Review

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Role of lncRNAs in prostate cancer development and progression

Abstract: Prostate cancer (PCa) is the second most common cause of cancer-related deaths in men. Despite advances in the characterization of genomic and epigenetic aberrations contributing to PCa, the etiology of PCa is still far from being understood. Research over the past decade demonstrated the role of long non-coding RNAs (lncRNAs) in deregulation of target genes mainly through epigenetic mechanisms. In PCa, evidence accumulated that hundreds of lncRNAs are dysregulated. Functional analyses revealed their contribution to prostate carcinogenesis by targeting relevant pathways and gene regulation mechanisms including PTEN/AKT and androgen receptor signaling as well as chromatin remodeling complexes. Here we summarize our current knowledge on the roles of lncRNAs in PCa and their potential use as biomarkers for aggressive PCa and as novel therapeutic targets.

Keywords: androgen receptor; chromatin remodeling; epigenetics; polycomb repressive complex (PRC); pseudogene; PTEN.

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Introduction

Prostate cancer (PCa) is the second most common cause of cancer-related deaths in men (Bray et al., 2013). PCa develops in a series of steps from prostatic intraepithelial neoplasia to localized tumors, which can further progress to metastatic castration-resistant prostate cancer (mCRPC). Integrative genomic profiling has identified an accumulation of genetic aberrations, including androgen-driven chromosomal rearrangements and oncogene fusion genes, deletions of tumor suppressor genes (TSG) such as *PTEN* (phosphatase and tensin homolog gene), and amplification and mutations of the androgen receptor (AR) contributing to prostate carcinogenesis (Taylor et al., 2010; Grasso et al., 2012; Weischenfeldt et al., 2013). Additionally, epigenetic modifications, including DNA methylation in the promoter region of TSGs, alterations in histone modifying enzymes, along with changes in the abundance and variety of non-coding RNAs (ncRNAs) have been shown to dysregulate cancer-relevant cellular pathways in PCa, such as cell cycle progression and hormonal response (Jeronimo et al., 2011).

Research over the past decade has accumulated evidence that genomic regions without protein-coding potential are transcribed into non-coding transcripts (ncRNAs) that are often altered during carcinogenesis (Esteller, 2011; Prensner and Chinnaiyan, 2011). Non-coding RNAs have been arbitrarily divided into short ncRNAs with a size <200 nt and long ncRNAs (lncRNAs) with a size >200 nt (Ponting et al., 2009). LncRNAs are associated with numerous roles, including alternative splicing, modulation of protein localization or mRNA decoy. A broad area of their function involves transcriptional regulation, notably through the recruitment of transcriptional regulators and chromatin modifiers (Maruyama and Suzuki, 2012). A common theme developing is that lncRNAs mediate the interactions of protein complexes with specific target sites in the genome. LncRNAs have the ability to recruit chromatin repressive complexes to target genes thus mediating gene silencing (Lee, 2012). More recent studies demonstrated also the recruitment of gene activating activities (Yang et al., 2013). As an example, a recent report describes TARID (TCF21 antisense RNA inducing demethylation) as a mediator of GADD45A/TDG/TET (Growth Arrest and DNA Damage-inducible 45/thymine-DNA glycosylase/Ten-eleven-translocation)-mediated DNA demethylation in the promoter of tumor suppressor *TCF21* (transcription factor 21) (Arab et al., 2014).

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Intriguingly, lncRNAs contribute to 'long-range' remodeling of the epigenetic landscape, for example as shown for the lncRNA Xist (X-inactive specific transcript), which was one of the earliest characterized lncRNAs. XIST is known to repress the inactive X-chromosome in females by recruitment of the polycomb repressive complex 2 (PRC2). The transcript is commonly depleted in female cancers while derepressed in male cancers, suggesting its role in tumor development (Weakley et al., 2011). Accordingly, chromosomal looping might enable recognition of distant lncRNA-targets, leading to their spatial proximity, thereby allowing widespread chromatin remodeling and coordinate regulation of the expression of genes or gene clusters (Mercer and Mattick, 2013).

Recent large scale RNA profiling projects have identified a multitude of novel lncRNAs dysregulated in PCa. RNA sequencing identified a set of 121 PCa-associated intergenic non-coding RNA transcripts termed PCAT family (Prensner et al., 2011). Transcriptional profiling of 14 tumors derived from Chinese PCa patients identified 406 lncRNAs differentially expressed during prostate carcinogenesis (Ren et al., 2012). Independent of RNA sequencing, Liu and colleagues developed a reannotation pipeline of the Affymetrix microarray probes mapping to lncRNAs. With this methodology and based on available expression data, they characterized a set of 102 novel lncRNAs upregulated in PCa (Du et al., 2013).

Despite this growing number of lncRNAs identified in PCa, only a few have been functionally characterized so far. Here we summarize the current knowledge on lncRNAs deregulated in PCa and the common emerging mechanisms in which they are implicated to trigger PCa development and progression, including PTEN/AKT and AR signaling as well as targeting of chromatin remodeling complexes (Table 1). We also outline their potential use as biomarkers for diagnosis of aggressive PCa and PCa progression, along with some novel approaches to target lncRNAs as a therapeutic strategy in the clinical management of PCa.

Key pathways dysregulated in prostate cancer by lncRNAs

Genetic alterations in the PTEN/AKT pathway and AR signaling are known to play causative roles in PCa development. Interestingly however, a series of studies has identified a complex network of mechanisms mediated by ncRNAs that regulate PTEN function at multiple levels (Figure 1). Also, various lncRNAs have been shown to function as AR co-activators or co-repressors (Figure 2).

