that prior binding by NKX3.1 was required to recruit TOP1 to enhancers following androgen treatment. siRNA-depletion of *NKX3.1* inhibited recruitment of TOP1 and reduced DHT-dependent upregulation of eRNA expression [166]. Strikingly, depletion of both *TOP1* and *NKX3.1* reduced DHT-mediated eRNA upregulation at the same AR-bound enhancers, apparently without affecting AR recruitment. This reveals that NKX3.1 and TOP1 occupy the same binding sites at enhancer elements and co-regulate an AR transcription program. The Y723F *TOP1* mutant did not block transcriptional activity in *TOP1*-depleted cells, suggesting that the nicking activity of *TOP1* is required for its effects on enhancer activation [166]. Given that single-strand nicks might lead to the formation of DNA double-strand breaks, several components of the DNA damage response pathway—MRE11, RAD50, and ATR—are recruited to AR-regulated enhancers after DHT treatment and are required for eRNA and protein-coding mRNA transcription. Taken together, these data suggest a common usage of the DNA damage repair machinery to regulate AR-mediated gene transcription, highlighting the complexity of PCa [166].

In a recent study, using Chem-seq [167], a compound that inhibits cell proliferation *in vitro* and tumor growth *in vivo*—SD70—was identified. SD70 binds to AR-bound functional enhancers, regulating DHT-induced gene transcriptional programs [167]. Moreover, it was found that KDM4C binds at AR-regulated enhancers and is recruited in a DHT-dependent fashion. *In vitro*, SD70 inhibits KDM4C demethylase activity causing elevated H3K9me2 levels at enhancer and promoter

regions—a plausible component of the inhibitory effects on DHT target gene expression [167]. These results suggest that targeting enhancer regions has potential therapeutic value for PCa.

3.2.3 lncRNA as master regulators of alternative splicing and translation

Recently, it has been shown that lncRNA are required to assemble nuclear domains specialized in RNA processing, such as the nuclear speckle and the paraspeckle. The oncogenic lncRNA *MALAT1* (also known as *NEAT2*, located at 11q13.1) is present within the nuclear speckle and spatially re-organizes the actively transcribed genes closed to the nuclear speckles, a domain know for its abundance in pre-mRNA splicing factors [10]. Knockdown of *MALAT1* disclosed that this lncRNA regulates alternative splicing of multiple genes by controlling the availability of serine/arginine-rich splicing factors in active transcription sites [168]. Interestingly, during post-transcriptional processing of *MALAT1*, a conserved 3′ tRNA-like sequence generates a short tRNA-like ncRNA called MALAT1-associated small cytoplasmic RNA (*MASCRNA*), whose function is still unclear. *MASCRNA* is a 61-bp short tRNA-like ncRNA of unknown function, generated by RNase-P cleavage and then exported to the cytoplasm [169].

On the other hand, the lncRNA *NEAT1* is an essential structural element to initiate *de novo* assembly of paraspeckles, which are believed to be nuclear domains specialized in retention of adenosine-to-inosine edited mRNAs [170]. Inducing *NEAT1* transcription locus is sufficient to form new paraspeckles at the integration locus. However, active transcription of *NEAT1* is necessary to tether the lncRNA to its own transcription locus and carry out this role [170]. Taking into account that *MALAT1* and *NEAT1* are separated by approximately 70 kb, it is conceivable that coordinated deregulation of both loci may hinder alternative splicing by controlling the nuclear localization of splicing factors as well the control of RNA editing and export, further contributing to prostate carcinogenesis [10]. Indeed, both *MALAT1* [171] and *NEAT1* [172] are overexpressed and possess pro-tumorigenic activity in PCa. *MALAT1* overexpression in primary PCa is associated with higher Gleason score, pathological stage, and serum PSA >20 ng/ml [173]. Besides its association with poor prognosis, MALAT1 expression is significantly increased in castration-resistant PCa (CRPC) compared to hormone-sensitive PCa [173]. Functional assays using siRNA specific to knock down *MALAT1* expression in 22RV1 and LNCaP-AI cells inhibited cell cycle at G0/G1 phase, migration, and invasion [173]. RNAi silencing of *MALAT1* in PCa xenografts of castrated male nude mice resulted in significant reduction of tumor volume and metastasis number, increasing survival time [173]. Whether these alterations are specific of

MALAT1 or are the combined effect in downstream genes (*e.g.*, RNA splicing deregulation) controlled by MALAT1 is still a matter under study. Using EZH2 antibody-based RNA immunoprecipitation combined with next-generation sequencing (RIP-seq), EZH2 was found to bind to *MALAT1* [171]. Both GST pull-down and RIP assays showed that the 3' end of *MALAT1* interacts with the N-terminal of EZH2. Moreover, *MALAT1* and *EZH2* are positively correlated in CRPC samples. Moreover, depletion of *MALAT1* impaired EZH2 recruitment to its target loci (*DAB2IP* and *BRACHYURY*) and caused its upregulation, suggesting that *MALAT1* mediates EZH2-enhanced migration and invasion in CRPC cell lines [171]. Moreover, *MALAT1* enhances expression of PRC2-independent target genes of EZH2 both *in vitro* and in patient-derived xenografts (TMEM48 and KIAA0101) [171].

NEAT1 is an ERα-regulated lncRNA, upregulated in PCa, producing two RNA isoforms that overlap completely at the 5′-end. The shorter isoform is 3.7 kB in length and more abundant than the longer, 23 kB, isoform (NEAT1_2) [172]. NEAT1 expression is a prognostic biomarker for aggressive PCa independent of standard clinical and pathologic parameters [172]. Estrogen treatment upregulates NEAT1 transcript levels in a time-dependent manner and in VCaP cells results in re-distribution of NEAT1 from paraspeckles to an enhanced distribution throughout the nucleus [172]. Knockout of NEAT1 compromised the expression of ERα target genes, suggesting that NEAT1 is not only a downstream target but also a mediator of ERα signalling in PCa cells. NEAT1 transcriptionally regulates a compendium of genes known to be involved in PCa progression, including *PSMA* and *GJB1* [172]. Overexpression of NEAT1_1 significantly increased active chromatin marks H3K4Me3 and H3AcK9 at the *PSMA* promoter and induced subsequent recruitment of NEAT1_1 and ERα to the same promoter. RNA immunoprecipitation revealed that NEAT1 directly interacts with histone H3, favoring a chromatin landscape for active transcription through active histone marks [172]. Phenotipically, knockdown of NEAT1 in VCaP cells significantly decreased proliferation and the invasive properties of cells. Overexpression of NEAT1 resulted in a significantly higher number of viable colonies, establishing an oncogenic role for NEAT1 [172]. In athymic nude mice, injection of either VCaP or NCI-H660 overexpressing NEAT1 resulted in a significantly higher tumour growth rate compared to scramble cells. Moreover, *in vitro* NEAT1 expression is inhibited when cells are treated with ERα antagonists in combination with E2. Similar results observed with AR antagonists enzalutamide and bicalutamide suggest that NEAT1 is associated with resistance to therapy [172]. Thus, these data suggest a role for paraspeckles in the lncRNA-mediated regulation of gene expression in PCa.

3.2.4 lincRNAs deregulation in PCa

Long intervening non-coding RNAs (lincRNAs) are emerging as key regulators of diverse cellular processes, but determining their individual function remains a challenge. lincRNAs are also called long intergenic non-coding RNAs, although lincRNAs derive from genes and are thus genic, which do not overlap with exons of either protein-coding or other non-lincRNA types of genes [19].

Ab initio transcriptome sequencing of polyA+ RNA from 102 PCa tissues and cell lines revealed a total of 1859 unannotated lincRNAs throughout the human genome [174]. A set of 121 of those transcripts accurately distinguished benign, localized and metastatic PCa by unsupervised clustering. *PCAT-1* (located in the 8q24 gene desert) is predominantly cytoplasmic and was upregulated in PCa samples especially in high-grade (GS ≥ 7) and metastatic tumors. Strikingly, *PCAT-1* and *EZH2* expression was nearly mutually exclusive, suggesting that their expression may define two subsets of high-grade disease. However, upregulation of *PCAT-1* was not dependent of 8q24 amplification [174]. Inhibiting *EZH2*, using either shRNAs or DZNep, caused a dramatic upregulation of *PCAT-1* in VCaP cells. ChIP assay showed that SUZ12, a core component of PRC2, directly binds to *PCAT-1* promoter ~1 kb upstream of TSS [174]. By RNA immunoprecipitation, it was demonstrated that *PCAT-1* binds to SUZ12 protein in VCaP cells, a feature that was abolished by RNase A, RNase H, or DNase I treatment. This suggests that *PCAT-1* exists primarily as a single-stranded RNA and secondarily as a RNA/DNA hybrid. Moreover, *PCAT-1* stable overexpression in RWPE cells promoted cell proliferation, and RNAi silencing decreased cell proliferation in LNCaP but not in DU145 (lacks *PCAT-1* expression) or VCaP cells (*PCAT-1* is repressed by PRC2) [174]. Genome-wide expression analysis of LNCaP cells after treatment with siRNAs against *PCAT-1* disclosed upregulation of 255 genes and repression of 115 genes, revealing that *PCAT-1* is predominantly repressive. Additionally, the upregulated genes showed enrichment for mitosis and cell cycle [174]. Specifically, PCAT-1 targets *BRCA2*, *CENPE*, and *CENPF*, whose expression is upregulated upon *PCAT-1* silencing in LNCaP cells. Further research demonstrated that PCAT-1 overexpression decreased RAD51 foci formation (a component of homologous recombination, HR) after therapy with PARP1 inhibitors and *PCAT-1* knockdown increased foci formation upon therapy, in PCa cells [175]. *BRCA2* inactivation impairs both HR and double-stranded DNA break repair (DSB). *PCAT-1* expression is correlated with decreased *BRCA2* levels, and *in vitro*, the 5′ end of *PCAT-1* is able to directly repress the activity of *BRCA2* 3′UTR [175]. PCAT-1 overexpression produces a functional deficiency in HR through post-transcriptional repression of *BRC2* tumor suppressor, which, in turn, reveals a high sensitivity to small molecule inhibitors

of PARP1, both *in vitro* and *in vivo* [175]. Whether PCAT-1 may act as predictive biomarker for patient response to PARP1 inhibitor therapy is still to be proved.

