CellPress
REVIEWS

Review

Landscape of Noncoding RNA in Prostate Cancer

Junjie T. Hua,[1,2] Sujun Chen,[1,2] and Housheng H. He[1,2,*]

The transcriptome of prostate cancer is highly heterogeneous, with noncoding transcripts being essential players. Long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) are two unique classes of noncoding RNA drawing increasing attention. Biologically, they have intriguing properties with important regulatory functions. Clinically, they present as promising biomarkers and therapeutic targets. Recent advancements in technologies have opened up new directions for noncoding RNA research, which include RNA–protein interaction, RNA secondary structure, and spatial transcriptomics. Furthermore, recent work has also evaluated the clinical applications of these noncoding RNAs in noninvasive liquid biopsy and RNA-based therapies. In this review, we summarize recent findings on lncRNAs and circRNAs in prostate cancer, discuss their clinical utilities, and highlight these exciting areas of research.

Highlights

Long noncoding RNA (lncRNA) and circular RNA (circRNA) are pervasive noncoding RNA classes with important functional roles, and are frequently dysregulated in prostate cancer.

While circRNAs share similarities with lncRNAs, the circular conformation leads to novel functional mechanisms, increased stability and unique clinical applications.

Recent technological advancements impel noncoding (nc)RNA research in RNA–protein interaction, RNA secondary structure, and spatial transcriptomics, which may reveal novel findings for clinical translation.

Both lncRNAs and circRNAs are promising biomarkers due to their expression specificity, and may be exploited for noninvasive liquid biopsy applications.

RNAi and antisense oligonucleotides (ASOs) are therapeutic strategies for targeting oncogenic ncRNAs, and can be delivered through nanoparticles or conjugated antibodies or receptors.

Noncoding Transcriptome of Prostate Cancer

Noncoding RNAs (ncRNAs) (see Glossary) are emerging as important regulators in different disease processes including cancer. Historically ncRNAs are classified into short and long ncRNAs based on the 200-nucleotide cutoff in mature transcript length [1]. Short ncRNAs include more well-defined species like **miRNAs** and small interfering RNAs (siRNAs). **Long noncoding RNAs (lncRNAs)** can be further classified into subgroups based on their genomic localization and evolutionary lineage, which includes long intergenic RNA (lincRNA), antisense RNAs, sense intronic RNAs, **enhancer RNAs (eRNAs)**, and **pseudogenes**. Recently, **circular RNAs (circRNAs)** were presented as a new class of pervasive RNA with regulatory potential [2–5]. Although there is sparse evidence that some lncRNAs and circRNAs can be translated [6–11], the majority of these RNA species are still considered noncoding.

In cancer, many ncRNAs are aberrantly expressed, and show evidence of function in oncogenesis or tumor progression [4,12–16]. In particular, miRNA has been studied extensively in different cancer types, including prostate cancer [17,18]. Recently, it was reported that many miRNAs are induced by hypoxia, and that hypoxia-suppressed miR-133a-3p can play a tumor suppressive role in prostate cancer [18]. In addition, many miRNAs are also being actively evaluated as biomarkers for prostate cancer progression, especially for noninvasive **liquid biopsy** application with blood and urine samples [19,20]. Aside from miRNAs, lncRNAs and circRNAs are relatively new players in the ncRNA field and are less well understood. Nonetheless they are gaining widespread attention for their abundance in number, expression specificity, functional roles in diseases, and potential clinical applications [2,4,13,21–24]. This review discusses recent developments in the ncRNA field in prostate cancer, with a focus on lncRNAs and circRNAs.

Emerging New ncRNA Players in Prostate Cancer

Long ncRNA

lncRNAs are a unique class of RNA that share some similarities with protein-coding mRNAs, but generally do not code for proteins. While once considered to be transcriptional noise, research data from the past decade have highlighted lncRNAs as a pervasive class of ncRNA with important biological roles [12–14,21–23].

Biogenesis and Identification

Similar to protein-coding mRNAs, lncRNAs are transcribed by RNA polymerase II and can undergo post-transcriptional splicing and modifications in the nucleus. In the genomic era, large transcriptomic studies driven by international efforts have revealed lncRNA to be one of the most pervasive classes of RNA in the human transcriptome, subsequently driving investigations into its functional

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada

[2]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

*Correspondence: Hansen.he@uhnresearch.ca

 https://doi.org/10.1016/j.tig.2019.08.004
© 2019 Elsevier Ltd. All rights reserved.

roles. In 2012, the **GENCODE Consortium** identified 9640 lncRNA loci and 15 512 transcripts [25]. With the development and adoption of newer technologies such as Capture Long Seq [26], the current version of GENCODE reports 16 193 lncRNA loci and 30 369 transcripts (https://www.gencodegenes.org/human/stats.html). The **FANTOM Consortium** recently profiled 1829 human tissues and cell lines using the Cap Analysis Gene Expression technique pairing with RNA sequencing (RNA-seq) to identify 27 919 high-confidence lncRNAs with annotated 5′ ends [27] (Figure 1A). With continuous improvements in sequencing and computational technologies, additional lowly or transiently expressed lncRNAs will likely be identified in the future, thus driving the increase in total number of lncRNAs in our transcriptome. These discoveries consequently resulted in the accompanying increase in scientific interest to functionally interrogate this large class of ncRNA.

Function

lncRNAs can regulate the expression of downstream target genes through different mechanisms, such as the recruitment of chromatin regulators and transcription factors [21,22,28]. Under normal physiological conditions, lncRNAs can play essential roles in processes such as development and cell differentiation [29–31]. However, not all lncRNAs are functional, as some may be byproducts of nearby transcription. As a result, different methods have been explored to prioritize functionally important candidates (Figure 1B), including conservation [32], differential expression [22,33], association with clinical features [21], and association with **SNPs** [22,23] or oncogenic mutations [34]. Notably, noncoding SNPs associated with prostate cancer can regulate gene expression by modulating transcription factor binding [35], and their association with lncRNAs can be exploited to reveal functional lncRNA candidates [22,23].

Genome-wide functional screens mediated by variants of **CRISPR** technology have also revealed important roles of hundreds of lncRNAs [36–39] (Figure 1C). While these screens did not focus on prostate cancer, similar approaches can be taken to reveal prostate-specific lncRNA candidates. It is important to note that the conventional CRISPR deletion approach through nonhomologous end joining (NHEJ) has limited efficacy in studying lncRNAs due to their lack of protein-coding potential. On the contrary, the paired-guide CRISPR approach deleting the promoters of lncRNAs can effectively modulate their abundance at the RNA level [40]. Alternatively, **CRISPR interference (CRISPRi)** and **CRISPR activation (CRISPRa)** can also efficiently suppress and enhance the expression of lncRNAs, respectively, and can be adapted for high-throughput screens [36,37,39]. In particular, the CRISPRa approach was recently coupled with drug treatment to reveal candidate drivers of drug resistance [39]. Aside from promoters, the splice sites of lncRNAs may also be suitable targets for CRISPR-based screens [38].

Prostate Cancer

lncRNAs are often aberrantly expressed in cancer. Possible mechanisms include oncogenic mutations, SNPs, copy number variation (CNV), and aberrant DNA methylation and histone modifications [22,23,41]. One of the most comprehensive lncRNA catalogs, particularly for cancer, is the miTranscriptome catalog generated from the meta-assembly of 7256 RNA-seq libraries from normal and tumor tissues and cell lines [13]. This catalog identified 58 648 lncRNAs across multiple tissues and cancer types, including 727 prostate-cancer-specific lncRNAs (Figure 1A). Transcriptomic studies comparing the RNA expression profiles of a large number of prostate cancer tumors and benign tissues have also revealed hundreds of prostate-cancer-associated lncRNAs, including *PCAT1*, *PCAT14*, *PVT1*, *PCAN-R1*, and *PCAN-R2* [12,42]. Moreover, several published prostate cancer RNA-seq datasets are available [43–47], and can be reinterpreted for lncRNA candidates [4,22].