PTEN/AKT pathway regulators

PTEN (phosphatase and tensin homolog gene) is a tumor suppressor gene commonly and gradually inactivated during PCa progression by somatic mutations as well as deletions (Phin et al., 2013). Loss of *PTEN* leads to activation of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway, stimulation of cell cycle progression and cell proliferation.

Besides genetic inactivation, PTEN is targeted by several microRNAs (miRNAs) from the miR-106b-93-25 cluster known to be overexpressed in PCa (Figure 1). Overexpression of the miR cluster in prostate epithelium reduced PTEN expression and initiated prostate tumorigenesis in a transgenic mouse model (Poliseno et al., 2010a). Interestingly, the same set of miRNAs was shown to target PTENpg1, a pseudogene-derived RNA highly homologous to PTEN (Figure 1A). In normal human tissues and PCa samples, PTEN and PTENpg1 expression are highly correlated. Overexpression of the PTENpg1 3′-untranslated region (UTR) in PCa cells enabled PTEN derepression (Poliseno et al., 2010b), indicating that PTENpg1 might function as a competitive endogenous RNA (ceRNA) and decoy for miRNAs targeting PTEN (Tay et al., 2011). In a series of colon cancer samples, PTENpg1 was downregulated and specifically deleted, and *PTENpg1* copy number variation correlated with PTEN expression. These data support the idea that loss of PTENpg1 contributes to PTEN inactivation (Poliseno et al., 2010b).

Recently, Johnsson et al. elegantly identified two PTENpg1 antisense ncRNAs designated as PTENpg1as α and β, with distinct functions in regulating PTEN expression (Johnsson et al., 2013). PTENpg1as α was shown to operate in *trans* by recruiting the polycomb repressive complex 2 (PRC2) and DNA methyltransferase 3a (DNMT3a) to the *PTEN* locus, thus antagonizing PTEN transcription by histone 3 lysine 27 tri-methylation (H3K27me3) (Figure 1B). Conversely, the variant β was shown to stabilize PTENpg1 transcript by the formation of a RNA duplex, enabling PTENpg1 to function as a miRNAs sponge (Figure 1C).

The PI3K/AKT pathway can be further activated by upregulation of Linc00963 (Figure 1D), demonstrated in the androgen-independent C4-2 PCa cell line compared to the androgen-sensitive LNCaP PCa cell line. The lincRNA was found to stimulate EGFR (epidermal growth factor

Table 1 lncRNAs associated with prostate carcinogenesis.

lncRNA	Locus	lncRNA type	Expression levels	Role in carcinogenesis	AR-reg.	References
PTEN/AKT pathway regulators						
PTENpg1 (PTENP1) (phosphatase and tensin homolog pseudogene 1)	chr9p13.3	pseudogene, trans regulation	↓ in colon cancer	↓ colony formation ↓ cell proliferation		(Poliseno et al., 2010a,b)
PTENpg1 AS (α and β form)	chr9p13.3	α form: trans β form: cis regulation				(Johnsson et al., 2013)
Linc00963 (long intergenic non-protein-coding RNA 963)	chr9q34.1	intergenic	↑ in metastatic androgen-independent vs. androgen-dependent cell line	↑ cell viability, migration, invasion ↓ apoptosis		(Wang et al., 2014)
Androgen receptor (AR) regulators						
PCGEM1 (LINC00071) (prostate cancer gene expression marker 1)	chr2q32.3	intergenic, trans regulation	↑ in PIN and localized PCa ↑ in African-American PCa patients vs. Caucasian-Americans	Oncogene ↑ cell proliferation ↓ apoptosis in AR-dependent cell lines	Yes	(Srikantan et al., 2000) (Petrovics et al., 2004) (Fu et al., 2006) (Yang et al., 2013) (Prensner et al., 2014b)
PRNCR1 (PCAT8) (prostate cancer non-coding RNA 1)	chr8q24.2	intergenic, trans regulation	↑ in PIN and PCa ↔ in PCa	Oncogene ↑ cell viability		(Chung et al., 2011) (Yang et al., 2013) (Prensner et al., 2014b)
CBR3-AS1 (PlncRNA-1) (carbonyl reductase 3 antisense RNA 1)	chr21q22.12	antisense	↑ in localized PCa	↑ cell viability ↓ apoptosis	Yes	(Cui et al., 2013)
PCA3 (DD3) (prostate cancer antigen 3)	chr9q21.3	antisense	↑ in localized PCa and metastatic samples	↑ cell survival ↑ cell growth ↓ apoptosis	Yes	(Bussemakers et al., 1999) (Auprich et al., 2011) (Ferreira et al., 2012)
CTBP1-AS (PCAT10) (C-terminal binding protein 1 antisense RNA)	chr4p16.3	antisense cis and trans regulation	↑ in localized PCa and metastases	Oncogene ↑ hormone-dependent and castration-resistant tumor growth ↑ cell cycle progression ↑ cell proliferation	Yes	(Takayama et al., 2013)
GAS5 (growth arrest-specific 5)	chr1q25.1	intergenic, trans regulation	↓ during androgen deprivation in an LNCaP hollow fiber model ↑ in PCa patients and metastases	↓ apoptosis ↑ cell survival Interaction with steroid receptor DNA binding site		(Mourtada-Maarabouni et al., 2009) (Romanuik et al., 2010) (Pickard et al., 2013) (Du et al., 2013)
Chromatin remodeling						
ANRIL (p15AS) (antisense non-coding RNA in the INK4 locus)	chr9p21.3	antisense cis regulation	↑ in localized PCa	↓ cell senescence ↑ proliferation		(Yap et al., 2010) (Kotake et al., 2011)
ANRASSF1 (antisense intronic non-coding RASSF1 RNA)	chr3p21.31	antisense cis regulation	↑ in LNCaP and DU145 vs. RWPE cell lines	↑ proliferation ↓ apoptosis		(Beckedorff et al., 2013)
PCAT-1 (prostate cancer-associated transcript-1)	chr8q24.21	intergenic, trans regulation	↑ in high-grade localized PCa and metastatic samples	Oncogene ↑ cell proliferation		(Prensner et al., 2011) (Prensner et al., 2014a)