PCAT-1 is located 725 kb upstream of the *MYC* oncogene [176]. Overexpression of *PCAT-1* in DU145 and RWPE increased c-MYC protein levels, while silencing of *PCAT-1* in LNCaP decreased c-MYC protein, suggesting a *cis*-regulation involving these loci [176]. Strikingly, c-MYC silencing fully abrogated the proliferative effects of *PCAT-1* overexpression in DU145 and RWPE, indicating that *PCAT-1* mediated cell proliferation is dependent of c-MYC overexpression. Luciferase assay revealed that *PCAT-1* overexpression increased *cMYC* 3′UTR activity, whereas silencing of *PCAT-1* decreased *c-MYC* 3′UTR activity. Mechanistically, this suggests that *PCAT-1* regulates *c-MYC* in a post-transcriptional manner by 3′UTR activation, which can result in gene activation and increased protein abundance [95].

Another important lincRNA in PCa is *SChLAP1* (second chromosome locus associated with prostate-1; also designated LINC00913) [177]. *SChLAP1* is located in a "gene desert" on chromosome 2q31.3 and is highly expressed in ~25 % of PCa, being more frequently expressed in metastatic compared to localized PCa. Its expression was associated with ETS gene fusions and *PTEN* deletions in localized PCa [177]. Moreover, *SChLAP1* levels independently predict poor outcome, including metastasization and PCa-specific mortality [177]. Knockdown of *SChLAP1* dramatically impaired cell invasion and proliferation *in vitro* and, in turn, overexpression of a siRNA-resistant *SChLAP1* isoform rescued the *in vitro* invasive phenotype of 22Rv1 cells treated with siRNA. Overexpression of the three *SChLAP1* isoforms in RWPE cells dramatically increased the ability of these cells to invade *in vitro* but did not affect cell proliferation. *In vivo*, *SChLAP1* depletion impaired metastatic seeding and growth. Overall, *SChLAP1* seems to control tumor invasion and metastasis by influencing cancer cell intravasation, extravasation, and subsequent tumor cell seeding [177]. Using Gene Set Enrichment Analysis of 22Rv1 and LNCaP cells with *SChLAP1* knockdown, *SChLAP1*-regulated genes were correlated with the SWI/SNF complex, a multiprotein complex known to physically rearrange nucleosomes at gene promoters, thus controlling transcription [177]. Mechanistically, *SChLAP1* co-immunoprecipitates with SNF5 and attenuates SNF5 genome-wide localization. Upon knockdown of *SChLAP1*, 9 of 12 target genes disclosed a substantial increase in SNF5 binding. These data sustain that oncogenic *SChLAP1* overexpression antagonizes the tumor-suppressive role of SWI/SNF complex function by attenuating the genomic binding of this complex, thereby impairing its ability to properly regulate gene expression [177].

Prostate cancer antigen 3 (*PCA3*) is a spliced intronic antisense lncRNA embedded within intron 6 of the corresponding sense gene *PRUNE2* and upregulated in PCa samples, holding

promise as biomarker for PCa detection [178]. *PCA3* controls *PRUNE2* levels *via* a unique regulatory mechanism involving formation of a *PRUNE2/PCA3* double-stranded RNA that undergoes ADAR-dependent adenosine-to-inosine RNA editing [178]. Because *Drosophila* behavior human splicing (DBHS) protein P54NRB binds to inosine-containing RNA (RNA-I), regulating gene expression, it was found, using RNA-ChIP, that *PCA3* and *PRUNE2* pre-mRNA species associate with P54NRB protein, suggesting that DBHS proteins also contribute for *PRUNE2/PCA3* regulation [178]. *In vitro* stimulation with a synthetic testosterone homolog induced *PCA3* expression and decrease PRUNE2 levels [97]. *PCA3* silencing or ectopic *PRUNE2* expression decreased cell proliferation and transformation *in vitro*; in contrast, *PRUNE2* silencing or ectopic *PCA3* expression increased cell proliferation and transformation [178]. PRUNE2-deficient PC3 cells stably expressing ectopic *PRUNE2* display lower levels of proliferation and transformation *in vitro*, consistent with the negative regulation of *PRUNE2* by *PCA3* [178]. In SCID mice, *PRUNE2* silencing and ectopic *PCA3* expression yielded markedly larger tumor xenografts than controls; in contrast, tumor growth was significantly diminished compared to controls when *PCA3* was silenced, further illustrating the oncogenic activity of *PCA3* [178]. Serum PSA was increased in SCID mice injected with LNCaP cells with ectopic PCA3 expression or *PRUNE2* silencing, compared to controls [178]. In human PCa samples, *PCA3* and *PRUNE2* levels inversely correlate. Moreover, A > G/T > C alterations were the most frequent substitutions, indicative of A-to-I editing in both *PCA3* and *PRUNE2* pre-mRNA strands [178]. These results establish *PCA3* as a dominant-negative oncogene and *PRUNE2* as a tumor suppressor gene in PCa, and their regulatory axis represents a putative target for clinical intervention [178].

3.2.5 Pseudogenes

CXADR-ψ, a processed pseudogene on chromosome 15, parental of the tumor-suppressor *CXADR*, was found overexpressed in PCa tissues compared to benign tissue samples [91]. CDNA cloning from two PCa samples positive for *CXADR-ψ* showed perfect sequence similarity to the pseudogene *CXADR-J* and only 84 % to CXADR wild-type gene [91]. No correlation was depicted for *CXADR* and *CXADR-ψ*. Interestingly, *CXADR-ψ* expression was nearly restricted to PCa lacking an ETS gene fusion, with few ETS-positive samples exhibiting expression of this pseudogene [91]. On the other hand, *CXADR* gene expression was found in both ETS-positive and ETS-negative samples [91]. In the same study, a PCa-specific readthrough transcript involving *KLK4*, an androgen-induced gene, and *KLKP1*, an adjacent pseudogene, was identified. *KLK4-KLKP1* transcript was highly expressed in 30–50 % of PCa tissues, and this expression was lineage and cancer specific, with low expression

detected in benign prostate and other tissues [91]. *KLK4-KLKP1* transcript was previously described in LNCaP as a *cis* sense-antisense chimeric transcript [91]. This chimeric transcript is composed of the first two exons of *KLK4* and the last two exons of *KLKP1*. It retains an open reading frame incorporating 54 amino acids encoded by the KLKP1 pseudogene in the putative chimeric protein [91]. Additional studies are needed to understand the biological role of the chimeric transcript *KLK4-KLKP1* in PCa biology.

Pseudogene transcription has also been shown to regulate cognate wild-type gene expression by sequestering miRNA acting endogenous miRNA sponges, or competing endogenous RNAs (ceRNAs) [79]. ceRNAs communicate and co-regulate each other by competing to bind to a common pool of miRNAs, thus altering miRNA availability and stoichiometry [79]. *PTENP1* pseudogene has been reported to regulate levels of its cognate gene, *PTEN*, by competing for shared miRNAs [79]. Both miR-19b and miR-20a (normally overexpressed in PCa) suppressed both *PTEN* and *PTENP1* mRNA abundance. Blocking miR-17 and miR-19 family increased *PTEN/PTENP1* levels, highlighting a shared miRNA-mediated regulation between these two genes and highlights the role of *PTENP1* as a tumor suppressor acting as a decoy for oncogenic miRNA-targeting of PTEN [79]. Additionally, *KRAS/KRAS1P* transcript levels are positively correlated in PCa and *KRAS1P* 3′UTR overexpression in DU145 cells resulted in increased *KRAS* mRNA abundance and cell growth. These data support a role for *KRAS1P* in PCa, being targeted by *KRAS*-targeting miRNAs. *In silico* analysis revealed that *KRAS1P* maintains the validated binding sites for miR-143 and let-7 family previously reported for *KRAS* [44]. These data provide a framework of pseudogenes as natural miRNA decoys in PCa development.

3.2.6 Transcribed ultraconserved region

Ultraconserved regions (UCR) are genomic sequences with 100 % conservation between human and rodent genomes, more than 200 base pairs in length but not harboring any known gene [179]. Due to the high levels of sequence conservation, UCR must have biological functions essential to mammalian cells, although still largely enigmatic. Some UCR have been functionally implicated in transcriptional enhancement, alternative splicing, nonsense mediated decay mechanisms, or miRNA-binding decoys [179]. There are 481 UCRs described, some of which overlap with coding exons, although it is believed that more than half of them do not encode any protein. Surprisingly, 68 % of UCRs (*i.e.*, 325) are transcribed, defining a new class of long non-coding RNA: transcribed ultraconserved region (T-UCRs) [180]. Many transcripts from T-UCRs are polyadenylated and enriched for H3K4me3 at the TSS [181]. Although UCRs range from 200 to 779 bp in length, the transcriptional units of T-UCRs (the non-spliced,

full-length cDNAs) are usually up to 2 kb for known T-UCRs [180, 182]. T-UCRs are expressed in normal tissues both ubiquitously or in a tissue-specific pattern.

The expression profile of the 481 known UCR revealed that particular T-UCRs are deregulated in PCa, including uc.106+, uc.477+, uc.363 + A, uc.454 + A, associating with cancer progression, Gleason score, and extraprostatic extension [179]. Modulation with the epigenetic drugs TSA and 5-AzaC increase uc.283 + A expression while treatment with R1881 increased the expression of uc.287+ and repressed uc.283 + A expression, indicating that both epigenetic factors and androgens are responsible for regulation of T-UCRs. Genome-wide expression analysis of LNCaP cells treated with a specific siRNA against uc.106+ or sicontrol indicated that uc.106+ might impair cellular transcription of genes involved in cell proliferation and cell death, as well as immune response. Although the experimental concept of this work [179] was not the most clear, it showed, for the first time, differential expression of T-UCR in prostate tissue samples.

The SNP rs8004379 in the UCR uc.368 is significantly associated with BCR [183]. Interestingly, the variant allele, C, for rs8004379 indicates a decreased risk of BCR in a dose-dependent manner after adjusting for age, PSA level, pathologic Gleason score, and stage [183]. RNA secondary structure prediction reveals that rs8004379 has a marked effect on uc.368 RNA structure, with a slight reduction in the free energy of the C allele compared to the A allele. Moreover, this SNP is located in the intron of *NPAS3* gene, and C allele in rs8004379 is correlated with increased *NPAS3* expression [183].

More detailed investigation is needed to establish a role for T-UCR in PCa.