In recent years, numerous studies have illustrated the functional roles of various individual lncRNAs in prostate cancer through *in vitro* cell line and *in vivo* xenograft models [21–23,33]. For instance, upregulation of lncRNA *PCAT1* in prostate cancer was found to recruit androgen receptor (AR) and Lysine-Specific Histone Demethylase 1A (LSD1) to upregulate late androgen response genes, thereby promoting prostate cancer cell growth in both cell line and xenograft models [22]. Another study reported that *PCAT1* can act as a **miRNA sponge** for *miR-34a* to stabilize the oncogene *MYC* [48].

Glossary

Antisense oligonucleotide (ASO): a single-stranded DNA or RNA molecule that can bind to target RNAs to mediate their silencing or degradation through the **endonuclease** RNase H.
Back-splicing: a noncanonical splicing process involving a downstream splice donor and an upstream splice acceptor, which ligates the RNA specie into a circular form.
Circular RNA (circRNA): covalently closed circular transcript generated through back-splicing.
CRISPR (clustered regularly interspaced short palindromic repeats): short segment of DNA repeats that, along with the endonuclease Cas9, forms the bacterial defense system against foreign genetic material, which is later adapted as a genome editing technology.
CRISPR activation (CRISPRa): variation of the CRISPR-Cas9 genome editing technology involving a Cas9 protein without its endonuclease activity (dCas9) fused to active domains such as VP64 to enhance gene expression.
CRISPR interference (CRISPRi): variation of the CRISPR-Cas9 genome editing technology involving dCas9 fused to repressive domains such as KRAB to suppress gene expression.
Endonuclease: enzyme that can cleave DNA or RNA molecules internally .
Enhancer RNA (eRNA): class of ncRNAs transcribed from active enhancers.
Exonuclease: enzyme that can degrade DNA or RNA molecules from their 5′ or 3′ ends.
Exosomes: extracellular vesicles released from cells that may contain DNA, RNA, or proteins.
FANTOM (Functional ANnoTation Of the Mammalian genome) Consortium: a large international research consortium led by the RIKEN research institute to identify functional elements in the human genome.
GENCODE Consortium: scientific consortium established by the National Human Genome Research Institute (NHGRI) that is a part of the ENCyclopedia Of DNA Elements (ENCODE)

With continual advancements in sequencing technologies and development of new molecular and cellular biology tools, it is currently an exciting time for the field of lncRNA research in prostate cancer. Discovery of additional lncRNAs dysregulated in prostate cancer pairing with genome-wide functional screens will undeniably yield a large repertoire of clinically actionable lncRNAs in prostate cancer.

circRNA

circRNA is a type of RNA that undergoes **back-splicing** and forms a covalently closed loop. Although once considered as sporadic events and mis-splicing byproducts, circRNAs are now believed to be a large class of regulatory RNAs [2,3]. The identification and functional characterization of CDR1as [3], coupled with the first circRNA profile [2], initiated a burst in the field of circRNA research. Accumulating evidence suggests important roles for circRNAs in various diseases, including cancers [49–53].

Biogenesis

circRNAs are the result of an inefficient back-splicing process, which largely shares the canonical machineries of RNA splicing (Figure 2A). Most current research focused on circRNAs derived from known genes. While escaping from debranching can result in intronic circRNAs [54–56], different mechanisms are implicated for the generation of circRNAs consisting of exonic sequences. Various regulatory sequences [57,58] and protein factors [58–62] are involved in the process and circRNAs can be produced co- [63] and/or post-transcriptionally [64,65].

Introns flanking the circularized exons are often enriched for complementary sequences such as inverted repeat Alu elements. Such sequences can bring the flanking introns into close proximity by forming a hairpin structure [57]. While certain levels of collaboration between the flanking intron and exon sequences were observed, the mechanism is yet to be defined [64]. Not all circularization requires complementary sequences. Insertion of quaking (QKI) binding motifs into linear RNA can induce back-splicing, possibly due to dimerization of QKI proteins that bind to the motifs. Other splicing factors like MBL [59], heterogeneous nuclear ribonucleoproteins (hnRNPs) [63], FUS [66], and NF90/NF110 [61] are also reported to modulate circRNA levels. In the *Drosophila* system, depletion of canonical splicing components, including SF3a and SF3b, can favor the generation of circRNAs over their linear counterparts [62].

circRNAs are mostly produced post-transcriptionally. Indirect evidence for post-transcriptional circularization is the requirement of polyadenylation (poly-A) on the linear transcripts for circRNA production [64]. Using 4-thiouridine (4sU) incorporation experiments, direct evidence that most circularization happens post-transcriptionally have been reported [65].

Function

While specific DNA sequences and protein factors are shown to facilitate RNA circularization [57,60,62,67], direct regulators for the tissue/cell type specific circRNA expression remains unclear. As such, an important question is whether the tissue/cell type-specific expression is a result of tight regulation or stochastic choice of different circular isoforms. Current evidences seem to support both hypotheses: on the one hand, most circRNAs are of low abundance, and global circRNAs level can be affected by a single event such as limitation in canonical splicing machinery [62] or viral infection [67]. On the other hand, hundreds of highly abundant circRNAs were identified, some with higher abundance than their linear counterparts [4,16,67]. Individual circRNAs can regulate specific gene expression, be oncogenic, and convey proliferative advantages [3,4,24,68,69].

Since most circRNAs are produced from the exons of known linear genes, they can regulate the abundance and function of these linear transcripts. Specifically, an increase in RNA circularization can lead to reduction of linear transcripts. The resultant circRNAs can further compete for RNA binding proteins through sequence homology with the corresponding linear transcripts. In addition, some mechanisms of circRNAs share similarities to that of lncRNAs (Figure 2A,B). They can activate gene expression by recruiting transcription factors to promoters [69], and act as protein/miRNA sponges or

Project, and aims to annotate the human and mouse genomes.
Liquid biopsy: noninvasive form of biopsy involving analysis of body fluid, such as blood and urine.
Long noncoding RNA (lncRNA): class of ncRNA over 200 nucleotides in length.
miRNA: large class of small ncRNA that is 22 nucleotides in length, and plays an important role in RNA interference pathways through the RNA-induced silencing complex (RISC).
miRNA sponge: biological molecule that can sequester specific miRNAs, and prevent them from targeting and degrading endogenous RNAs.
Noncoding RNAs (ncRNAs): RNA molecules that do not contain canonical open reading frames, and are thus not normally translated into proteins.
Pseudogenes: class of ncRNAs with strong homology to protein coding genes, that are not translated into proteins.
RNA interference (RNAi): biological process involving RNA-dependent cleavage of target RNA molecules, typically mediated by the RISC complex composed of miRNAs or small interference RNAs (siRNAs) and an endonuclease Augonaute.
Single cell RNA sequencing (scRNA-seq): type of RNA sequencing technology that captures and sequences the RNA molecules of each individual cell.
SNP: inheritable genetic variation at a single nucleotide base pair between individuals of a population.