(**Table 1** Continued)

lncRNA	Locus	lncRNA type	Expression levels	Role in carcinogenesis	AR-reg.	References
SChLAP1 (PCAT114) (second chromosome locus associated with prostate 1)	chr2q31	intergenic, trans regulation	↑ in localized PCa and metastatic samples	Oncogene ↑ cell invasiveness and metastasis		(Prensner et al., 2013)
LncRNAs regulated by genomic imprinting						
H19 (imprinted maternally expressed transcript)	chr11p15.5	intergenic	↓ with aging ↑ in PCa patients	LOI in normal PCa tissue is associated with cancer susceptibility	Yes	(Fu et al., 2008) (Fu et al., 2004) (Berteaux et al., 2004) (Ribarska et al., 2014)
Meg3 (maternally expressed 3)	chr14q32.2	intergenic	↓ in localized PCa vs. normal samples	↑ proliferation (other cancer types) ↓ apoptosis (other cancer types)		(Zhou et al., 2012) (Du et al., 2013) (Ribarska et al., 2014)
Putative biomarkers						
MALAT-1 (NEAT2) (metastasis-associated lung adenocarcinoma transcript 1)	chr11q13.1	intergenic	↑ progressively from low to high Gleason score, serum PSA and tumor stage ↑ in CRPC than localized tumors	Oncogene ↑ proliferation ↑ invasion, migration, metastasis		(Ren et al., 2013a)
PCAN-R1 (PCAT6 or KDM5B-AS1) (prostate cancer-associated non-coding RNA 1)	chr1q32.1	intergenic	↑ progressively from localized PCa to metastatic samples	↑ proliferation		(Du et al., 2013)
PCAN-R2 (PCAT7) (prostate cancer-associated non-coding RNA 2)	chr9q22.32	antisense	↑ progressively from localized PCa to metastatic samples	↑ proliferation		(Du et al., 2013)
PCAT-18 (LOC728606) (prostate cancer-associated transcript-18)	chr18q11.2	intergenic	↑ in high and low Gleason score patients vs. BPH samples ↑ progressively from normal to mCRPC plasma samples	↑ cell migration, invasion, proliferation ↓ apoptosis	Yes	(Crea et al., 2014)
PCAT-29 (prostate cancer-associated transcript-29)	chr15q23	intergenic	↑ in androgen-dependent vs. androgen-independent and normal cell lines ↓ in response to DHT ↓ in PCa patients with higher biochemical recurrence	Tumor suppressor ↓ cell migration ↓ proliferation	Yes	(Malik et al., 2014)
TRPM2-AS (transient receptor potential cation channel, subfamily M, member 2, antisense RNA)	chr21q22.3	antisense cis regulation	↑ in TMPRSS2:ERG positive and metastatic PCa patients ↑ in PCa patients with higher biochemical recurrence	Oncogene ↓ apoptosis ↓ cellular stress		(Orfanelli et al., 2014)

↑ upregulation, higher expression, enhanced activity; ↓ downregulated, lower expression, reduced activity; AR-reg., androgen receptor regulated; BPH, benign prostatic hyperplasia; LOI, loss of imprinting; (m)CRPC, (metastatic) castration-resistant prostate cancer; PCa, prostate cancer; PIN, prostate intraepithelial neoplasia.