4 Clinical utility of ncRNA in PCa management

4.1 Diagnostic and prognostic biomarkers

The emergence of regulatory RNA offers several putative benefits due to its tissue- and cancer-specific expression and involvement in the regulation of PCa hallmarks (Fig. 6). Serum PSA is currently in widespread clinical use, increasing prostate cancer early detection. However, its lack of specificity results in high negative biopsy rate, overdiagnosis, and overtreatment of PCa [184]. NcRNAs may, thus, provide new biomarkes to accurately diagnose PCa, improve disease management, and reduce overtreatment. Given that sncRNAs are resistant to variations in temperature and pH as well as to endogenous RNase activity, they offer unprecedented potential to become blood/urine-based biomarkers [185]. Serum samples from men with low-risk, localized PCa, and metastatic CRPC have been shown to exhibit distinct circulating miRNA signatures [186]. Indeed, miR-21 [187], miR-141 [185, 186], and miR-375 [186] expression levels are increased in the plasma/sera and discriminate patients with advanced PCa from healthy controls, associating with poor prognosis. Moreover, miR-21 serum levels are particularly elevated in patients resistant to docetaxel-based chemotherapy [187]. In two independent cohorts, promoter hypermethylation of GABRE~miR-452~miR-224 predicted biochemical recurrence after radical prostatectomy [188]. Moreover, GABRE~miR-452~miR-224 methylation levels also accurately distinguished non-malignant from PCa samples (AUC: 0.98), suggesting that this locus might be suitable for urine-based PCa detection. Not only GABRE~miR-452~miR-224 has biomarker potential, but also re-expression

Fig. 6 lncRNAs as master regulators of PCa phenotype. Alterations in genomic sequence and/or expression levels in PCa cells led to initial identification of PCa-associated lncRNAs. Subsequent functional studies directly connected some of the identified lncRNAs with prostate carcinogenesis. Those not only control some of the hallmarks of cancer but also contribute to androgen-independent growth, transcriptional regulation and may be of value for clinical management of PCa patients
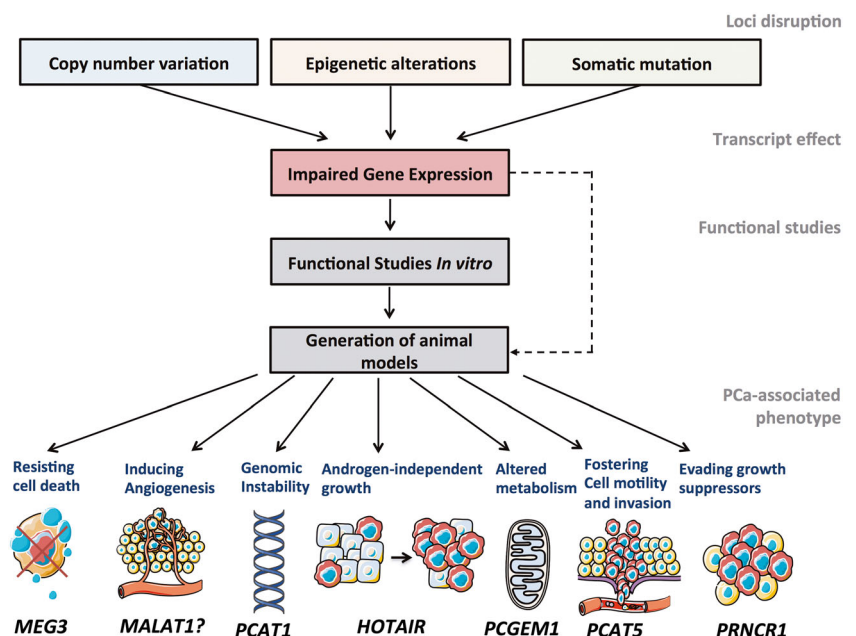
of miR-224 and miR-452 impaired cell viability, migration, and invasion capabilities [188].

The lncRNA *PCA3* is markedly overexpressed in more than 95 % of primary PCa [189]. Due to its PCa specificity, urinary detection of *PCA3* has been developed as a PCa detection test with superior tumor specificity compared to PSA [184]. FDA approved this test for clinical use under the name of Progensa *PCA3* with the ultimate goal of aiding in the decision of repeat prostate biopsy. However, correlations between *PCA3* expression and clinical and pathological parameters are conflicting, although some studies reported that *PCA3* test is negative in men with indolent PCa [190]. To improve its performance as a prognostic biomarker, *PCA3* was combined with other de-regulated genes, such as *TMPRSS2-ERG*. In two independent prospective, multicentric, evaluations the panel composed of *PCA3* and *TMPRSS2-ERG* showed superior PCa specificity over serum PSA. This finding might help reduce the number of excessive prostate biopsies [191] and could also have utility for risk stratification in an active surveillance setting [192].

ncRNAs may also be detected in exosomes secreted into blood stream or urine. Exosomes are membranous vesicles containing various biomolecules, including lncRNAs, involved in cellular communication and are secreted from many cells, including cancer cells. Combining sncRNA-sequencing and qPCR validation in exosomes derived from CRPC patients, increased expression of miR-1290 and miR-375 was found in exosomes and associated with decreased overall survival in CRPC patients [193]. A multivariate model that included miR-1290 and miR-375 levels, ADT failure time, and PSA levels at the time of CRPC stage, concluded that patients with a high risk score had a 2.58-fold higher risk of death than patients with a low-risk score (HR: 2.58; 95 % CI, 1.51–4.41) [193]. In exosomes purified from urine samples either from PCa patients or individuals with benign prostatic hyperplasia (BPH), the expression levels of lincRNA-p21 were significantly higher in PCa, discriminating from BPH [194]. The biomarker performance of lincRNA-p21, however, was disappointing (67 % sensitivity and 63 % specificity). Combination with serum PSA increased specificity to 94 %, but sensitivity decreased to 52 %. Testing in larger cohorts is needed to fully disclose the biomarker potential of exosomal ncRNAs in PCa.

4.2 ncRNAs as tools for genomic epidemiology and risk prediction

Over the last years, genome-wide association studies have become a routine tool to identify germline SNPs and cancer-associated genetic variations that map to non-coding coordinates [195]. The vast majority of those SNPs are located within enhancers, but others are localized within ncRNA-gene body [196]. Although PCa risk-related loci were enriched in lncRNAs, the SNP density in regions of lncRNA was similar

to that of protein-coding regions [197]. The 8q24 region has been identified as the most important susceptibility region for PCa [198]. This 1.2 Mb stretch of the genome is enriched for lncRNAs, including *PCAT1*, *PRNCR1*, and *PVT1* and it also harbors the *c-MYC* gene. The eight SNPs detected at 8q24 account for approximately 8 % of the 2-fold increased risk of PCa in first-degree relatives of men with the disease [198]. The link between 8q24 SNPs and PCa risk is, however, not clear, although the proximity to *c-MYC* oncogene suggests that these SNPs might be involved in long-range control of *MYC* expression, notwithstanding the lack of experimental data to support this speculation [198].

Mapping of DNase I hypersensitive sites identified a variant called rs378854, which is in complete linkage disequilibrium with rs620861, as a novel functional PCa-specific genetic variant [199]. *In vitro*, the risk allele (G) of rs378854 reduces binding of the transcription factor YY1 (a putative tumor-suppressor in PCa). Chromatin conformation capture experiments depicted that the region surrounding rs378854 interacts with *MYC* and *PVT1* promoters. Moreover, expression of the *PVT1* oncogene in normal prostate tissue increased with the presence of the risk allele of rs378854, whereas expression of *MYC* was not affected [199].

Collectively, clinical use of some SNPs may help to identify patients at risk for PCa and may stratify patient phenotypes (such as clinically aggressive *vs.* indolent) and outcome. The use of specific SNPs may also be useful to predict patients' response to therapy.

5 Discussion and conclusions

RNA is not only functional as a messenger between DNA and protein but it is also involved in the regulation of genome organization and gene expression, which is extremely elaborated in complex organisms. Among the challenges in the coming years, depiction of the crosstalk between different types of structural RNAs as well as the hierarchy of RNA- and protein-mediated regulation of gene expression that contribute to PCa are capital. Additionally, characterization of the mechanisms mediating RNA communication between PCa cells and mapping the genomic locations of RNA-binding sites [66] are mandatory to further understand the how gene expression control and cell state decisions are accomplished in PCa. Will ncRNA help on achieving a better definition of PCa as single pathological entity or ncRNA profiling may render a subclassifcation of PCa?

Cellular RNAs contain more than a hundred structurally distinct post-transcriptional modifications at different sites [200]. These RNA modifications may play an adaptive role that can fine-tune the structures and functions of mature RNAs to influence gene expression [200]. Some post-transcriptional RNA modifications can be dynamic and might have regulatory roles

equivalent to those of post-translational protein modifications. Therefore, *RNA epigenetics* will help determine both mechanisms and functions of these dynamic RNA modifications and ultimately define the "prostate cancer epitranscriptome."

Genome editing using CRISPR approaches will offer the capability to dissect ncRNAs functions. Moreover, it will provide the ability to directly modify or correct critical PCa-associated alterations by targeting a genomic locus with an engineered guide RNA, offering new therapeutic options for PCa.

During prostate epithelial transformation, AR cistrome undergoes extensive reprogramming. Accordingly, androgen-induced eRNA scaffolds AR-associated protein complexes that modulate chromosomal architecture, suggesting that eRNAs are the most critical RNAs involved in PCa.

Translating the developments in RNA biology and technology updates into deeper understanding of prostate carcinogenesis may assist in the advance of precision medicine, providing not only new and more robust biomarkers (either single or panel ncRNA) but also paving the way for patient-tailored RNA-based therapies, as an alternative to currently available therapeutic strategies. The age of RNA has come.

References

1. Ling, H., Vincent, K., Pichler, M., Fodde, R., Berindan-Neagoe, I., Slack, F. J., et al. (2015). Junk DNA and the long non-coding RNA twist in cancer genetics. *Oncogene*. doi:10.1038/onc.2014.456.

2. Rinn, J. L., & Chang, H. Y. (2012). Genome regulation by long noncoding RNAs. *Annual Review of Biochemistry, 81*, 145–166. doi:10.1146/annurev-biochem-051410-092902.

3. Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., & Zamecnik, P. C. (1958). A soluble ribonucleic acid intermediate in protein synthesis. *Journal of Biological Chemistry, 231*(1), 241–257.

4. Palade, G. E. (1955). A small particulate component of the cytoplasm. *Journal of Biophysical and Biochemical Cytology, 1*(1), 59–68.

5. Consortium, E P, Bernstein, B. E., Birney, E., Dunham, I., Green, E. D., Gunter, C., et al. (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature, 489*(7414), 57–74. doi:10.1038/nature11247.

6. Ma, W., Ay, F., Lee, C., Gulsoy, G., Deng, X., Cook, S., et al. (2015). Fine-scale chromatin interaction maps reveal the cis-regulatory landscape of human lincRNA genes. *Nature Methods, 12*(1), 71–78. doi:10.1038/nmeth.3205.

7. Kapranov, P., Cheng, J., Dike, S., Nix, D. A., Duttagupta, R., Willingham, A. T., et al. (2007). RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science, 316*(5830), 1484–1488. doi:10.1126/science.1138341.