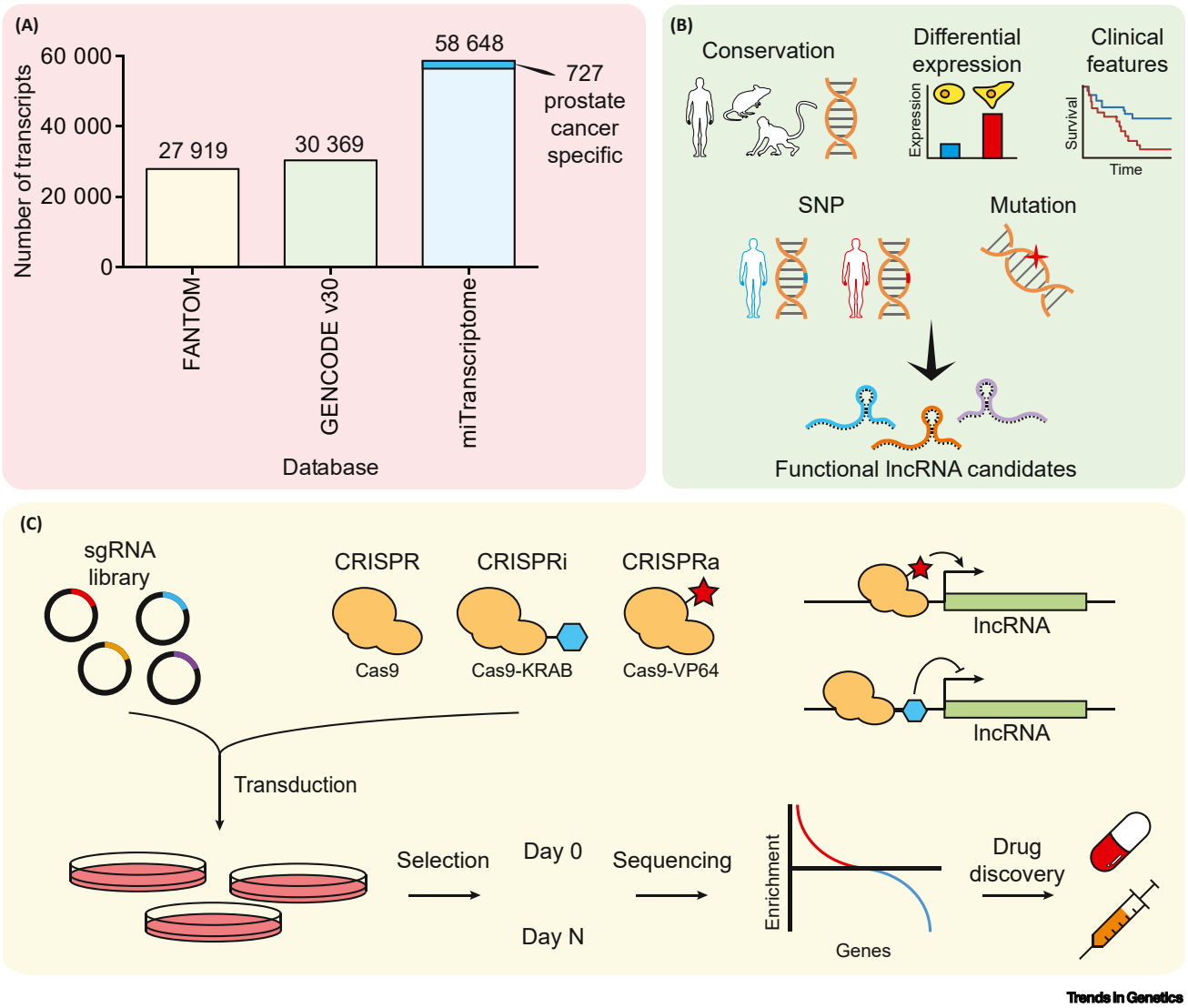

Figure 1. Current Landscape of Long Noncoding RNA (lncRNA) Research.

(A) Number of annotated lncRNA transcripts from publicly available databases. (B) Nonscreening-based methodologies for identifying functional lncRNAs. (C) CRISPR-based screening approaches for identifying functional lncRNA candidates. Abbreviations: CRISPRa, CRISPR activation; CRISPRi, CRISPR interference; sgRNA, single guide RNA.

scaffolds [70]. The circular form can also lead to circRNA specific properties. circRNAs are resistant to miRNA recruited **exonuclease** due to the lack of open ends, and this in turn can stabilize miRNAs upon binding [4,70]. It is worth noting that, despite being the most frequently studied mechanism, regulating miRNA might not be as unique and potent as was deduced from the CDR1as example [3,71].

Prostate Cancer

The lack of 3′ poly-A, low abundance, and overlapping sequence with linear transcripts previously underestimated the number of circRNAs in the high-throughput sequencing era. Recent studies have highlighted the prevalence and highly tissue/cell type-specific expression of circRNAs across various disease conditions [2]. Over 400 000 unique circRNAs have been identified to date [4,16],

which is more than twice the number of linear transcripts. However, this number is still likely underestimated because we do not fully understand the regulation of circRNA biogenesis.

Recent studies have revealed a widespread and functional RNA circularization landscape in prostate cancer [4,16]. Hundreds of circRNAs that are differentially expressed among normal tissue, primary, metastatic castration-resistant, and neuroendocrine prostate cancer have been identified, and some can convey proliferation advantage independently. The overall level of RNA circularization is tightly correlated to prostate cancer aggressiveness, and individual circRNAs usually harbor prognostic information that differs from their linear counterparts. Moreover, circRNAs have higher stability compared with their linear counterparts, and thousands are detected in human blood plasma and patient urine **exosomes**, making them ideal candidates for minimum to noninvasive biomarkers (Figure 2C). These studies together have revealed a remarkably complex layer rich in diagnostic and therapeutic opportunities in the prostate cancer transcriptome.