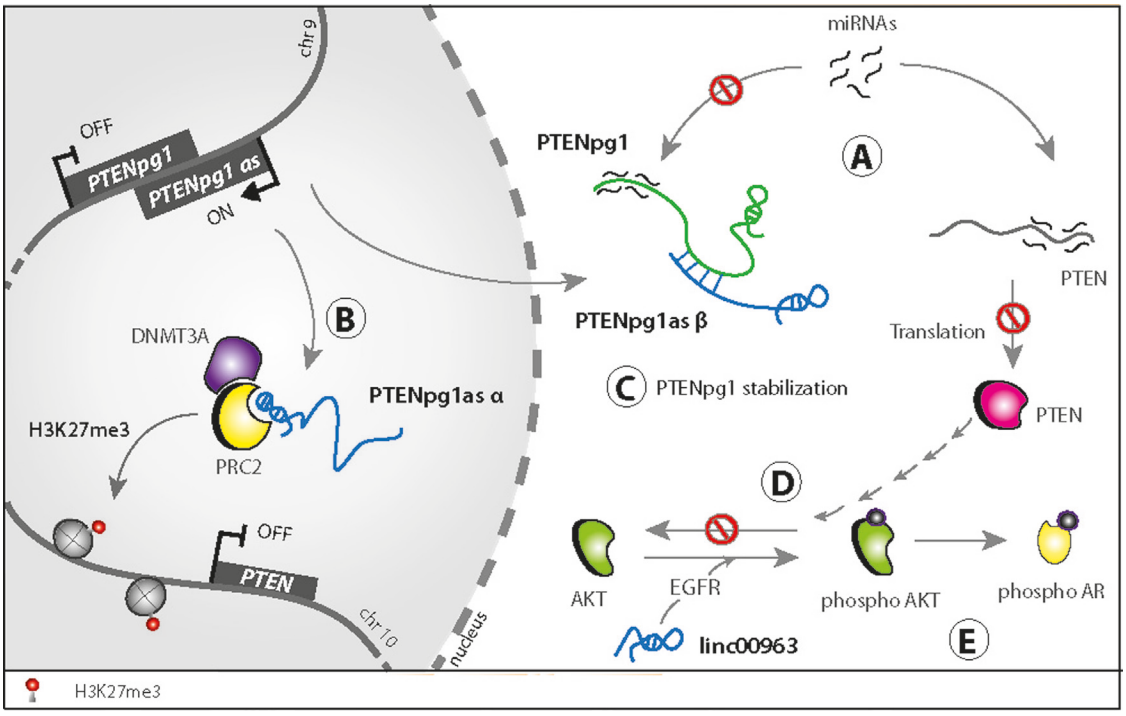

Figure 1 Non-coding RNA-mediated PTEN/AKT pathway regulation.
DNMT3A, DNA methyltransferase 3A; EGFR: epidermal growth factor receptor; H3K27me3, histone 2 lysine 27 tri-methylation; miRNAs, microRNAs; phosphoAKT, phosphorylated protein kinase B; phosphor AR, phosphorylated androgen receptor; PRC2, Polycomb repressive complex 2; PTEN, phosphatase and tensin homolog gene; PTENpg1, phosphatase and tensin homolog pseudogene 1; PTENpg1as α and β, phosphatase and tensin homolog pseudogene 1 antisense α and β. The details are given in the text.

receptor) expression and promote AKT signaling, thus facilitating the development of androgen-independent PCa with enhanced cell proliferation, migration and invasion (Wang et al., 2014).

Regulation of androgen receptor (AR) activity

The physiological development of the prostate gland as well as the malignant progression of PCa is dependent on the AR. AR activation by steroid hormones promotes nuclear translocation of the dimerized receptor, which in turn leads to the expression of AR target genes, such as *NKX3.1* or *PSA* (prostate-specific antigen). AR transcriptional activation is mediated by the recognition of androgen response elements (ARE) on the DNA, and its specificity is regulated by multiple cofactors such as CREB-binding protein (CBP)/p300 or through the interplay of lncRNAs (Fu et al., 2000).

To maintain a constant level of AR, the cell uses several tricks to overcome AR deprivation therapy. *AR* gene amplification and upregulation is a common feature of castration-resistant PCa. The expression of splice variants and somatic mutations of AR have also been reported and result in constant activation of the receptor or activation by alternative ligands than steroids, respectively (Mills, 2014). PTEN inactivation was shown to activate AR as a consequence of AKT activation (Figure 1E), which can interact with and phosphorylate AR, leading to AR-mediated signaling in a ligand-independent way (Phin et al., 2013).

In addition to these mechanisms, lncRNA-dependent AR activation strategies have been brought to light recently, which either co-activate or co-repress AR function (Figure 2).

PCGEM1 and *PRNCR1*

The best studied examples of lncRNAs with AR co-activator functions are PCGEM1 (prostate cancer gene expression marker 1) and PRNCR1 (prostate cancer non-coding RNA 1), both overexpressed in PCa (Petrovics et al., 2004; Chung et al., 2011). They have been shown to activate the AR even in the absence of its ligand or in presence of a truncated version of AR (AR-V7) present in castration-resistant