8. Nord, A. S., Blow, M. J., Attanasio, C., Akiyama, J. A., Holt, A., Hosseini, R., et al. (2013). Rapid and pervasive changes in genome-wide enhancer usage during mammalian development. *Cell, 155*(7), 1521–1531. doi:10.1016/j.cell.2013.11.033.

9. Flynn, R. A., & Chang, H. Y. (2012). Active chromatin and noncoding RNAs: an intimate relationship. *Current Opinion in Genetics and Development, 22*(2), 172–178. doi:10.1016/j.gde.2011.11.002.

10. Rinn, J., & Guttman, M. (2014). RNA Function. RNA and dynamic nuclear organization. *Science, 345*(6202), 1240–1241. doi:10.1126/science.1252966.

11. Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., et al. (2012). Landscape of transcription in human cells. *Nature, 489*(7414), 101–108. doi:10.1038/nature11233.

12. Mattick, J. S., & Rinn, J. L. (2015). Discovery and annotation of long noncoding RNAs. *Nature Structural & Molecular Biology, 22*(1), 5–7. doi:10.1038/nsmb.2942.

13. Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., et al. (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Research, 22*(9), 1775–1789. doi:10.1101/gr.132159.111.

14. Le Thomas, A., Rogers, A. K., Webster, A., Marinov, G. K., Liao, S. E., Perkins, E. M., et al. (2013). Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state. *Genes and Development, 27*(4), 390–399. doi:10.1101/gad.209841.112.

15. Fang, W., & Bartel, D. P. (2015). The menu of features that define primary MicroRNAs and enable de novo design of MicroRNA genes. *Molecular Cell, 60*(1), 131–145. doi:10.1016/j.molcel.2015.08.015.

16. Mercer, T. R., & Mattick, J. S. (2013). Structure and function of long noncoding RNAs in epigenetic regulation. *Nature Structural & Molecular Biology, 20*(3), 300–307. doi:10.1038/nsmb.2480.

17. Raj, A., & van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell, 135*(2), 216–226. doi:10.1016/j.cell.2008.09.050.

18. Graur, D., Zheng, Y., Price, N., Azevedo, R. B., Zufall, R. A., & Elhaik, E. (2013). On the immortality of television sets: "function" in the human genome according to the evolution-free gospel of ENCODE. *Genome Biology and Evolution, 5*(3), 578–590. doi:10.1093/gbe/evt028.

19. Ulitsky, I., & Bartel, D. P. (2013). lincRNAs: genomics, evolution, and mechanisms. *Cell, 154*(1), 26–46. doi:10.1016/j.cell.2013.06.020.

20. Hezroni, H., Koppstein, D., Schwartz, M. G., Avrutin, A., Bartel, D. P., & Ulitsky, I. (2015). Principles of long noncoding RNA evolution derived from direct comparison of transcriptomes in 17 species. *Cell Reports, 11*(7), 1110–1122. doi:10.1016/j.celrep.2015.04.023.

21. Qureshi, I. A., & Mehler, M. F. (2012). Emerging roles of noncoding RNAs in brain evolution, development, plasticity and disease. *Nature Review Neuroscience, 13*(8), 528–541. doi:10.1038/nrn3234.

22. Gupta, R. A., Shah, N., Wang, K. C., Kim, J., Horlings, H. M., Wong, D. J., et al. (2010). Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature, 464*(7291), 1071–1076. doi:10.1038/nature08975.

23. Guil, S., Soler, M., Portela, A., Carrere, J., Fonalleras, E., Gomez, A., et al. (2012). Intronic RNAs mediate EZH2 regulation of epigenetic targets. *Nature Structural & Molecular Biology, 19*(7), 664–670. doi:10.1038/nsmb.2315.

24. Boque-Sastre, R., Soler, M., Oliveira-Mateos, C., Portela, A., Moutinho, C., Sayols, S., et al. (2015). Head-to-head antisense transcription and R-loop formation promotes transcriptional activation. *Proceedings of the National Academy of Sciences of the United States of America, 112*(18), 5785–5790. doi:10.1073/pnas.1421197112.

25. Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., et al. (2002). Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America, 99*(24), 15524–15529. doi:10.1073/pnas.242606799.

26. Fu, A., Jacobs, D. I., Hoffman, A. E., Zheng, T., & Zhu, Y. (2015). PIWI-interacting RNA 021285 is involved in breast tumorigenesis possibly by remodeling the cancer epigenome. *Carcinogenesis, 36*(10), 1094–1102. doi:10.1093/carcin/bgv105.

27. Bolton, E. M., Tuzova, A. V., Walsh, A. L., Lynch, T., & Perry, A. S. (2014). Noncoding RNAs in prostate cancer: the long and the short of it. *Clinical Cancer Research, 20*(1), 35–43. doi:10.1158/1078-0432.CCR-13-1989.

28. Steitz, J. A., & Tycowski, K. T. (1995). Small RNA chaperones for ribosome biogenesis. *Science, 270*(5242), 1626–1627.

29. Kelly, B. D., Miller, N., Sweeney, K. J., Durkan, G. C., Rogers, E., Walsh, K., et al. (2015). A circulating MicroRNA signature as a biomarker for prostate cancer in a high risk group. *Journal of Clinical Medicine, 4*(7), 1369–1379. doi:10.3390/jcm4071369.

30. Prensner, J. R., & Chinnaiyan, A. M. (2011). The emergence of lncRNAs in cancer biology. *Cancer Discovery, 1*(5), 391–407. doi:10.1158/2159-8290.CD-11-0209.

31. Affymetrix, E. T. P., & Cold Spring Harbor Laboratory, E. T. P. (2009). Post-transcriptional processing generates a diversity of 5'-modified long and short RNAs. *Nature, 457*(7232), 1028–1032. doi:10.1038/nature07759.

32. Ghildiyal, M., & Zamore, P. D. (2009). Small silencing RNAs: an expanding universe. *Nature Reviews Genetics, 10*(2), 94–108. doi:10.1038/nrg2504.

33. Nguyen, T. A., Jo, M. H., Choi, Y. G., Park, J., Kwon, S. C., Hohng, S., et al. (2015). Functional anatomy of the human microprocessor. *Cell, 161*(6), 1374–1387. doi:10.1016/j.cell.2015.05.010.

34. Wilson, R. C., Tambe, A., Kidwell, M. A., Noland, C. L., Schneider, C. P., & Doudna, J. A. (2015). Dicer-TRBP complex formation ensures accurate mammalian microRNA biogenesis. *Molecular Cell, 57*(3), 397–407. doi:10.1016/j.molcel.2014.11.030.

35. Ha, M., & Kim, V. N. (2014). Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology, 15*(8), 509–524. doi:10.1038/nrm3838.

36. Fromm, B., Billipp, T., Peck, L. E., Johansen, M., Tarver, J. E., King, B. L., et al. (2015). A uniform system for the annotation of vertebrate microRNA genes and the evolution of the human microRNAome. *Annual Review of Genetics, 49*, 213–242. doi:10.1146/annurev-genet-120213-092023.

37. Yang, J. S., & Lai, E. C. (2011). Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. *Molecular Cell, 43*(6), 892–903. doi:10.1016/j.molcel.2011.07.024.

38. Cheloufi, S., Dos Santos, C. O., Chong, M. M., & Hannon, G. J. (2010). A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature, 465*(7298), 584–589. doi:10.1038/nature09092.

39. Schirle, N. T., Sheu-Gruttadauria, J., & MacRae, I. J. (2014). Structural basis for microRNA targeting. *Science, 346*(6209), 608–613. doi:10.1126/science.1258040.

40. Hafner, M., Landthaler, M., Burger, L., Khorshid, M., Hausser, J., Berninger, P., et al. (2010). Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell, 141*(1), 129–141. doi:10.1016/j.cell.2010.03.009.

41. Baek, D., Villen, J., Shin, C., Camargo, F. D., Gygi, S. P., & Bartel, D. P. (2008). The impact of microRNAs on protein output. *Nature, 455*(7209), 64–71. doi:10.1038/nature07242.

42. Hausser, J., & Zavolan, M. (2014). Identification and consequences of miRNA-target interactions—beyond repression of gene expression. *Nature Reviews Genetics, 15*(9), 599–612. doi:10.1038/nrg3765.

43. Wyman, S. K., Knouf, E. C., Parkin, R. K., Fritz, B. R., Lin, D. W., Dennis, L. M., et al. (2011). Post-transcriptional generation of miRNA variants by multiple nucleotidyl transferases contributes to miRNA transcriptome complexity. *Genome Research, 21*(9), 1450–1461. doi:10.1101/gr.118059.110.

44. Koppers-Lalic, D., Hackenberg, M., Bijnsdorp, I. V., van Eijndhoven, M. A., Sadek, P., Sie, D., et al. (2014). Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Reports, 8*(6), 1649–1658. doi:10.1016/j.celrep.2014.08.027.

45. Chiang, H. R., Schoenfeld, L. W., Ruby, J. G., Auyeung, V. C., Spies, N., Baek, D., et al. (2010). Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes and Development, 24*(10), 992–1009. doi:10.1101/gad.1884710.

46. Berezikov, E., van Tetering, G., Verheul, M., van de Belt, J., van Laake, L., Vos, J., et al. (2006). Many novel mammalian microRNA candidates identified by extensive cloning and RAKE analysis. *Genome Research, 16*(10), 1289–1298. doi:10.1101/gr.5159906.

47. Kim, V. N., Han, J., & Siomi, M. C. (2009). Biogenesis of small RNAs in animals. *Nature Reviews Molecular Cell Biology, 10*(2), 126–139. doi:10.1038/nrm2632.

48. Ghildiyal, M., Seitz, H., Horwich, M. D., Li, C., Du, T., Lee, S., et al. (2008). Endogenous siRNAs derived from transposons and mRNAs in Drosophila somatic cells. *Science, 320*(5879), 1077–1081. doi:10.1126/science.1157396.

49. Okamura, K., Chung, W. J., Ruby, J. G., Guo, H., Bartel, D. P., & Lai, E. C. (2008). The Drosophila hairpin RNA pathway generates endogenous short interfering RNAs. *Nature, 453*(7196), 803–806. doi:10.1038/nature07015.

50. Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D., & Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes and Development, 12*(23), 3715–3727.

51. Peng, J. C., & Lin, H. (2013). Beyond transposons: the epigenetic and somatic functions of the Piwi-piRNA mechanism. *Current Opinion in Cell Biology, 25*(2), 190–194. doi:10.1016/j.ceb.2013.01.010.

52. Grimson, A., Srivastava, M., Fahey, B., Woodcroft, B. J., Chiang, H. R., King, N., et al. (2008). Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. *Nature, 455*(7217), 1193–1197. doi:10.1038/nature07415.