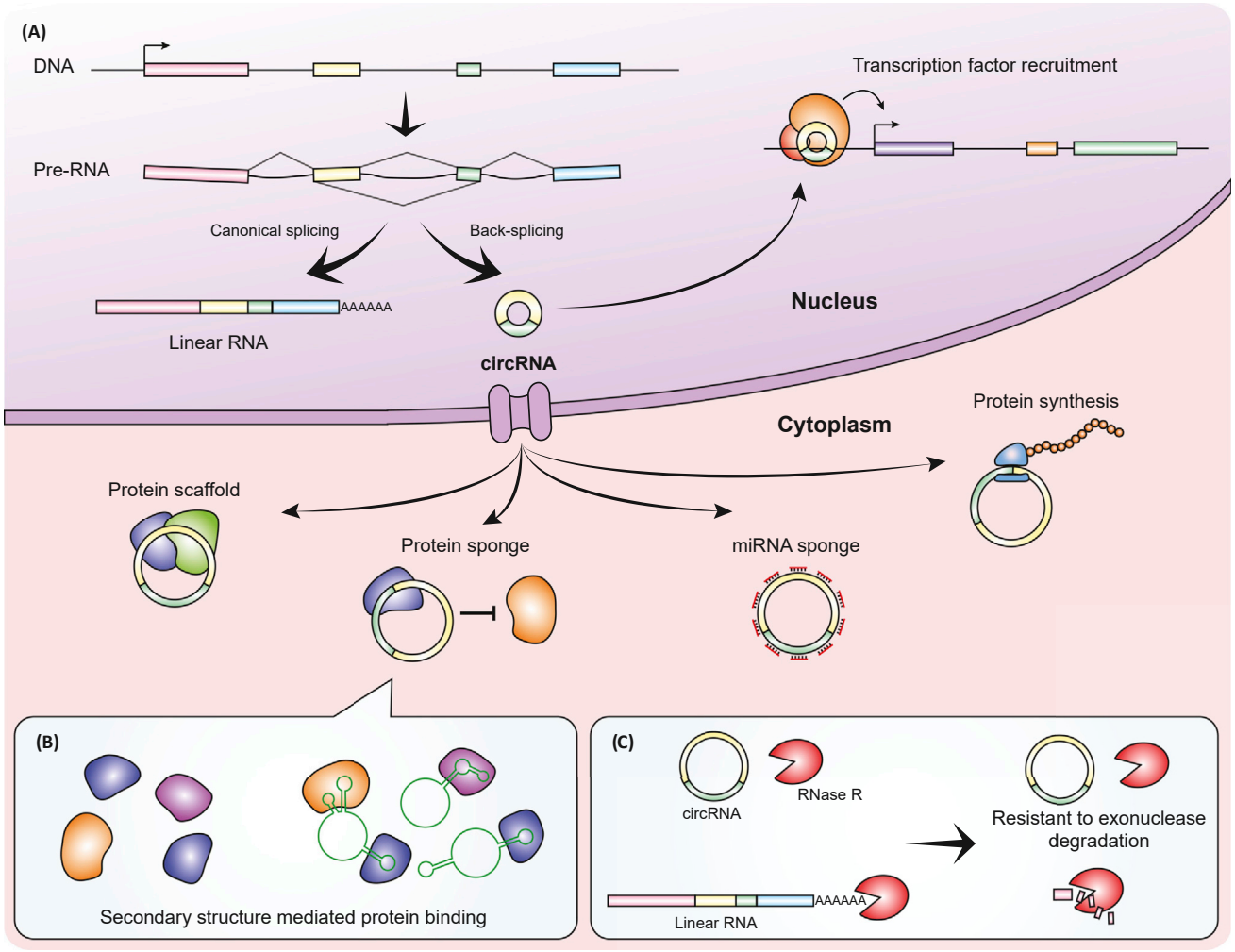

Figure 2. Biogenesis and Functional Mechanisms of Circular RNA (circRNA).
(A) A schematic showing biogenesis of circRNAs through the noncanonical back-splicing process, and their reported functional mechanisms. (B) A panel highlighting the importance of RNA secondary structure in mediating protein binding. (C) A panel depicting superior stability of circRNA compared with their linear counterparts due to its exonuclease-resistant properties. Abbreviation: RNase R, Ribonuclease R.

New Areas of ncRNA Research

It has become evident that investigating the binding partners and structures of ncRNAs are crucial for understanding their functional mechanisms. In addition, given that prostate cancer is a highly heterogeneous cancer [72,73], it would be important to explore these cell type specific ncRNAs at an intratumor level. Recent development of new technologies will allow us to address these key areas of ncRNA research that were not possible decades ago.

ncRNA–Protein Interaction

Identifying the interacting partners is one of the first steps of lncRNA/circRNA functional research, from which we can gain insights into related pathways and mechanisms. For example, lncRNA *SChLAP1* was found to interact with the SWItch/sucrose nonfermentable (SWI/SNF) complex, suggesting that it may function through modulating chromatin remodeling and accessibility [21]. Recently, a panel of circRNAs were found to interact with nucleic acid receptors with antiviral activity, suggesting their role in regulating the immune response [5]. To uncover binding partners, various *in vitro* and *in vivo* RNA pulldown methods have been developed [74–76].

Traditional RNA pulldown methods rely on the use of modified DNA or RNA probes targeting the RNA of interest in a complex. *In vitro* methods involve applying cellular extract containing proteins to preformed RNA–probe complexes [75]. On the contrary, *in vivo* methods require crosslinking proteins and RNA endogenously before adding modified probes for subsequent pulldown [76]. Compared with *in vivo* methods, *in vitro* methods are relatively simple and easy to perform, and can result in good signals, albeit the lack of endogenous context. These methods can be coupled with mass spectrometry to identify global RNA–protein interacting profiles. Alternatively, bound DNA and RNA can be extracted for next-generation sequencing to uncover binding sites of lncRNAs and circRNAs on DNA and RNA. Nonetheless, these traditional probe-based methods may suffer from potential off-targeting issues of the nucleic acid probes. A newer method involving direct biotinylation of interacting proteins has recently been developed [77], but has yet to be adapted for circRNA studies.

Secondary Structures of ncRNAs

Another new area of ncRNA research is understanding the secondary and tertiary structures of ncRNAs. While the interactions between ncRNAs and their nucleic acid targets largely depend on sequence complementarity, their interactions with proteins are affected by their structures. This is most well exemplified in miRNAs, where the stem loop structure of primary miRNAs regulate processing factors binding [77]. It has also been shown that local stem loop structures of lncRNAs and circRNAs can modulate the binding of proteins [5,78]. Therefore, it is not surprising that ncRNAs are more conserved at specific functional modules or sequence motifs [79], which is analogous to proteins and their functional domains. Despite this, investigations into the structure of ncRNAs have largely relied on computational prediction [80] until recently.

Advancements in sequencing and computational technologies, coupled with the use of small chemical probes or specific enzymes, have innovated the RNA structure field, allowing for direct probing of RNA structures [81–83]. Notably, the selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) technique was later improved upon (SHAPE-MaP and icSHAPE), and has been used in studies to interrogate the structures of lncRNAs such as *XIST* and *NEAT1* and circRNAs [5,84,85]. circRNAs exhibit different secondary structures than their linear counterparts despite sequence similarities, which resulted in differences in functionalities [5]. In addition, different structures have also been reported for short and long isoforms of *NEAT1* [86]. These studies highlight the importance of understanding the structures of different ncRNA transcripts, which can provide important insights into their potential functional mechanisms.

Single Cell and Spatial Transcriptomics

Expression of ncRNAs is highly specific and often tightly regulated. In cancer, numerous lncRNA and circRNA expressions are specific to cancer types or subtypes [4,12,13,16]. While the expression of

ncRNAs is generally low in bulk transcriptomic analyses, recent studies have found higher levels of expression in subpopulations of cells [86,87], providing opportunities to interrogate intratumoral heterogeneity. Intraductal carcinoma and cribriform architecture (IDC/CA) is a subpathology of prostate cancer that is associated with unfavorable patient outcomes [74]. Remarkably, the expression of lncRNA *SChLAP1* was found to be over threefold higher in prostate tumors with this subpathology, and may be a useful biomarker for this aggressive feature [74]. Therefore, studying ncRNA expression profiles at single cell level or spatially across tumor samples may reveal unique molecular subtypes or rare cell populations of prostate cancer and provide molecular insights into tumor heterogeneity.

Recent advancement in **single cell (sc)RNA-seq** technology provides insights into the tumor transcriptome at single cell resolution [87,88]. Novel scRNA-seq protocols also allow investigation of non-poly(A) transcripts [89]. While scRNA-seq has been performed on prostate cancer cells to reveal subpopulations of androgen-independent cells [90], there are currently no reported studies on ncRNA profiles in prostate cancer. In addition to scRNA-seq, spatial transcriptomic profiling techniques can provide expression profiles across tumor sections through the use of spatially unique barcodes [91,92]. Using this technique, unbiased spatial transcriptomic maps of prostate tumors with multiple cancerous regions surrounded by normal prostate, stromal, and immune cells have been generated [92]. Together, these novel techniques present us with unprecedented opportunities to capture precise transcriptomic profiles of ncRNAs and to investigate molecular features of tumor heterogeneity.

Clinical Relevance of lncRNAs and circRNAs

In prostate cancer, hundreds of lncRNAs and circRNAs are reported to be dysregulated [4,12,13,16,42]; some to a greater extent than protein-coding mRNAs [74]. Subsequent studies have demonstrated roles of these ncRNAs in prostate cancer development and progression [4,21–23,33]. Given their prevalence, expression specificity and functional importance, there are immense interests in evaluating lncRNAs and circRNAs as novel biomarkers and therapeutic targets in clinical settings.