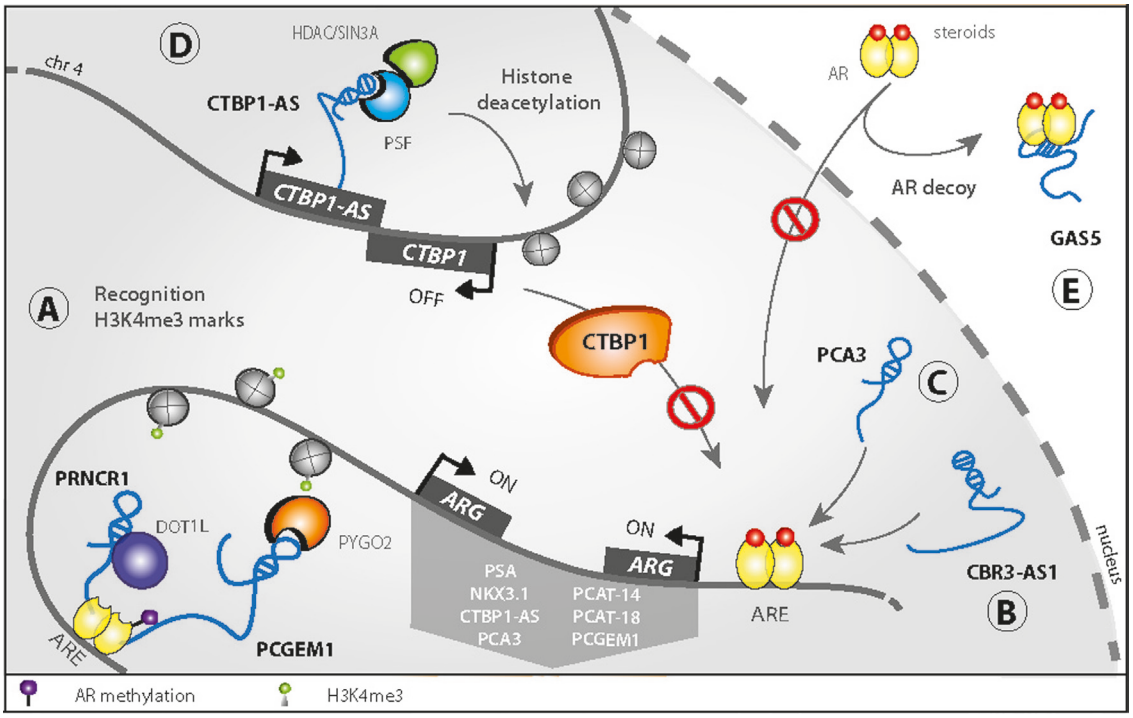

Figure 2 Long non-coding RNA-mediated androgen receptor regulation.
AKT, protein kinase B; AR, androgen receptor; ARE, androgen response elements; *ARG*, androgen regulated gene; CBR3-AS1, carbonyl reductase 3 antisense RNA 1; CTBP1, C-terminal binding protein 1; CTBP1-AS, C-terminal binding protein 1 antisense RNA; DOT1L, DOT1-like histone H3K79 methyltransferase; GAS5, growth arrest-specific 5; HDAC/SIN3A, histone deacetylase and sin3A complex; PCGEM1, prostate cancer gene expression marker 1; phospho, phosphorylated; PRNCR1, prostate cancer non-coding RNA 1; PSF, PTB-associated splicing factor; PTEN, phosphatase and tensin homolog gene; PYGO2, pygopus 2. For a detailed description see text.

PCa cells. RNA immunoprecipitation (RIP) experiments demonstrated the interaction between PRNCR1 and the AR. This binding leads to recruitment of DOT1L methyltransferase, which methylates AR and allows the subsequent interaction of PCGEM1 with the methylated AR. In turn, PCGEM1 recruits PYGO2 (Pygopus 2), which enables the binding of AR to H3K4me3 chromatin marks in the promoter regions of AR-regulated genes and leads to their activation (Figure 2A) (Yang et al., 2013).

Notably, an independent team recently failed to recapitulate the interaction between both lncRNAs and AR in LNCaP cells under the same RIP conditions as used in the previous study. Moreover, based on four PCa RNA-seq studies PRNCR1 was not overexpressed in aggressive PCa and was not associated with prostate cancer-specific mortality (Prensner et al., 2014b).

CBR3-AS (PlncRNA-1)

Using RNA sequencing, Cui et al. identified that CBR3-AS (CBR3-antisense) was upregulated in localized PCa samples relative to normal prostate epithelial cells. In reporter experiments, CBR3-AS knockdown reduced activation of an ARE reporter. Moreover, AR and NKX3.1 expression were reduced after CBR3-AS silencing. These findings suggests that CBR3-AS participates in the activation of the AR through regulation of *AR* expression itself (Figure 2B). However, a direct interaction between these two factors was not demonstrated (Cui et al., 2013).

PCA3

This lncRNA was originally described in 1999 by Isaacs and colleagues as being highly upregulated in PCa tissues compared to normal and benign prostate hyperplasia (Bussemakers et al., 1999), leading to its development as a PCa biomarker (see section “Detection of aggressive PCa”). More recent studies indicate that PCA3 expression might play a functional role in PCa progression. Indeed, PCA3 silencing in DHT-stimulated LNCaP cells reduced the expression of AR-regulated genes, and reduced cell growth and viability through induction of cell cycle arrest and apoptosis (Figure 2C) (Ferreira et al., 2012).

CTBP1-AS

CTBP1 (C-terminal binding protein 1) is a transcriptional co-repressor of AR. The binding of CTBP1 to the G9A histone methyltransferase leads to the transcriptional repression of AR-regulated genes via deposition of H3K9me3 histone marks. The expression of this protein-coding gene is inversely correlated with the increased expression of its antisense transcript CTBP1-AS from localized tumors to metastatic samples. Interaction of the nascent lncRNA with the transcriptional repressor PSF (PTB-associated splicing factor) and HDAC/Sin3A complex leads to the repression of the sense transcript *CTBP1* in AR-dependent and independent cells. The effect of CTBP1-AS on AR target genes and cell cycle promotion works indirectly via the repression of *CTBP1* (Figure 2D). Nevertheless, the lncRNA was suggested to also directly repress expression of other genes by acting in trans through targeting PSF and HDACs/Sin3a to these sites. Altogether, CTBP1-AS promotes the oncogenic growth of PCa highlighted in a xenograft model of CTBP1-AS overexpression cells (Takayama et al., 2013).

GAS5

Downregulation of GAS5 (growth arrest-specific 5) has been reported in several malignancies, such as breast or lung cancer. Also, GAS5 is downregulated in PCa cell lines derived from metastases vs. primary tissue (Mourtada-Maarabouni et al., 2009). In an *in vivo* LNCaP hollow fiber mouse model which is reminiscent of the progression of PCa cells to androgen independence, GAS5 expression decreased in castration-resistant cells (Romanuik et al., 2010). However, in a recent study on gene expression related to PCa progression in patient samples, GAS5 was identified as an lncRNA rather upregulated in PCa tissue and metastasis vs. normal prostate tissue, similar to PCAT-1 (Du et al., 2013).

Chrousos and colleagues demonstrated that GAS5 interacts with several steroid receptors, including the ligand-bound AR. GAS5 may act as a decoy by interacting with the AR DNA binding domain in form of a double stranded RNA mimic, thus preventing the binding of AR to its target AREs, and restricts the expression of cell survival genes (Figure 2E) (Kino et al., 2010). Indeed, overexpression of this lncRNA in 22Rv1 prostate carcinoma cells was associated with the promotion of apoptosis (Pickard et al., 2013). However, more mechanistic studies are needed to confirm the binding of GAS5 to AR and to assess the impact in the context of prostate tissue.

lncRNAs regulated by AR activation

Besides protein-coding RNAs, ncRNAs were reported to be regulated by AR (Takayama et al., 2011). AR localization by ChIP-on-chip techniques combined with 5′-CAGE (cap analysis of gene expression) sequencing of dihydrotestosterone (DHT)-treated LNCaP PCa cells enabled the identification of AR-regulated lncRNAs such as CTBP1-AS (Takayama et al., 2013). Similarly, PCAT-14 (prostate cancer-associated transcript-14) positively responded to AR activation by the synthetic androgen methyltrienolone (R1881) in LNCaP cells (Prensner et al., 2011). Another member of the PCAT family, PCAT-18 was upregulated after DHT treatment, although this effect was suggested to be due to the activation of upstream transcription factors (Crea et al., 2014). In contrast, the lncRNA H19 was downregulated after DHT treatment in LNCaP cells (Berteaux et al., 2004). Also, expression of PCAT-29 (prostate cancer-associated transcript 29) under the control of AR binding to its promoter was reduced after DHT treatment in AR-positive cell lines (Malik et al., 2014).