53. Kim, V. N. (2006). Small RNAs just got bigger: Piwi-interacting RNAs (piRNAs) in mammalian testes. *Genes and Development, 20*(15), 1993–1997. doi:10.1101/gad.1456106.

54. Martinez, V. D., Vucic, E. A., Thu, K. L., Hubaux, R., Enfield, K. S., Pikor, L. A., et al. (2015). Unique somatic and malignant expression patterns implicate PIWI-interacting RNAs in cancer-type specific biology. *Science Reports, 5*, 10423. doi:10.1038/srep10423.

55. Ferreira, H. J., Heyn, H., Garcia del Muro, X., Vidal, A., Larriba, S., Munoz, C., et al. (2014). Epigenetic loss of the PIWI/piRNA machinery in human testicular tumorigenesis. *Epigenetics, 9*(1), 113–118. doi:10.4161/epi.27237.

56. Tycowski, K. T., Shu, M. D., & Steitz, J. A. (1996). A mammalian gene with introns instead of exons generating stable RNA products. *Nature, 379*(6564), 464–466. doi:10.1038/379464a0.

57. Williams, G. T., & Farzaneh, F. (2012). Are snoRNAs and snoRNA host genes new players in cancer? *Nature Reviews Cancer, 12*(2), 84–88. doi:10.1038/nrc3195.

58. Kiss-Laszlo, Z., Henry, Y., Bachellerie, J. P., Caizergues-Ferrer, M., & Kiss, T. (1996). Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell, 85*(7), 1077–1088.

59. Decatur, W. A., & Fournier, M. J. (2002). rRNA modifications and ribosome function. *Trends in Biochemical Sciences, 27*(7), 344–351.

60. Weinstein, L. B., & Steitz, J. A. (1999). Guided tours: from pre-cursor snoRNA to functional snoRNP. *Current Opinion in Cell Biology, 11*(3), 378–384. doi:10.1016/S0955-0674(99)80053-2.

61. Ni, J., Tien, A. L., & Fournier, M. J. (1997). Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA. *Cell, 89*(4), 565–573.

62. Darzacq, X., Jady, B. E., Verheggen, C., Kiss, A. M., Bertrand, E., & Kiss, T. (2002). Cajal body-specific small nuclear RNAs: a novel class of 2'-O-methylation and pseudouridylation guide RNAs. *EMBO Journal, 21*(11), 2746–2756. doi:10.1093/emboj/21.11.2746.

63. Ono, M., Scott, M. S., Yamada, K., Avolio, F., Barton, G. J., & Lamond, A. I. (2011). Identification of human miRNA precursors that resemble box C/D snoRNAs. *Nucleic Acids Research, 39*(9), 3879–3891. doi:10.1093/nar/gkq1355.

64. Brameier, M., Herwig, A., Reinhardt, R., Walter, L., & Gruber, J. (2011). Human box C/D snoRNAs with miRNA like functions: expanding the range of regulatory RNAs. *Nucleic Acids Research, 39*(2), 675–686. doi:10.1093/nar/gkq776.

65. Su, H., Xu, T., Ganapathy, S., Shadfan, M., Long, M., Huang, T. H., et al. (2014). Elevated snoRNA biogenesis is essential in breast cancer. *Oncogene, 33*(11), 1348–1358. doi:10.1038/onc.2013.89.

66. Morris, K. V., & Mattick, J. S. (2014). The rise of regulatory RNA. *Nature Reviews Genetics, 15*(6), 423–437. doi:10.1038/nrg3722.

67. Goodarzi, H., Liu, X., Nguyen, H. C., Zhang, S., Fish, L., & Tavazoie, S. F. (2015). Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement. *Cell, 161*(4), 790–802. doi:10.1016/j.cell.2015.02.053.

68. Gebetsberger, J., & Polacek, N. (2013). Slicing tRNAs to boost functional ncRNA diversity. *RNA Biology, 10*(12), 1798–1806. doi:10.4161/rna.27177.

69. Taft, R. J., Hawkins, P. G., Mattick, J. S., & Morris, K. V. (2011). The relationship between transcription initiation RNAs and CCCTC-binding factor (CTCF) localization. *Epigenetics & Chromatin, 4*, 13. doi:10.1186/1756-8935-4-13.

70. Kowalczyk, M. S., Higgs, D. R., & Gingeras, T. R. (2012). Molecular biology: RNA discrimination. *Nature, 482*(7385), 310–311. doi:10.1038/482310a.

71. Zhao, Z., Dammert, M. A., Grummt, I., & Bierhoff, H. (2016). lncRNA-induced nucleosome repositioning reinforces transcriptional repression of rRNA genes upon hypotonic stress. *Cell Reports, 14*(8), 1876–1882. doi:10.1016/j.celrep.2016.01.073.

72. Wang, D., Garcia-Bassets, I., Benner, C., Li, W., Su, X., Zhou, Y., et al. (2011). Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature, 474*(7351), 390–394. doi:10.1038/nature10006.

73. Wang, K. C., Yang, Y. W., Liu, B., Sanyal, A., Corces-Zimmerman, R., Chen, Y., et al. (2011). A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature, 472*(7341), 120–124. doi:10.1038/nature09819.

74. Nagano, T., Mitchell, J. A., Sanz, L. A., Pauler, F. M., Ferguson-Smith, A. C., Feil, R., et al. (2008). The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science, 322*(5908), 1717–1720. doi:10.1126/science.1163802.

75. Maruyama, A., Mimura, J., & Itoh, K. (2014). Non-coding RNA derived from the region adjacent to the human HO-1 E2 enhancer selectively regulates HO-1 gene induction by modulating Pol II binding. *Nucleic Acids Research, 42*(22), 13599–13614. doi:10.1093/nar/gku1169.

76. Li, W., Notani, D., Ma, Q., Tanasa, B., Nunez, E., Chen, A. Y., et al. (2013). Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature, 498*(7455), 516–520. doi:10.1038/nature12210.

77. Faghihi, M. A., Modarresi, F., Khalil, A. M., Wood, D. E., Sahagan, B. G., Morgan, T. E., et al. (2008). Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. *Nature Medicine, 14*(7), 723–730. doi:10.1038/nm1784.

78. Yoon, J. H., Abdelmohsen, K., Srikantan, S., Yang, X., Martindale, J. L., De, S., et al. (2012). LincRNA-p21 suppresses target mRNA translation. *Molecular Cell, 47*(4), 648–655. doi:10.1016/j.molcel.2012.06.027.

79. Poliseno, L., Salmena, L., Zhang, J., Carver, B., Haveman, W. J., & Pandolfi, P. P. (2010). A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature, 465*(7301), 1033–1038. doi:10.1038/nature09144.

80. Hansen, T. B., Jensen, T. I., Clausen, B. H., Bramsen, J. B., Finsen, B., Damgaard, C. K., et al. (2013). Natural RNA circles function as efficient microRNA sponges. *Nature, 495*(7441), 384–388. doi:10.1038/nature11993.

81. Hansen, T. B., Wiklund, E. D., Bramsen, J. B., Villadsen, S. B., Statham, A. L., Clark, S. J., et al. (2011). miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular anti-sense RNA. *EMBO Journal, 30*(21), 4414–4422. doi:10.1038/emboj.2011.359.

82. Fatica, A., & Bozzoni, I. (2014). Long non-coding RNAs: new players in cell differentiation and development. *Nature Reviews Genetics, 15*(1), 7–21. doi:10.1038/nrg3606.

83. Willingham, A. T., Orth, A. P., Batalov, S., Peters, E. C., Wen, B. G., Aza-Blanc, P., et al. (2005). A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. *Science, 309*(5740), 1570–1573. doi:10.1126/science.1115901.

84. Montes, M., Nielsen, M. M., Maglieri, G., Jacobsen, A., Hojfeldt, J., Agrawal-Singh, S., et al. (2015). The lncRNA MIR31HG regulates p16(INK4A) expression to modulate senescence. *Nature Communications, 6*, 6967. doi:10.1038/ncomms7967.

85. Pelechano, V., & Steinmetz, L. M. (2013). Gene regulation by antisense transcription. *Nature Reviews Genetics, 14*(12), 880–893. doi:10.1038/nrg3594.

86. Balbin, O. A., Malik, R., Dhanasekaran, S. M., Prensner, J. R., Cao, X., Wu, Y. M., et al. (2015). The landscape of antisense gene expression in human cancers. *Genome Research, 25*(7), 1068–1079. doi:10.1101/gr.180596.114.

87. Lam, M. T., Li, W., Rosenfeld, M. G., & Glass, C. K. (2014). Enhancer RNAs and regulated transcriptional programs. *Trends in Biochemical Sciences, 39*(4), 170–182. doi:10.1016/j.tibs.2014.02.007.

88. Heinz, S., Romanoski, C. E., Benner, C., & Glass, C. K. (2015). The selection and function of cell type-specific enhancers. *Nature Reviews Molecular Cell Biology, 16*(3), 144–154. doi:10.1038/nrm3949.

89. Hsieh, C. L., Fei, T., Chen, Y., Li, T., Gao, Y., Wang, X., et al. (2014). Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proceedings of the National Academy of Sciences of the United States of America, 111*(20), 7319–7324. doi:10.1073/pnas.1324151111.

90. Korkmaz, G., Lopes, R., Ugalde, A. P., Nevedomskaya, E., Han, R., Myacheva, K., et al. (2016). Functional genetic screens for enhancer elements in the human genome using CRISPR-Cas9. *Nature Biotechnology, 34*(2), 192–198. doi:10.1038/nbt.3450.

91. Kalyana-Sundaram, S., Kumar-Sinha, C., Shankar, S., Robinson, D. R., Wu, Y. M., Cao, X., et al. (2012). Expressed pseudogenes in the transcriptional landscape of human cancers. *Cell, 149*(7), 1622–1634. doi:10.1016/j.cell.2012.04.041.

92. Pei, B., Sisu, C., Frankish, A., Howald, C., Habegger, L., Mu, X. J., et al. (2012). The GENCODE pseudogene resource. *Genome Biology, 13*(9), R51. doi:10.1186/gb-2012-13-9-r51.

93. Han, Y. J., Ma, S. F., Yourek, G., Park, Y. D., & Garcia, J. G. (2011). A transcribed pseudogene of MYLK promotes cell proliferation. *FASEB Journal, 25*(7), 2305–2312. doi:10.1096/fj.10-177808.

94. Watanabe, T., Totoki, Y., Toyoda, A., Kaneda, M., Kuramochi-Miyagawa, S., Obata, Y., et al. (2008). Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. *Nature, 453*(7194), 539–543. doi:10.1038/nature06908.

95. Hawkins, P. G., & Morris, K. V. (2010). Transcriptional regulation of Oct4 by a long non-coding RNA antisense to Oct4-pseudogene 5. *Transcription, 1*(3), 165–175. doi:10.4161/trns.1.3.13332.