Biomarker Potentials

The utility of lncRNAs as cancer biomarkers is most well exemplified by lncRNA *PCA3*, the first lncRNA-based biomarker approved by the FDA for prostate cancer diagnosis. *PCA3* is highly prostate cancer specific, with a 34-fold higher expression in prostate tumors compared with normal tissues [93], and can be detected in urine samples [94]. These properties led to the establishment of a urine-based *PCA3* biomarker test for determining the need for rebiopsy in ambiguous cases with high prostate-specific antigen (PSA) levels but negative initial biopsies. Aside from *PCA3*, several additional lncRNAs, including *MALAT1*, *PCAT18*, and *SChLAP1*, are showing promise as prostate cancer diagnostic and prognostic biomarkers [33,95,96]. Notably, *SChLAP1* has been evaluated in several independent studies as a prognostic biomarker for metastatic prostate cancer [74,97].

Clinically, there has been increasing interest in liquid biopsy because it is less invasive than conventional tissue biopsy and is suitable for real-time disease monitoring (Figure 3). Due to their specificity in expression levels, lncRNAs are attractive candidates for liquid-biopsy-based biomarkers, and indeed they have been tested in both plasma [33] and urine [76–79] (Table 1, Key Table). Exosomes present another source for extracellular lncRNAs, and numerous exosomal lncRNAs have been evaluated in preclinical settings [97–99]. Expression of exosomal *lncRNA-p21* was elevated in the urine of prostate cancer patients compared with the control group [100]. It has been reported that the uptake of lncRNAs into prostate cancer exosomes is selective, but the exact mechanisms are currently unclear [100].

circRNAs are a new attractive group of ncRNA biomarkers for liquid biopsy due to their resistance to exonuclease degradation and enhanced stability in circulation. Thousands of circRNAs have been identified to be more enriched in blood and urine samples [16], with individual ones being characterized in different cancer types, including the androgen responsive *circSMARCA5* in prostate cancer

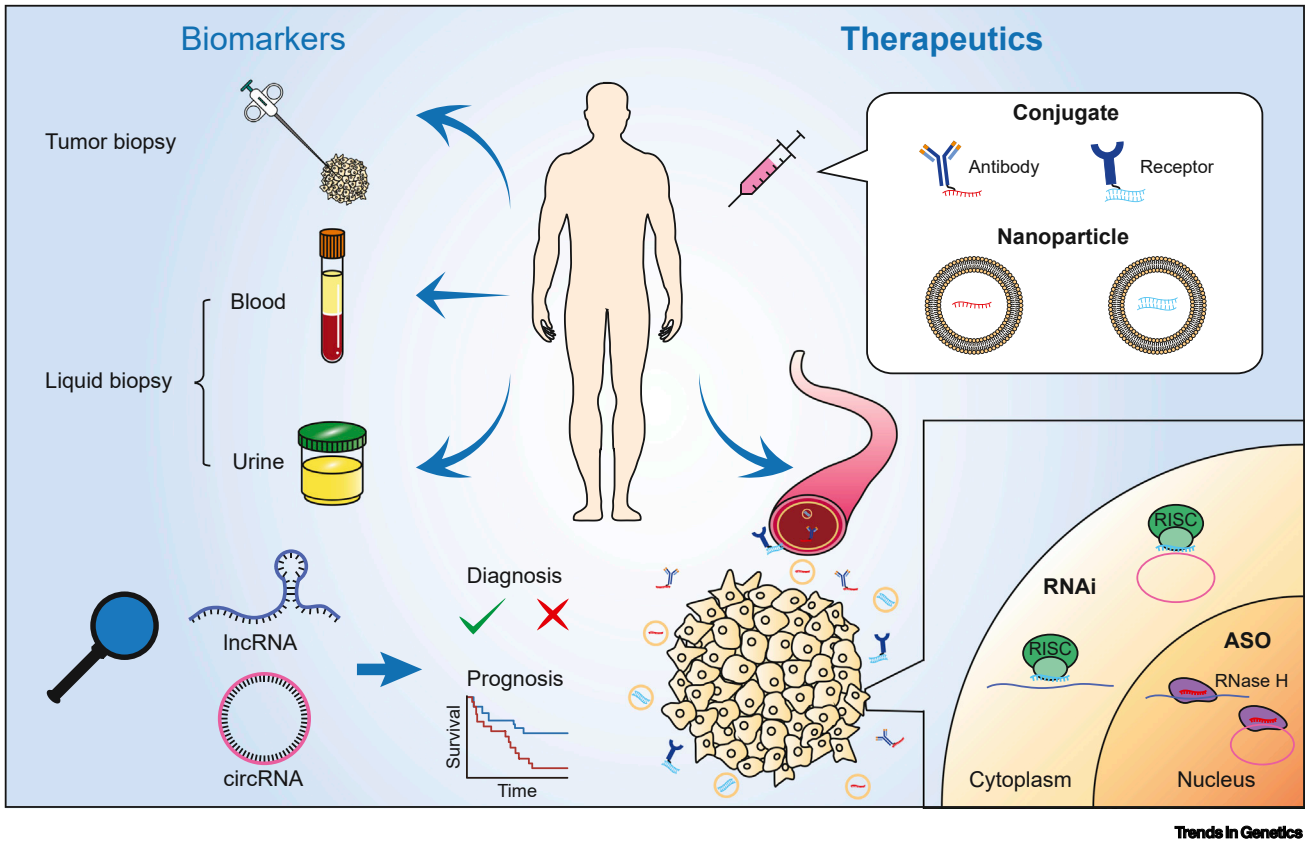

Figure 3. Clinical Applications of Long Noncoding RNA (lncRNA) and Circular RNA (circRNA).
Biomarker (left panel) and therapeutic (right panel) potential of lncRNAs and circRNAs in prostate cancer. These noncoding RNA (ncRNA) species can be measured from conventional tumor biopsy or liquid biopsy samples (blood and urine), and are potential diagnostic and prognostic biomarkers. RNAi and antisense oligonucleotide (ASO) agents can effectively target ncRNAs in the cytoplasm and nucleus, respectively, and can be delivered through nanoparticles or antibody/receptor conjugates *in vivo*. Abbreviation: RISC, RNA-induced silencing complex; RNase H, Ribonuclease H.

[101]. The study of circRNAs as biomarkers is in its infancy but has already shown promising potential, and more work is advocated for it to be applied in clinical settings.

Therapeutic Potential

ncRNAs with important oncogenic functions in prostate cancer may serve as novel therapeutic targets. In preclinical studies, lncRNAs *SChLAP1*, *PCAT1*, *PCAT18*, and *PCAT19* were found to contribute to prostate cancer cell growth and aggressiveness [21–23,33]. In addition, lncRNAs *NEAT1* and *HOXD-AS2* are associated with antiandrogen and chemotherapy sensitivity of prostate cancer [102,103]. lncRNA-mediated drug resistance may even be transmitted between cancer cells through exosomes [104]. circRNAs such as *circSMARCA5* promote prostate cancer cell growth and inhibit apoptosis [102]. Given their important functional roles, there is a strong rationale to target these oncogenic ncRNAs as novel therapeutic options.