The AR-cofactor PCGEM1 was shown to trigger its own expression via a positive feedback loop through AR. Indeed, PCGEM1 was originally described to be expressed after AR stimulation of LNCaP cells and absent in AR-negative cell lines (Srikantan et al., 2000). Similarly, AR activity directly controls the expression of PCA3. PCA3 upregulation by DHT in LNCaP cells was abolished by application of an AR antagonist (Ferreira et al., 2012).

lncRNA-mediated chromatin remodeling

Chromatin modifiers are often the target of deficiencies during tumorigenesis. EZH2 (enhancer of zeste 2), one component of the PRC2 complex, is commonly overexpressed in PCa, resulting in aberrant silencing of TSGs. Additionally, lncRNA-dependent recruitment of chromatin modifiers could be related to the altered chromatin patterns reported in cancer cells (Gregory and Shiekhattar, 2004), leading to the inactivation of TSGs and activation of oncogenes. As an example, the lncRNAs HOTAIR and KCNQ10T1 exert their function through binding to the polycomb complex, but this was not shown in the context of PCa so far (Lee, 2012). Coupling of lncRNAs with this chromatin modifier complex is a common feature for numerous lncRNAs, indicating a fine-tuning of lncRNA-dependent gene expression (Figure 3).

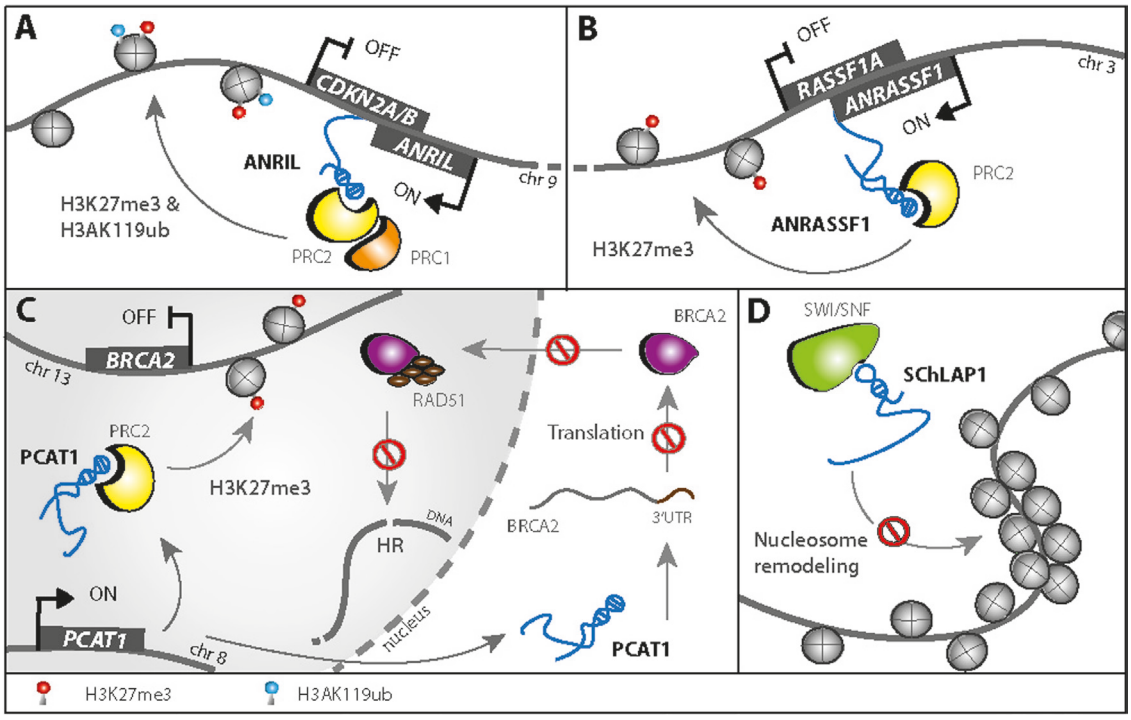

Figure 3 Long non-coding RNA-mediated chromatin remodeling.
ANRASSF1, antisense intronic non-coding RASSF1 RNA; ANRIL, antisense non-coding RNA in the INK4 locus; BRCA2: breast cancer 2, early onset; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A; DNMT3A, DNA methyltransferase 3A; HR, homologous recombination; PCAT-1, prostate cancer-associated transcript 1; PRC1/2, Polycomb repressive complex 1 and 2; PSF, PTB-associated splicing factor; RAD51, RAD51 homolog A SChLAP1, second chromosome locus associated with prostate 1; *RASSF1A*, Ras association domain family 1, isoform A; SWI/SNF, Switch/sucrose non-fermentable complex. Details are given in the text.

lncRNAs associated with epigenetic silencing

In addition to the lncRNAs described below, PTENpg1as α, CTBP1-AS, PRNCR1 and PCGEM1 have been shown to interact with chromatin remodeling complexes (for details see section "Key pathways dysregulated in PCa").