96. Siegel, R., Naishadham, D., & Jemal, A. (2013). Cancer statistics, 2013. *CA: A Cancer Journal for Clinicians, 63*(1), 11–30. doi:10.3322/caac.21166.

97. Bill-Axelson, A., Holmberg, L., Garmo, H., Rider, J. R., Taari, K., Busch, C., et al. (2014). Radical prostatectomy or watchful waiting in early prostate cancer. *New England Journal of Medicine, 370*(10), 932–942. doi:10.1056/NEJMoa1311593.

98. Tomlins, S. A., Laxman, B., Dhanasekaran, S. M., Helgeson, B. E., Cao, X., Morris, D. S., et al. (2007). Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature, 448*(7153), 595–599. doi:10.1038/nature06024.

99. Abd Elmageed, Z. Y., Yang, Y., Thomas, R., Ranjan, M., Mondal, D., Moroz, K., et al. (2014). Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. *Stem Cells, 32*(4), 983–997. doi:10.1002/stem.1619.

100. Hudson, R. S., Yi, M., Esposito, D., Watkins, S. K., Hurwitz, A. A., Yfantis, H. G., et al. (2012). MicroRNA-1 is a candidate tumor suppressor and prognostic marker in human prostate cancer. *Nucleic Acids Research, 40*(8), 3689–3703. doi:10.1093/nar/gkr1222.

101. Lin, P. C., Chiu, Y. L., Banerjee, S., Park, K., Mosquera, J. M., Giannopoulou, E., et al. (2013). Epigenetic repression of miR-31 disrupts androgen receptor homeostasis and contributes to prostate cancer progression. *Cancer Research, 73*(3), 1232–1244. doi:10.1158/0008-5472.CAN-12-2968.

102. Benassi, B., Flavin, R., Marchionni, L., Zanata, S., Pan, Y., Chowdhury, D., et al. (2012). MYC is activated by USP2a-mediated modulation of microRNAs in prostate cancer. *Cancer Discovery, 2*(3), 236–247. doi:10.1158/2159-8290.CD-11-0219.

103. Majid, S., Dar, A. A., Saini, S., Shahryari, V., Arora, S., Zaman, M. S., et al. (2013). miRNA-34b inhibits prostate cancer through demethylation, active chromatin modifications, and AKT pathways. *Clinical Cancer Research, 19*(1), 73–84. doi:10.1158/1078-0432.CCR-12-2952.

104. Suh, S. O., Chen, Y., Zaman, M. S., Hirata, H., Yamamura, S., Shahryari, V., et al. (2011). MicroRNA-145 is regulated by DNA methylation and p53 gene mutation in prostate cancer. *Carcinogenesis, 32*(5), 772–778. doi:10.1093/carcin/bgr036.

105. Hart, M., Wach, S., Nolte, E., Szczyrba, J., Menon, R., Taubert, H., et al. (2013). The proto-oncogene ERG is a target of microRNA miR-145 in prostate cancer. *FEBS Journal, 280*(9), 2105–2116. doi:10.1111/febs.12236.

106. Larne, O., Hagman, Z., Lilja, H., Bjartell, A., Edsjo, A., & Ceder, Y. (2015). miR-145 suppress the androgen receptor in prostate cancer cells and correlates to prostate cancer prognosis. *Carcinogenesis, 36*(8), 858–866. doi:10.1093/carcin/bgv063.

107. Wang, C., Tao, W., Ni, S., Chen, Q., Zhao, Z., Ma, L., et al. (2015). Tumor-suppressive microRNA-145 induces growth arrest by targeting SENP1 in human prostate cancer cells. *Cancer Science, 106*(4), 375–382. doi:10.1111/cas.12626.

108. Kaukoniemi, K. M., Rauhala, H. E., Scaravilli, M., Latonen, L., Annala, M., Vessella, R. L., et al. (2015). Epigenetically altered miR-193b targets cyclin D1 in prostate cancer. *Cancer Medicine, 4*(9), 1417–1425. doi:10.1002/cam4.486.

109. Rauhala, H. E., Jalava, S. E., Isotalo, J., Bracken, H., Lehmusvaara, S., Tammela, T. L., et al. (2010). miR-193b is an epigenetically regulated putative tumor suppressor in prostate cancer. *International Journal of Cancer, 127*(6), 1363–1372. doi:10.1002/ijc.25162.

110. Xie, C., Jiang, X. H., Zhang, J. T., Sun, T. T., Dong, J. D., Sanders, A. J., et al. (2013). CFTR suppresses tumor progression through miR-193b targeting urokinase plasminogen activator (uPA) in prostate cancer. *Oncogene, 32*(18), 2282–2291, 2291 e2281-2287, doi:10.1038/onc.2012.251.

111. Saini, S., Majid, S., Yamamura, S., Tabatabai, L., Suh, S. O., Shahryari, V., et al. (2011). Regulatory role of mir-203 in prostate cancer progression and metastasis. *Clinical Cancer Research, 17*(16), 5287–5298. doi:10.1158/1078-0432.CCR-10-2619.

112. Viticchie, G., Lena, A. M., Latina, A., Formosa, A., Gregersen, L. H., Lund, A. H., et al. (2011). MiR-203 controls proliferation, migration and invasive potential of prostate cancer cell lines. *Cell Cycle, 10*(7), 1121–1131.

113. Hailer, A., Grunewald, T. G., Orth, M., Reiss, C., Kneitz, B., Spahn, M., et al. (2014). Loss of tumor suppressor mir-203 mediates overexpression of LIM and SH3 Protein 1 (LASP1) in high-risk prostate cancer thereby increasing cell proliferation and migration. *Oncotarget, 5*(12), 4144–4153. doi:10.18632/oncotarget.1928.

114. Xiang, J., Bian, C., Wang, H., Huang, S., & Wu, D. (2015). MiR-203 down-regulates Rap1A and suppresses cell proliferation, adhesion and invasion in prostate cancer. *Journal of Experimental & Clinical Cancer Research, 34*, 8. doi:10.1186/s13046-015-0125-x.

115. Siu, M. K., Abou-Kheir, W., Yin, J. J., Chang, Y. S., Barrett, B., Suau, F., et al. (2014). Loss of EGFR signaling regulated miR-203 promotes prostate cancer bone metastasis and tyrosine kinase inhibitors resistance. *Oncotarget, 5*(11), 3770–3784. doi:10.18632/oncotarget.1994.

116. Hulf, T., Sibbritt, T., Wiklund, E. D., Patterson, K., Song, J. Z., Stirzaker, C., et al. (2013). Epigenetic-induced repression of microRNA-205 is associated with MED1 activation and a poorer prognosis in localized prostate cancer. *Oncogene, 32*(23), 2891–2899. doi:10.1038/onc.2012.300.

117. Gandellini, P., Folini, M., Longoni, N., Pennati, M., Binda, M., Colecchia, M., et al. (2009). miR-205 Exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase Cepsilon. *Cancer Research, 69*(6), 2287–2295. doi:10.1158/0008-5472.CAN-08-2894.

118. Hagman, Z., Haflidadottir, B. S., Ceder, J. A., Larne, O., Bjartell, A., Lilja, H., et al. (2013). miR-205 negatively regulates the androgen receptor and is associated with adverse outcome of prostate cancer patients. *British Journal of Cancer, 108*(8), 1668–1676. doi:10.1038/bjc.2013.131.

119. Tucci, P., Agostini, M., Grespi, F., Markert, E. K., Terrinoni, A., Vousden, K. H., et al. (2012). Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America, 109*(38), 15312–15317. doi:10.1073/pnas.1110977109.

120. Lin, Z. Y., Huang, Y. Q., Zhang, Y. Q., Han, Z. D., He, H. C., Ling, X. H., et al. (2014). MicroRNA-224 inhibits progression of human prostate cancer by downregulating TRIB1. *International Journal of Cancer, 135*(3), 541–550. doi:10.1002/ijc.28707.

121. Goto, Y., Nishikawa, R., Kojima, S., Chiyomaru, T., Enokida, H., Inoguchi, S., et al. (2014). Tumour-suppressive microRNA-224 inhibits cancer cell migration and invasion via targeting oncogenic TPD52 in prostate cancer. *FEBS Letters, 588*(10), 1973–1982. doi:10.1016/j.febslet.2014.04.020.

122. Ribas, J., Ni, X., Haffner, M., Wentzel, E. A., Salmasi, A. H., Chowdhury, W. H., et al. (2009). miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. *Cancer Research, 69*(18), 7165–7169. doi:10.1158/0008-5472.CAN-09-1448.

123. Coppola, V., Musumeci, M., Patrizii, M., Cannistraci, A., Addario, A., Maugeri-Sacca, M., et al. (2013). BTG2 loss and miR-21 up-regulation contribute to prostate cell transformation by inducing luminal markers expression and epithelial-mesenchymal transition. *Oncogene, 32*(14), 1843–1853. doi:10.1038/onc.2012.194.

124. Yang, C. H., Pfeffer, S. R., Sims, M., Yue, J., Wang, Y., Linga, V. G., et al. (2015). The oncogenic microRNA-21 inhibits the tumor suppressive activity of FBXO11 to promote tumorigenesis. *Journal of Biological Chemistry, 290*(10), 6037–6046. doi:10. 1074/jbc.M114.632125.

125. Jalava, S. E., Urbanucci, A., Latonen, L., Waltering, K. K., Sahu, B., Janne, O. A., et al. (2012). Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer. *Oncogene, 31*(41), 4460–4471. doi:10.1038/onc.2011.624.

126. Liao, H., Xiao, Y., Hu, Y., Xiao, Y., Yin, Z., & Liu, L. (2015). microRNA-32 induces radioresistance by targeting DAB2IP and regulating autophagy in prostate cancer cells. *Oncology Letters, 10*(4), 2055–2062. doi:10.3892/ol.2015.3551.

127. Ma, Y., Yang, H. Z., Dong, B. J., Zou, H. B., Zhou, Y., Kong, X. M., et al. (2014). Biphasic regulation of autophagy by miR-96 in prostate cancer cells under hypoxia. *Oncotarget, 5*(19), 9169–9182. doi:10.18632/oncotarget.2396.

128. Haflidadottir, B. S., Larne, O., Martin, M., Persson, M., Edsjo, A., Bjartell, A., et al. (2013). Upregulation of miR-96 enhances cellular proliferation of prostate cancer cells through FOXO1. *PLoS ONE, 8*(8), e72400. doi:10.1371/journal.pone.0072400.