Two main categories of RNA-based therapeutic approaches are **RNAi** and **antisense oligonucleotides (ASOs)**, which can be designed to target almost any RNA region (Figure 3). However, there is a difference in mechanism of action. RNAi involves the use of exogenous double-stranded siRNAs or miRNAs to target oncogenic mRNAs and ncRNAs through the same pathway as endogenous miRNA. On the contrary, ASOs involve the use of single-stranded nucleic acid molecules and result

Key Table

Table 1. Liquid Biopsy lncRNA Biomarker Candidates for Prostate Cancer Reported in the Past 5 Years

lncRNA	Biomarker type	Expression	Sample	Refs
SAP30L-AS1[a]	Diagnostic	Down	Plasma exosome	[80]
lncRNA-p21	Diagnostic	Up	Urine exosome	[81]
MALAT1	Diagnostic	Up	Urine	[76]
FR0348383	Diagnostic	Up	Urine	[78]
SChLAP1	Prognostic	Up	Urine, plasma exosome	[77,80]
lncAPP	Prognostic	Up	Urine	[79]
PCAT18	Prognostic	Up	Plasma	[34]

[a]Decreased expression in plasma exosome (PMID: 29614511), but increased expression in tumor tissue (PMID: 30599235) is observed in prostate cancer.

in gene silencing through RNase H-mediated degradation of target RNAs. In recent years, there have been significant advancements in the design and delivery of RNA-based therapeutics, with chemical modifications resulting in improved target specificity and stability, as well as decreased toxicity [105]. The first RNAi-based drug Patisiran was approved by the FDA last year for the treatment of hereditary transthyretin amyloidosis [106]. ASOs have also showed promise in targeting lncRNAs in a recent study on patients with Angelman syndrome [107]. The *in vivo* delivery of these therapeutic agents has been improved recently through conjugation with receptor ligands and packing in nanoparticles [108,109].

In prostate cancer, ASOs Oblimersen targeting Bcl-2 and Custirsen targeting clusterin have been evaluated in Phase II and III clinical trials, respectively, albeit with lack of efficacy [110,111]. Additional ASO agents, such as Apatorsen targeting Hsp27 [112], ARRx targeting AR (NCT03300505), and RNAi targeting AR (NCT02866916) are currently in Phase I clinical trials. While these current trials are focusing on protein-coding genes, functional lncRNAs and circRNAs from preclinical studies will provide a novel repertoire of candidates for RNA-based therapeutics.

Besides exploiting vulnerabilities conveyed by lncRNA and circRNA, exploring the unique properties of circRNAs is a new frontier. Engineered RNA circles can be more effective in delivering therapeutic proteins. For example, high-quality protein translation with threefold longer production half-life using artificial circRNAs has been achieved [113]. Additionally, a tornado system to effectively achieve high-level cellular circRNA aptamers and biosensor expression has been developed [114]. circRNAs as therapeutic agents show intriguing potential. While advances in other disease models can pave the way for their utilization in prostate cancer, research in prostate cancer is warranted to address tissue-specific challenges.

Concluding Remarks and Future Perspectives

lncRNAs and circRNAs are two promising new players in the prostate cancer field with attractive biomarker and therapeutic potentials. Recent advancements in technologies provide exciting opportunities for ncRNA research and its translation into the clinic. We are now starting to unravel their detailed functional mechanisms, explore their expression at the single cell level for biomarker applications, and screen for therapeutic targets through CRISPR-based screening approaches.

Despite these exciting breakthroughs and promises, the field has yet to overcome some challenges and limitations. For studies into interacting partners of ncRNAs, probe-based pulldown remains the gold standard for lncRNAs and the only viable method for circRNAs, which is susceptible to off-targeting and signal-to-noise ratio issues. Newer methods involving the recruitment of biotin ligase birA require the addition of recruitment sequences flanking the endogenous lncRNA, which can result in unexpected changes in RNA structure or localization. These methods are also not adapted for circRNAs because their 5′ and 3′ ends are back-spliced together. For the study of ncRNA structures, SHAPE techniques have changed the field drastically, but lack scalability and are not fully optimized for transcriptome-wide studies. In addition, these techniques measure the local flexibility of nucleotides to predict the overall RNA structure computationally. Techniques providing precise and direct measurement on overall RNA structure are still lacking. Although powerful, scRNA-seq and spatial transcriptomic approaches suffer from inevitable random variations and are currently limited to the detection of highly expressed genes, which may not be ideal for the study of ncRNAs with relatively low expression.

Despite these challenges and limitations, significant findings in these new areas of ncRNA research are nonetheless being revealed actively (see Outstanding Questions). Currently, we have only just begun to explore the biogenesis and functional mechanisms of lncRNAs and circRNAs in great detail, and to appreciate their clinical utilities.

Outstanding Questions

How is circRNA biogenesis regulated, are there common sequences and/or structures that facilitate the circularization of transcripts not flanked by short repeats? In particular, what are the upstream direct regulators and determining factors of circRNA isoform expression?

It is challenging to identify circRNA specific interacting partners due to their highly overlapping sequence with linear transcripts, can we develop novel techniques to identify these interacting partners effectively?

Can different lncRNAs and circRNAs be classified into groups based on their secondary structures? Are there recurring functional structure modules? Do RNA structures change in response to pathological processes or treatment conditions?

What are the specific mechanisms for lncRNA and circRNA sorting into exosomes, and how reproducible will exosome-based biomarkers be?

Advancements in technologies provided opportunities to investigate the noncoding transcriptome in unprecedented detail. How does the subtype specific expression of lncRNAs and circRNAs relate and contribute to the intratumoral heterogeneity of prostate cancer?

Current clinical trials showed modest efficacy for RNA-based therapeutics in prostate cancer. How can we improve this for better clinical utility? What roles can lncRNAs and circRNAs play in this process? Can we also explore circRNA-specific characteristics for better delivery of therapeutic agents?

References

1. Cech, T.R. and Steitz, J.A. (2014) The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* 157, 77–94
2. Memczak, S. *et al.* (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495, 333–338
3. Hansen, T.B. *et al.* (2013) Natural RNA circles function as efficient microRNA sponges. *Nature* 495, 384–388
4. Chen, S. *et al.* (2019) Widespread and functional RNA circularization in localized prostate cancer. *Cell* 176, 831–843.e22
5. Liu, C.-X. *et al.* (2019) Structure and degradation of circular RNAs regulate PKR activation in innate immunity. *Cell* 177, 865–880.e21
6. Anderson, D.M. *et al.* (2015) A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell* 160, 595–606
7. Nelson, B.R. *et al.* (2016) A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science* 351, 271–275
8. Cai, B. *et al.* (2017) LncRNA-Six1 encodes a micropeptide to activate Six1 in cis and is involved in cell proliferation and muscle growth. *Front. Physiol.* 8, 230
9. Legnini, I. *et al.* (2017) Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. *Mol. Cell* 66, 22–37.e9
10. Pamudurti, N.R. *et al.* (2017) Translation of circRNAs. *Mol. Cell* 66, 9–21.e7
11. Zhang, M. *et al.* (2018) A peptide encoded by circular form of LINC-PINT suppresses oncogenic transcriptional elongation in glioblastoma. *Nat. Commun.* 9, 141
12. Du, Z. *et al.* (2013) Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. *Nat. Struct. Mol. Biol.* 20, 908–913
13. Iyer, M.K. *et al.* (2015) The landscape of long noncoding RNAs in the human transcriptome. *Nat. Genet.* 47, 199–208
14. Chiu, H.-S. *et al.* (2018) Pan-cancer analysis of lncRNA regulation supports their targeting of cancer genes in each tumor context. *Cell Rep.* 23, 297–312.e12
15. Hansen, T.B. *et al.* (2013) Circular RNA and miR-7 in cancer. *Cancer Res.* 73, 5609–5612
16. Vo, J.N. *et al.* (2019) The landscape of circular RNA in cancer. *Cell* 176, 869–881.e13
17. Hoey, C. *et al.* (2018) miRNA-106a and prostate cancer radioresistance: a novel role for LITAF in ATM regulation. *Mol. Oncol.* 12, 1324–1341
18. Bhandari, V. *et al.* (2019) Molecular landmarks of tumor hypoxia across cancer types. *Nat. Genet.* 51, 308–318
19. Fredsøe, J. *et al.* (2018) Diagnostic and prognostic microRNA biomarkers for prostate cancer in cell-free urine. *Eur. Urol. Focus* 4, 825–833
20. Matin, F. *et al.* (2018) A plasma biomarker panel of four microRNAs for the diagnosis of prostate cancer. *Sci. Rep.* 8, 6653
21. Prensner, J.R. *et al.* (2013) The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. *Nat. Genet.* 45, 1392–1398
22. Guo, H. *et al.* (2016) Modulation of long noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. *Nat. Genet.* 48, 1142–1150
23. Hua, J.T. *et al.* (2018) Risk SNP-mediated promoter-enhancer switching drives prostate cancer through lncRNA PCAT19. *Cell* 174, 564–575.e18
24. Guarnerio, J. *et al.* (2016) Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. *Cell* 165, 289–302
25. Harrow, J. *et al.* (2012) GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res.* 22, 1760–1774
26. Lagarde, J. *et al.* (2017) High-throughput annotation of full-length long noncoding RNAs with capture long-read sequencing. *Nat. Genet.* 49, 1731–1740
27. Hon, C.-C. *et al.* (2017) An atlas of human long non-coding RNAs with accurate 5′ ends. *Nature* 543, 199–204
28. Dimitrova, N. *et al.* (2014) LincRNA-p21 Activates p21 in cis to promote polycomb target gene