ANRIL

The *INK4/ARF* locus located on chromosome 9 encodes not only for the three TSGs cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A* and *CDKN2B*) and *ARF* (alternate reading frame of the *INK4a/ARF* locus), but also for the antisense transcript ANRIL (antisense non-coding RNA in the INK4 locus). Elevated expression of ANRIL in localized tumors was associated with the repression of *CDKN2A* and *CDKN2B*, while *ARF* expression remained unaffected (Yap et al., 2010). CDKN2B (also known as *p15* or *INK4b*) and CDKN2A (known as *p16* or *INK4a*) block retinoblastoma (Guttman et al., 2009) protein phosphorylation through inhibition of cyclin-dependent kinase (CDK) complexes (Sherr and Roberts, 1999). Consequently, repression of *CDKN2A* and *CDKN2B* triggers uncontrolled proliferation, a hallmark of cancerous cells. Repression of the TSGs is mediated by binding of ANRIL to CBX7 (chromobox 7), a component of the polycomb repressive complex 1 (PRC1), promoting mono-ubiquitination of histone 2A lysine 119 (H2AK119). Additionally, chromatin immunoprecipitation (ChIP) experiments revealed decreased association of PRC2 component EZH2 and loss of H3K27me3 marks at the *INK4* locus after ANRIL silencing. This insight was further confirmed by Xiong and colleagues, who proved by RNA immunoprecipitation the interaction between SUZ12, another PRC2 component, and ANRIL, leading to *cis*-repression of the *INK4* locus by H3K27me3 deposition (Kotake et al., 2011) (Figure 3A).

ANRASSF1

The unspliced antisense intronic non-coding RASSF1A (ANRASSF1) was identified by RNA sequencing to be overexpressed in LNCaP and DU145 PCa cell lines vs. normal prostate cells (Beckedorff et al., 2013). The nascent

ANRASSF1 transcript was shown to repress the tumor suppressor gene *RASSF1A* (Ras Association Domain family 1, isoform A) through recruitment of the PRC2 complex in *cis*. *RASSF1A* was previously reported to be epigenetically silenced by DNA methylation via homeobox 3B (HOX3B)-dependent recruitment of DNA methyltransferase 3B (DNMT3B) to the *RASSF1A* promoter (Palakurthy et al., 2009). However, after knockdown of ANRASSF1, Beckedorff et al. did not detect a change in DNA methylation or DNMT3B recruitment to the *RASSF1A* locus (Beckedorff et al., 2013) **(**Figure 3B).

PCAT-1

PCAT-1 (prostate cancer-associated transcript 1) is a lincRNA (long intergenic non-coding RNA) expressed from a commonly amplified locus on chromosome 8. PCAT-1 is upregulated in metastatic PCa samples and to a lesser extent in localized tumors. Elevated nuclear expression of PCAT-1 was associated with downregulation of BRCA2 (breast cancer 2, early onset) and the centromere-associated proteins E and F (CENPE and CENPF) and promoted cell proliferation. In a study by Prensner et al., the nuclear fraction of PCAT-1 was shown to interact with the PRC2 complex and mediated the *trans* repression (Prensner et al., 2011). In a recent follow-up study, the same group demonstrated that the bulk of PCAT-1 was detectable in the cytoplasm. The authors suggest that BRCA2 repression by cytoplasmic PCAT-1 arises at the post-transcriptional level by a mechanism involving the 3′UTR of BRCA2. BRCA2 is involved in DNA repair by recruiting RAD51 recombinase to the site of DNA double-strand breaks. Thus, BRCA2 repression by PCAT-1 leads to impaired homologous recombination repair in PCa (Prensner et al., 2014a) **(**Figure 3C).

Nucleosome remodeling

Gene expression is regulated by chromatin modifiers in concert with the remodeling of nucleosomes along the chromatin. Nucleosome motion in promoter regions is mediated by ATP-dependent chromatin remodeling complexes, such as the SWI/SNF (SWItch/Sucrose NonFermentable) complex. Components of the SWI/SNF complex are frequently mutated in cancer, notably the transcription activator *BRG1* (Brahma-related gene 1, also known as ATP-dependent helicase SMARCA4, SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4) in PCa cells (Roberts and Orkin, 2004) and in small cell carcinoma of the ovary (Jelinic et al., 2014).

A second layer of regulation stem from the recently described lincRNA *SChLAP1* (second chromosome locus associated with prostate 1, also called LINC00913) upregulated in about 25% of aggressive PCa. SChLAP1 interacts with the SWI/SNF subunit SNF5/INI1 (also known as SMARCB1). Upregulation of this lincRNA in PCa cells was associated with decreased SNF5 recruitment to DNA, thus antagonizing the genome-wide localization and regulatory functions of the SWI/SNF complex **(**Figure 3D). SChLAP1 silencing in castration-resistant 22Rv1 cells reduced the propensity to form secondary lesions *in vivo*, further supporting its role in cancer progression to metastasis (Prensner et al., 2013). A recent report supports the potential use of SChLAP1 expression as a predictor of PCA-specific mortality (Prensner et al., 2014b).

LncRNA expression regulated by genomic imprinting

Beyond working as scaffolds to recruit chromatin remodeling complexes to regulate specific genomic loci, lncRNA expression can in turn be modulated by the deposition of epigenetic modifications, such as DNA methylation.