129. Siu, M. K., Tsai, Y. C., Chang, Y. S., Yin, J. J., Suau, F., Chen, W. Y., et al. (2015). Transforming growth factor-beta promotes prostate bone metastasis through induction of microRNA-96 and activation of the mTOR pathway. *Oncogene, 34*(36), 4767–4776. doi:10.1038/onc.2014.414.

130. Mihelich, B. L., Khramtsova, E. A., Arva, N., Vaishnav, A., Johnson, D. N., Giangreco, A. A., et al. (2011). miR-183-96-182 cluster is overexpressed in prostate tissue and regulates zinc homeostasis in prostate cells. *Journal of Biological Chemistry, 286*(52), 44503–44511. doi:10.1074/jbc.M111.262915.

131. Ueno, K., Hirata, H., Shahryari, V., Deng, G., Tanaka, Y., Tabatabai, Z. L., et al. (2013). microRNA-183 is an oncogene targeting Dkk-3 and SMAD4 in prostate cancer. *British Journal of Cancer, 108*(8), 1659–1667. doi:10.1038/bjc.2013.125.

132. Larne, O., Ostling, P., Haflidadottir, B. S., Hagman, Z., Aakula, A., Kohonen, P., et al. (2015). miR-183 in prostate cancer cells positively regulates synthesis and serum levels of prostate-specific antigen. *European Urology, 68*(4), 581–588. doi:10.1016/j.eururo. 2014.12.025.

133. Costa-Pinheiro, P., Ramalho-Carvalho, J., Vieira, F. Q., Torres-Ferreira, J., Oliveira, J., Goncalves, C. S., et al. (2015). MicroRNA-375 plays a dual role in prostate carcinogenesis. *Clinical Epigenetics, 7*(1), 42. doi:10.1186/s13148-015-0076-2.

134. Szczyrba, J., Nolte, E., Wach, S., Kremmer, E., Stohr, R., Hartmann, A., et al. (2011). Downregulation of Sec23A protein by miRNA-375 in prostate carcinoma. *Molecular Cancer Research, 9*(6), 791–800. doi:10.1158/1541-7786.MCR-10-0573.

135. Dong, X. Y., Rodriguez, C., Guo, P., Sun, X., Talbot, J. T., Zhou, W., et al. (2008). SnoRNA U50 is a candidate tumor-suppressor gene at 6q14.3 with a mutation associated with clinically significant prostate cancer. *Human Molecular Genetics, 17*(7), 1031–1042. doi:10.1093/hmg/ddm375.

136. Sieron, P., Hader, C., Hatina, J., Engers, R., Wlazlinski, A., Muller, M., et al. (2009). DKC1 overexpression associated with prostate cancer progression. *British Journal of Cancer, 101*(8), 1410–1416. doi:10.1038/sj.bjc.6605299.

137. Thomson, E., Ferreira-Cerca, S., & Hurt, E. (2013). Eukaryotic ribosome biogenesis at a glance. *Journal of Cell Science, 126*(Pt 21), 4815–4821. doi:10.1242/jcs.111948.

138. Martens-Uzunova, E. S., Hoogstrate, Y., Kalsbeek, A., Pigmans, B., Vredenbregt-van den Berg, M., Dits, N., et al. (2015). C/D-box snoRNA-derived RNA production is associated with malignant transformation and metastatic progression in prostate cancer. *Oncotarget, 6*(19), 17430–17444.

139. Uemura, M., Zheng, Q., Koh, C. M., Nelson, W. G., Yegnasubramanian, S., & De Marzo, A. M. (2012). Overexpression of ribosomal RNA in prostate cancer is common but not linked to rDNA promoter hypomethylation. *Oncogene, 31*(10), 1254–1263. doi:10.1038/onc.2011.319.

140. McStay, B., & Grummt, I. (2008). The epigenetics of rRNA genes: from molecular to chromosome biology. *Annual Review of Cell and Developmental Biology, 24*, 131–157. doi:10.1146/annurev. cellbio.24.110707.175259.

141. Li, S., Hu, M. G., Sun, Y., Yoshioka, N., Ibaragi, S., Sheng, J., et al. (2013). Angiogenin mediates androgen-stimulated prostate cancer growth and enables castration resistance. *Molecular Cancer Research, 11*(10), 1203–1214. doi:10.1158/1541-7786. MCR-13-0072.

142. Koh, C. M., Gurel, B., Sutcliffe, S., Aryee, M. J., Schultz, D., Iwata, T., et al. (2011). Alterations in nucleolar structure and gene expression programs in prostatic neoplasia are driven by the MYC oncogene. *American Journal of Pathology, 178*(4), 1824–1834. doi:10.1016/j.ajpath.2010.12.040.

143. Lee, Y. S., Shibata, Y., Malhotra, A., & Dutta, A. (2009). A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). *Genes and Development, 23*(22), 2639–2649. doi:10.1101/gad. 1837609.

144. Olvedy, M., Scaravilli, M., Hoogstrate, Y., Visakorpi, T., Jenster, G., & Martens-Uzunova, E. (2016). A comprehensive repertoire of tRNA-derived fragments in prostate cancer. *Oncotarget*. doi:10. 18632/oncotarget.8293.

145. Honda, S., Loher, P., Shigematsu, M., Palazzo, J. P., Suzuki, R., Imoto, I., et al. (2015). Sex hormone-dependent tRNA halves enhance cell proliferation in breast and prostate cancers. *Proceedings of the National Academy of Sciences of the United States of America, 112*(29), E3816–3825. doi:10.1073/pnas.1510077112.

146. Ivanov, P., Emara, M. M., Villen, J., Gygi, S. P., & Anderson, P. (2011). Angiogenin-induced tRNA fragments inhibit translation initiation. *Molecular Cell, 43*(4), 613–623. doi:10.1016/j.molcel. 2011.06.022.

147. Maute, R. L., Schneider, C., Sumazin, P., Holmes, A., Califano, A., Basso, K., et al. (2013). tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. *Proceedings of the National Academy of Sciences of the United States of America, 110*(4), 1404–1409. doi:10.1073/pnas.1206761110.

148. Siomi, M. C., Sato, K., Pezic, D., & Aravin, A. A. (2011). PIWI-interacting small RNAs: the vanguard of genome defence. *Nature Reviews Molecular Cell Biology, 12*(4), 246–258. doi:10.1038/ nrm3089.

149. Boormans, J. L., Korsten, H., Ziel-van der Made, A. J., van Leenders, G. J., de Vos, C. V., Jenster, G., et al. (2013). Identification of TDRD1 as a direct target gene of ERG in primary prostate cancer. *International Journal of Cancer, 133*(2), 335–345. doi:10.1002/ijc.28025.

150. Yang, Y., Zhang, X., Song, D., & Wei, J. (2015). Piwil2 modulates the invasion and metastasis of prostate cancer by regulating the expression of matrix metalloproteinase-9 and epithelial-mesenchymal transitions. *Oncology Letters, 10*(3), 1735–1740. doi:10.3892/ol.2015.3392.

151. Luo, G., Wang, M., Wu, X., Tao, D., Xiao, X., Wang, L., et al. (2015). Long non-coding RNA MEG3 inhibits cell proliferation and induces apoptosis in prostate cancer. *Cellular Physiology and Biochemistry, 37*(6), 2209–2220. doi:10.1159/000438577.

152. Malik, R., Patel, L., Prensner, J. R., Shi, Y., Iyer, M. K., Subramaniyan, S., et al. (2014). The lncRNA PCAT29 inhibits oncogenic phenotypes in prostate cancer. *Molecular Cancer Research, 12*(8), 1081–1087. doi:10.1158/1541-7786.MCR-14-0257.

153. Kawamura, N., Nimura, K., Nagano, H., Yamaguchi, S., Nonomura, N., & Kaneda, Y. (2015). CRISPR/Cas9-mediated gene knockout of NANOG and NANOGP8 decreases the malignant potential of prostate cancer cells. *Oncotarget, 6*(26), 22361–22374. doi:10.18632/oncotarget.4293.

154. Sakurai, K., Reon, B. J., Anaya, J., & Dutta, A. (2015). The lncRNA DRAIC/PCAT29 locus constitutes a tumor-suppressive nexus. *Molecular Cancer Research, 13*(5), 828–838. doi:10.1158/1541-7786.MCR-15-0016-T.

155. Pickard, M. R., Mourtada-Maarabouni, M., & Williams, G. T. (2013). Long non-coding RNA GAS5 regulates apoptosis in prostate cancer cell lines. *Biochimica et Biophysica Acta, 1832*(10), 1613–1623. doi:10.1016/j.bbadis.2013.05.005.

156. Hung, C. L., Wang, L. Y., Yu, Y. L., Chen, H. W., Srivastava, S., Petrovics, G., et al. (2014). A long noncoding RNA connects c-Myc to tumor metabolism. *Proceedings of the National Academy of Sciences of the United States of America, 111*(52), 18697–18702. doi:10.1073/pnas.1415669112.

157. Yang, L., Lin, C., Jin, C., Yang, J. C., Tanasa, B., Li, W., et al. (2013). lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. *Nature, 500*(7464), 598–602. doi:10.1038/nature12451.

158. Cui, Z., Ren, S., Lu, J., Wang, F., Xu, W., Sun, Y., et al. (2013). The prostate cancer-up-regulated long noncoding RNA PlncRNA-1 modulates apoptosis and proliferation through reciprocal regulation of androgen receptor. *Urologic Oncology, 31*(7), 1117–1123. doi:10.1016/j.urolonc.2011.11.030.

159. Zhang, A., Zhao, J. C., Kim, J., Fong, K. W., Yang, Y. A., Chakravarti, D., et al. (2015). LncRNA HOTAIR enhances the androgen-receptor-mediated transcriptional program and drives castration-resistant prostate cancer. *Cell Reports, 13*(1), 209–221. doi:10.1016/j.celrep.2015.08.069.

160. Ylipaa, A., Kivinummi, K., Kohvakka, A., Annala, M., Latonen, L., Scaravilli, M., et al. (2015). Transcriptome sequencing reveals PCAT5 as a novel ERG-regulated long noncoding RNA in prostate cancer. *Cancer Research, 75*(19), 4026–4031. doi:10.1158/0008-5472.CAN-15-0217.

161. Orfanelli, U., Jachetti, E., Chiacchiera, F., Grioni, M., Brambilla, P., Briganti, A., et al. (2015). Antisense transcription at the TRPM2 locus as a novel prognostic marker and therapeutic target in prostate cancer. *Oncogene, 34*(16), 2094–2102. doi:10.1038/onc.2014.144.

162. Yap, K. L., Li, S., Munoz-Cabello, A. M., Raguz, S., Zeng, L., Mujtaba, S., et al. (2010). Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Molecular Cell, 38*(5), 662–674. doi:10.1016/j.molcel.2010.03.021.