expression and to enforce the G1/S checkpoint. *Mol. Cell* 54, 777–790
29. Ulitsky, I. *et al.* (2011) Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell* 147, 1537–1550
30. Delás, M.J. *et al.* (2019) lncRNA Spehd regulates hematopoietic stem and progenitor cells and is required for multilineage differentiation. *Cell Rep.* 27, 719–729.e6
31. Sarropoulos, I. *et al.* (2019) Developmental dynamics of lncRNAs across mammalian organs and species. *Nature* 25, 1915
32. Hosono, Y. *et al.* (2017) Oncogenic role of THOR, a conserved cancer/testis long non-coding RNA. *Cell* 171, 1559–1572.e20
33. Crea, F. *et al.* (2014) Identification of a long non-coding RNA as a novel biomarker and potential therapeutic target for metastatic prostate cancer. *Oncotarget* 5, 764–774
34. Ashouri, A. *et al.* (2016) Pan-cancer transcriptomic analysis associates long non-coding RNAs with key mutational driver events. *Nat. Commun.* 7, 13197
35. Huang, Q. *et al.* (2014) A prostate cancer susceptibility allele at 6q22 increases RFX6 expression by modulating HOXB13 chromatin binding. *Nat. Genet.* 46, 126–135
36. Liu, S.J. *et al.* (2017) CRISPRi-based genome-scale identification of functional long noncoding RNA loci in human cells. *Science* 355, eaah7111
37. Joung, J. *et al.* (2017) Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. *Nature* 548, 343–346
38. Liu, Y. *et al.* (2018) Genome-wide screening for functional long noncoding RNAs in human cells by Cas9 targeting of splice sites. *Nat. Biotechnol.* 36, 1203–1210
39. Bester, A.C. *et al.* (2018) An integrated genome-wide CRISPRa approach to functionalize lncRNAs in drug resistance. *Cell* 173, 649–664.e20
40. Zhu, S. *et al.* (2016) Genome-wide deletion screening of human long non-coding RNAs using a paired-guide RNA CRISPR-Cas9 library. *Nat. Biotechnol.* 34, 1279–1286
41. Kumegawa, K. *et al.* (2016) A genomic screen for long noncoding RNA genes epigenetically silenced by aberrant DNA methylation in colorectal cancer. *Sci. Rep.* 6, 26699
42. Prensner, J.R. *et al.* (2011) Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. *Nat. Biotechnol.* 29, 742–749
43. Abeshouse, A. *et al.* (2015) The molecular taxonomy of primary prostate cancer. *Cell* 163, 1011–1025
44. Robinson, D. *et al.* (2015) Integrative clinical genomics of advanced prostate cancer. *Cell* 162, 454
45. Fraser, M. *et al.* (2017) Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* 541, 359–364
46. Robinson, D.R. *et al.* (2017) Integrative clinical genomics of metastatic cancer. *Nature* 548, 297–303
47. Quigley, D.A. *et al.* (2018) Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell* 174, 758–769.e9
48. Prensner, J.R. *et al.* (2014) The long non-coding RNA PCAT-1 promotes prostate cancer cell proliferation through cMyc. *Neoplasia* 16, 900–908
49. Du, W.W. *et al.* (2016) Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. *Cell Death Differ.* 24, 357–370
50. Zhou, X. *et al.* (2017) Identification and functional characterization of circRNA-0008717 as an oncogene in osteosarcoma through sponging miR-203. *Oncotarget* 9, 22288–22300
51. Guarnerio, J. *et al.* (2016) Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. *Cell* 166, 1055–1056
52. Yang, Q. *et al.* (2017) A circular RNA promotes tumorigenesis by inducing c-myc nuclear translocation. *Cell Death Differ.* 24, 1609–1620
53. Du, W.W. *et al.* (2018) A circular RNA circ-DNMT1 enhances breast cancer progression by activating autophagy. *Oncogene* 44, 1370
54. Jeck, W.R. and Sharpless, N.E. (2014) Detecting and characterizing circular RNAs. *Nat. Biotechnol.* 32, 453–461
55. Zhang, Y. *et al.* (2013) Circular intronic long noncoding RNAs. *Mol. Cell* 51, 792–806
56. Barrett, S.P. *et al.* (2015) Circular RNA biogenesis can proceed through an exon-containing lariat precursor. *eLife* 4, 55
57. Zhang, X.-O. *et al.* (2014) Complementary sequence-mediated exon circularization. *Cell* 159, 134–147
58. Ivanov, A. *et al.* (2015) Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals. *Cell Rep.* 10, 170–177
59. Ashwal-Fluss, R. *et al.* (2014) circRNA biogenesis competes with pre-mRNA splicing. *Mol. Cell* 56, 55–66
60. Conn, S.J. *et al.* (2015) The RNA binding protein quaking regulates formation of circRNAs. *Cell* 160, 1125–1134
61. Li, X. *et al.* (2017) Coordinated circRNA biogenesis and function with NF90/NF110 in viral infection. *Mol. Cell* 67, 214–227.e7
62. Liang, D. *et al.* (2017) The output of protein-coding genes shifts to circular RNAs when the pre-mRNA processing machinery is limiting. *Mol. Cell* 68, 940–954.e3
63. Kramer, M.C. *et al.* (2015) Combinatorial control of *Drosophila* circular RNA expression by intronic repeats, hnRNPs, and SR proteins. *Genes Dev.* 29, 2168–2182
64. Liang, D. and Wilusz, J.E. (2014) Short intronic repeat sequences facilitate circular RNA production. *Genes Dev.* 28, 2233–2247
65. Zhang, Y. *et al.* (2016) The biogenesis of nascent circular RNAs. *Cell Rep.* 15, 611–624
66. Errichelli, L. *et al.* (2017) FUS affects circular RNA expression in murine embryonic stem cell-derived motor neurons. *Nat. Commun.* 8, 14741
67. Salzman, J. *et al.* (2012) Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 7, e30733
68. Li, Z. *et al.* (2015) Exon-intron circular RNAs regulate transcription in the nucleus. *Nat. Struct. Mol. Biol.* 22, 256–264
69. Piwecka, M. *et al.* (2017) Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. *Science* 8, eaam8526
70. Kristensen, L.S. *et al.* (2017) Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene* 7, 155–565
71. Stagsted, L.V. *et al.* (2019) Noncoding AUG circRNAs constitute an abundant and conserved subclass of circles. *Life Sci. Alliance* 2, e201900398
72. Boutros, P.C. *et al.* (2015) Spatial genomic heterogeneity within localized, multifocal prostate cancer. *Nat. Genet.* 47, 736–745
73. Chua, M.L.K. *et al.* (2017) A prostate cancer "nimbosus": genomic instability and SChLAP1 dysregulation underpin aggression of intraductal