163. Takayama, K., Horie-Inoue, K., Katayama, S., Suzuki, T., Tsutsumi, S., Ikeda, K., et al. (2013). Androgen-responsive long noncoding RNA CTBP1-AS promotes prostate cancer. *EMBO Journal, 32*(12), 1665–1680. doi:10.1038/emboj.2013.99.

164. Beckedorff, F. C., Ayupe, A. C., Crocci-Souza, R., Amaral, M. S., Nakaya, H. I., Soltys, D. T., et al. (2013). The intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation. *PLoS Genetics, 9*(8), e1003705. doi:10.1371/journal.pgen.1003705.

165. Loven, J., Hoke, H. A., Lin, C. Y., Lau, A., Orlando, D. A., Vakoc, C. R., et al. (2013). Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell, 153*(2), 320–334. doi:10.1016/j.cell.2013.03.036.

166. Puc, J., Kozbial, P., Li, W., Tan, Y., Liu, Z., Suter, T., et al. (2015). Ligand-dependent enhancer activation regulated by topoisomerase-I activity. *Cell, 160*(3), 367–380. doi:10.1016/j.cell.2014.12.023.

167. Jin, C., Yang, L., Xie, M., Lin, C., Merkurjev, D., Yang, J. C., et al. (2014). Chem-seq permits identification of genomic targets of drugs against androgen receptor regulation selected by functional phenotypic screens. *Proceedings of the National Academy of Sciences of the United States of America, 111*(25), 9235–9240. doi:10.1073/pnas.1404303111.

168. Tripathi, V., Ellis, J. D., Shen, Z., Song, D. Y., Pan, Q., Watt, A. T., et al. (2010). The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Molecular Cell, 39*(6), 925–938. doi:10.1016/j.molcel.2010.08.011.

169. Wilusz, J. E., Freier, S. M., & Spector, D. L. (2008). 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. *Cell, 135*(5), 919–932. doi:10.1016/j.cell.2008.10.012.

170. Clemson, C. M., Hutchinson, J. N., Sara, S. A., Ensminger, A. W., Fox, A. H., Chess, A., et al. (2009). An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Molecular Cell, 33*(6), 717–726. doi:10.1016/j.molcel.2009.01.026.

171. Wang, D., Ding, L., Wang, L., Zhao, Y., Sun, Z., Karnes, R. J., et al. (2015). LncRNA MALAT1 enhances oncogenic activities of EZH2 in castration-resistant prostate cancer. *Oncotarget, 6*(38), 41045–41055. doi:10.18632/oncotarget.5728.

172. Chakravarty, D., Sboner, A., Nair, S. S., Giannopoulou, E., Li, R., Hennig, S., et al. (2014). The oestrogen receptor alpha-regulated lncRNA NEAT1 is a critical modulator of prostate cancer. *Nature Communications, 5*, 5383. doi:10.1038/ncomms6383.

173. Ren, S., Liu, Y., Xu, W., Sun, Y., Lu, J., Wang, F., et al. (2013). Long noncoding RNA MALAT-1 is a new potential therapeutic target for castration resistant prostate cancer. *Journal of Urology, 190*(6), 2278–2287. doi:10.1016/j.juro.2013.07.001.

174. Prensner, J. R., Iyer, M. K., Balbin, O. A., Dhanasekaran, S. M., Cao, Q., Brenner, J. C., et al. (2011). Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. *Nature Biotechnology, 29*(8), 742–749. doi:10.1038/nbt.1914.

175. Prensner, J. R., Chen, W., Iyer, M. K., Cao, Q., Ma, T., Han, S., et al. (2014). PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. *Cancer Research, 74*(6), 1651–1660. doi:10.1158/0008-5472.CAN-13-3159.

176. Prensner, J. R., Chen, W., Han, S., Iyer, M. K., Cao, Q., Kothari, V., et al. (2014). The long non-coding RNA PCAT-1 promotes prostate cancer cell proliferation through cMyc. *Neoplasia, 16*(11), 900–908. doi:10.1016/j.neo.2014.09.001.

177. Prensner, J. R., Iyer, M. K., Sahu, A., Asangani, I. A., Cao, Q., Patel, L., et al. (2013). The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. *Nature Genetics, 45*(11), 1392–1398. doi:10.1038/ng.2771.

178. Salameh, A., Lee, A. K., Cardo-Vila, M., Nunes, D. N., Efstathiou, E., Staquicini, F. I., et al. (2015). PRUNE2 is a human prostate cancer suppressor regulated by the intronic long noncoding RNA PCA3. *Proceedings of the National Academy of Sciences of the*

United States of America, 112(27), 8403–8408. doi:10.1073/pnas.1507882112.

179. Hudson, R. S., Yi, M., Volfovsky, N., Prueitt, R. L., Esposito, D., Volinia, S., et al. (2013). Transcription signatures encoded by ultraconserved genomic regions in human prostate cancer. Molecular Cancer, 12, 13. doi:10.1186/1476-4598-12-13.

180. Calin, G. A., Liu, C. G., Ferracin, M., Hyslop, T., Spizzo, R., Sevignani, C., et al. (2007). Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas. Cancer Cell, 12(3), 215–229. doi:10.1016/j.ccr.2007.07.027.

181. Mestdagh, P., Fredlund, E., Pattyn, F., Rihani, A., Van Maerken, T., Vermeulen, J., et al. (2010). An integrative genomics screen uncovers ncRNA T-UCR functions in neuroblastoma tumours. Oncogene, 29(24), 3583–3592. doi:10.1038/onc.2010.106.

182. Lujambio, A., Portela, A., Liz, J., Melo, S. A., Rossi, S., Spizzo, R., et al. (2010). CpG island hypermethylation-associated silencing of non-coding RNAs transcribed from ultraconserved regions in human cancer. Oncogene, 29(48), 6390–6401. doi:10.1038/onc.2010.361.

183. Bao, B. Y., Lin, V. C., Yu, C. C., Yin, H. L., Chang, T. Y., Lu, T. L., et al. (2016). Genetic variants in ultraconserved regions associate with prostate cancer recurrence and survival. Science Reports, 6, 22124. doi:10.1038/srep22124.

184. Hessels, D., & Schalken, J. A. (2009). The use of PCA3 in the diagnosis of prostate cancer. Nature Reviews. Urology, 6(5), 255–261. doi:10.1038/nrurol.2009.40.

185. Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., et al. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America, 105(30), 10513–10518. doi:10.1073/pnas.0804549105.

186. Nguyen, H. C., Xie, W., Yang, M., Hsieh, C. L., Drouin, S., Lee, G. S., et al. (2013). Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. Prostate, 73(4), 346–354. doi:10.1002/pros.22572.

187. Zhang, H. L., Yang, L. F., Zhu, Y., Yao, X. D., Zhang, S. L., Dai, B., et al. (2011). Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. Prostate, 71(3), 326–331. doi:10.1002/pros.21246.

188. Kristensen, H., Haldrup, C., Strand, S., Mundbjerg, K., Mortensen, M. M., Thorsen, K., et al. (2014). Hypermethylation of the GABRE~miR-452~miR-224 promoter in prostate cancer predicts biochemical recurrence after radical prostatectomy. Clinical Cancer Research, 20(8), 2169–2181. doi:10.1158/1078-0432.CCR-13-2642.

189. Bussemakers, M. J., van Bokhoven, A., Verhaegh, G. W., Smit, F. P., Karthaus, H. F., Schalken, J. A., et al. (1999). DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Research, 59(23), 5975–5979.

190. Auprich, M., Chun, F. K., Ward, J. F., Pummer, K., Babaian, R., Augustin, H., et al. (2011). Critical assessment of preoperative urinary prostate cancer antigen 3 on the accuracy of prostate cancer staging. European Urology, 59(1), 96–105. doi:10.1016/j.eururo.2010.10.024.

191. Leyten, G. H., Hessels, D., Jannink, S. A., Smit, F. P., de Jong, H., Cornel, E. B., et al. (2014). Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. European Urology, 65(3), 534–542. doi:10.1016/j.eururo.2012.11.014.

192. Lin, D. W., Newcomb, L. F., Brown, E. C., Brooks, J. D., Carroll, P. R., Feng, Z., et al. (2013). Urinary TMPRSS2: ERG and PCA3 in an active surveillance cohort: results from a baseline analysis in the Canary Prostate Active Surveillance Study. Clinical Cancer Research, 19(9), 2442–2450. doi:10.1158/1078-0432.CCR-12-3283.

193. Huang, X., Yuan, T., Liang, M., Du, M., Xia, S., Dittmar, R., et al. (2015). Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. European Urology, 67(1), 33–41. doi:10.1016/j.eururo.2014.07.035.

194. Isin, M., Uysaler, E., Ozgur, E., Koseoglu, H., Sanli, O., Yucel, O. B., et al. (2015). Exosomal lncRNA-p21 levels may help to distinguish prostate cancer from benign disease. Frontiers in Genetics, 6, 168. doi:10.3389/fgene.2015.00168.

195. Hindorff, L. A., Sethupathy, P., Junkins, H. A., Ramos, E. M., Mehta, J. P., Collins, F. S., et al. (2009). Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences of the United States of America, 106(23), 9362–9367. doi:10.1073/pnas.0903103106.

196. Glinskii, A. B., Ma, S., Ma, J., Grant, D., Lim, C. U., Guest, I., et al. (2011). Networks of intergenic long-range enhancers and snpRNAs drive castration-resistant phenotype of prostate cancer and contribute to pathogenesis of multiple common human disorders. Cell Cycle, 10(20), 3571–3597. doi:10.4161/cc.10.20.17842.

197. Jin, G., Sun, J., Isaacs, S. D., Wiley, K. E., Kim, S. T., Chu, L. W., et al. (2011). Human polymorphisms at long non-coding RNAs (lncRNAs) and association with prostate cancer risk. Carcinogenesis, 32(11), 1655–1659. doi:10.1093/carcin/bgr187.

198. Al Olama, A. A., Kote-Jarai, Z., Giles, G. G., Guy, M., Morrison, J., Severi, G., et al. (2009). Multiple loci on 8q24 associated with prostate cancer susceptibility. Nature Genetics, 41(10), 1058–1060. doi:10.1038/ng.452.

199. Meyer, K. B., Maia, A. T., O'Reilly, M., Ghoussaini, M., Prathalingam, R., Porter-Gill, P., et al. (2011). A functional variant at a prostate cancer predisposition locus at 8q24 is associated with PVT1 expression. PLoS Genetics, 7(7), e1002165. doi:10.1371/journal.pgen.1002165.

200. He, C. (2010). Grand challenge commentary: RNA epigenetics? Nature Chemical Biology, 6(12), 863–865. doi:10.1038/nchembio.482.