and cribriform subpathologies. *Eur. Urol.* 72, 665–674
74. Bai, Q. *et al.* (2016) Detection of RNA-binding proteins by in vitro RNA pull-down in adipocyte culture. *JoVE*. https://doi.org/10.3791/54207
75. Chu, C. *et al.* (2015) Systematic discovery of Xist RNA binding proteins. *Cell* 161, 404–416
76. Ramanathan, M. *et al.* (2018) RNA-protein interaction detection in living cells. *Nat. Methods* 15, 207–212
77. Nguyen, T.A. *et al.* (2015) Functional anatomy of the human microprocessor. *Cell* 161, 1374–1387
78. Liu, N. and Pan, T. (2016) N^6-methyladenosine–encoded epitranscriptomics. *Nat. Struct. Mol. Biol.* 23, 98–102
79. Kirk, J.M. *et al.* (2018) Functional classification of long non-coding RNAs by k -mer content. *Nat. Genet.* 50, 1474–1482
80. Reuter, J.S. and Mathews, D.H. (2010) RNAstructure: software for RNA secondary structure prediction and analysis. *BMC Bioinformatics* 11, 919
81. Wilkinson, K.A. *et al.* (2006) Selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE): quantitative RNA structure analysis at single nucleotide resolution. *Nat. Protoc.* 1, 1610–1616
82. Siegfried, N.A. *et al.* (2014) RNA motif discovery by SHAPE and mutational profiling (SHAPE-MaP). *Nat. Methods* 11, 959–965
83. Flynn, R.A. *et al.* (2016) Transcriptome-wide interrogation of RNA secondary structure in living cells with icSHAPE. *Nat. Protoc.* 11, 273–290
84. Smola, M.J. *et al.* (2016) SHAPE reveals transcript-wide interactions, complex structural domains, and protein interactions across the Xist lncRNA in living cells. *Proc. Natl. Acad. Sci.* 113, 10322–10327
85. Lin, Y. *et al.* (2018) Structural analyses of NEAT1 lncRNAs suggest long-range RNA interactions that may contribute to paraspeckle architecture. *Nucleic Acids Res.* 46, 3742–3752
86. Liu, S.J. *et al.* (2016) Single-cell analysis of long non-coding RNAs in the developing human neocortex. *Genome Biol.* 17, 67
87. Lv, D. *et al.* (2016) Systematic characterization of lncRNAs' cell-to-cell expression heterogeneity in glioblastoma cells. *Oncotarget* 7, 18403–18414
88. Li, X. *et al.* (2018) Dissecting lncRNA roles in renal cell carcinoma metastasis and characterizing genomic heterogeneity by single-cell RNA-seq. *Mol. Cancer Res.* 16, 1879–1888
89. Hayashi, T. *et al.* (2018) Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. *Nat. Commun.* 9, 619
90. Miyamoto, D.T. *et al.* (2015) RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science* 349, 1351–1356
91. Berglund, E. *et al.* (2018) Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity. *Nat. Commun.* 9, 1133
92. Rodriques, S.G. *et al.* (2019) Slide-seq: a scalable technology for measuring genome-wide expression at high spatial resolution. *Science* 363, 1463–1467
93. de Kok, J.B. *et al.* (2002) DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. *Cancer Res.* 62, 2695–2698
94. Deras, I.L. *et al.* (2008) PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J. Urol.* 179, 1587–1592
95. Wang, F. *et al.* (2014) Development and prospective multicenter evaluation of the long noncoding RNA MALAT-1 as a diagnostic urinary biomarker for prostate cancer. *Oncotarget* 5, 11091–11102
96. Prensner, J.R. *et al.* (2014) RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. *Lancet Oncol.* 15, 1469–1480
97. Wang, Y.-H. *et al.* (2018) Tumor-derived exosomal long noncoding RNAs as promising diagnostic biomarkers for prostate cancer. *Cell. Physiol. Biochem.* 46, 532–545
98. Işın, M. *et al.* (2015) Exosomal lncRNA-p21 levels may help to distinguish prostate cancer from benign disease. *Front. Genet.* 6, 168
99. Zhan, Y. *et al.* (2018) Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer. *Mol. Cancer* 17, 142
100. Ahadi, A. *et al.* (2016) Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes. *Sci. Rep.* 6, 24922
101. Kong, Z. *et al.* (2017) Androgen-responsive circular RNA circSMARCA5 is up-regulated and promotes cell proliferation in prostate cancer. *Biochem. Biophys. Res. Commun.* 493, 1217–1223
102. Chakravarty, D. *et al.* (2014) The oestrogen receptor alpha-regulated lncRNA NEAT1 is a critical modulator of prostate cancer. *Nat. Commun.* 5, 5383
103. Gu, P. *et al.* (2017) lncRNA HOXD-AS1 regulates proliferation and chemo-resistance of castration-resistant prostate cancer via recruiting WDR5. *Mol. Ther.* 25, 1959–1973
104. Qu, L. *et al.* (2016) Exosome-transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. *Cancer Cell* 29, 653–668
105. Verma, A. (2018) Recent advances in antisense oligonucleotide therapy in genetic neuromuscular diseases. *Ann. Indian Acad. Neurol.* 21, 3–8
106. Adams, D. *et al.* (2018) Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N. Engl. J. Med.* 379, 11–21
107. Meng, L. *et al.* (2015) Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. *Nature* 518, 409–412
108. Ämmälä, C. *et al.* (2017) Targeted delivery of antisense oligonucleotides to pancreatic b-cells. *Sci. Adv.* 4, eaat3386
109. Gao, S. *et al.* (2009) The effect of chemical modification and nanoparticle formation on stability and biodistribution of siRNA in mice. *Mol. Ther.* 17, 1225–1233
110. Sternberg, C.N. *et al.* (2009) Docetaxel plus oblimersen sodium (Bcl-2 antisense oligonucleotide): an EORTC multicenter, randomized phase II study in patients with castration-resistant prostate cancer. *Ann. Oncol.* 20, 1264–1269
111. Beer, T.M. *et al.* (2017) Custirsen (OGX-011) combined with cabazitaxel and prednisone versus cabazitaxel and prednisone alone in patients with metastatic castration-resistant prostate cancer previously treated with docetaxel (AFFINITY): a randomised, open-label, international, phase 3 trial. *Lancet Oncol.* 18, 1532–1542
112. Chi, K.N. *et al.* (2016) A phase I dose-escalation study of apatorsen (OGX-427), an antisense inhibitor targeting heat shock protein 27 (Hsp27), in patients with castration-resistant prostate cancer and other advanced cancers. *Ann. Oncol.* 27, 1116–1122
113. Wesselhoeft, R.A. *et al.* (2018) Engineering circular RNA for potent and stable translation in eukaryotic cells. *Nat. Commun.* 9, 1838
114. Litke, J.L. and Jaffrey, S.R. (2019) Highly efficient expression of circular RNA aptamers in cells using autocatalytic transcripts. *Nat. Biotechnol.* 37, 667–675