H19

LncRNA H19 is the lncRNA that was first described in mammals. This lncRNA is specifically expressed from the maternal allele, whereas the flanking gene *IGF2* (insulin-like growth factor 2) is expressed from the paternal allele. Both genes are under the control of an imprinting center (IC) located upstream of *H19* and an enhancer. The IC acts as an insulator, recognized by the transcriptional regulator CTCF [CCCTC-Binding Factor (Zinc Finger Protein)] on the maternal allele, leading to *IGF2* repression. Conversely, IC is methylated on the paternal allele, preventing CTCF binding and allowing *IGF2* expression through the downstream enhancer. Loss of *H19*/*IGF2* imprinting is a common phenomenon in tissue susceptible to aging, such as prostate, and is further accentuated during carcinogenesis. In this case, decreased CTCF expression and binding to IC might allow methylation of the IC. Consequently, IGF2 is expressed from both alleles (Fu et al., 2004, 2008). In a recent study, Ribarska et al. reported downregulation of both IGF2 and H19 in PCa samples versus benign prostate tissue, although methylation of

the IC and surrounding regions was not altered (Ribarska et al., 2014).

MEG3

The paternally imprinted lncRNA MEG3 (maternally expressed gene 3) is downregulated in numerous cancer types. Overexpression of this lncRNA is associated with decreased cell growth. MEG3 reduces the expression of the E3 ubiquitin protein ligase MDM2, thereby activating p53 by preventing its proteasomal degradation (Zhou et al., 2012). MEG3 is downregulated in PCa relative to benign prostate samples. Hypermethylation of the *MEG3* promoter region was detected by pyrosequencing in tumor samples, suggesting that DNA methylation is involved in the loss of expression of MEG3 in PCa (Ribarska et al., 2014). Reduced expression of MEG3 in localized and metastatic PCa samples vs. normal prostate was corroborated in an independent dataset (Du et al., 2013).

Diagnostic and therapeutic potential of lncRNAs in PCa

Detection of aggressive PCa

Beside surgery and radiation therapy, androgen deprivation therapy is one of the options in PCa treatment to control growth and spreading of PCa cells. However, tumor cells often overcome hormone deprivation and become androgen-independent. Aberrant activation of the AR pathway is involved in this transition. Indeed, the previously mentioned lncRNAs PCGEM1, PRNCR1, GAS5 and Linc00963 are thought to play a critical role in the progression from androgen-dependent to -independent growth of cancer cell lines, which might subsequently trigger the development of metastases. The role of PCGEM1 and PRNCR1 in this transition has recently been challenged (Prensner et al., 2014b).

Unlike protein-coding RNAs or miRNAs, the majority of lncRNAs exhibits a tissue or physiological-specific expression pattern. For instance, PCAT-1 and -18 share a restricted elevated expression pattern in high-grade PCa patients (Prensner et al., 2011; Crea et al., 2014). This limited expression pattern of lncRNAs supports the notion that they represent great potential for a specific and accurate diagnosis of PCa and for the identification of patients with an increased risk to progress to metastasis. So far, stratification of PCa risk is mostly based on the detection of PSA levels in serum. Nevertheless, this test fails to discriminate benign from malignant PCa and is also detectable in other pathological conditions of the prostate, such as benign prostatic hyperplasia (BPH) and prostatitis.

In search for more reliable non-invasive biomarkers, the Progensa™ PCA3 (prostate cancer gene 3) test was approved by the FDA (Food and Drug Administration) for the detection of PCA3 in urine after transrectal massage (Groskopf et al., 2006). PCA3 was described as a better diagnostic marker for localized and indolent tumors than PSA levels. Nevertheless, its expression does not improve PCa risk stratification (Auprich et al., 2011). In combination with the detection of the TMPRSS2:ERG gene fusion transcript in urine, the reliability of PCa detection based on PCA3 increased and improved the detection of high-risk tumors (Leyten et al., 2014).

The lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) was originally described as a metastasis biomarker in lung carcinoma (Ji et al., 2003). In the effort to develop tools for the non-invasive diagnosis of PCa, Ren et al. examined the presence of a MALAT1-derived miniRNA in plasma samples. MD miniRNA levels were significantly elevated in PCa patients vs. non-PCa patients. So far, it was not tested whether MALAT-1 or MD miniRNA expression levels in plasma samples could distinguish metastatic PCa from indolent cases (Ren et al., 2013b). The same group reported that expression of MALAT1 was elevated in castration-resistant PCa (CRPC) samples vs. localized tumors and was associated with the disposition of cells to metastasize *in vivo* (Ren et al., 2013a).

With the aim to identify PCa driver lncRNAs, Du et al. searched for lncRNAs progressively upregulated from normal to metastatic prostate samples. Among them, PCAN-R1 and PCAN-R2 expression positively correlate with the amplification of their respective genomic loci. Both lncRNAs participate in PCa progression through an influence on cell proliferation. However, the functional mechanisms of PCAN-R1 and R2 action are not known so far. Du et al. excluded a regulatory effect on the expression of their neighboring genes *KDM5B* (lysine-specific demethylase 5B, also known as *JARID1B*, a H3K4 demethylase) and *FBP2* (fructose-1,6-biphosphatase 2, involved in cell metabolism), respectively (Du et al., 2013).

More recently, Helgason and colleagues detected the PCa-specific lncRNA PCAT-18 in plasma samples. Detection levels correlated with the tumor stage and were higher in metastatic CRPC plasma samples than in plasma samples of healthy individuals and patients with localized PCa. PCAT-18 was detectable in a higher proportion

of high-grade PCa patients than PCA3 and therefore might be a better predictor of outcome. In a PCa xenograft model with androgen-dependent LTL331 cells, androgen deprivation after castration initially reduced PCAT-18 levels. Prolonged low castrate levels of androgens led to the recurrence of a CRPC subline LTL313BR with enhanced PCAT-18 levels (Crea et al., 2014).

To improve PCA diagnosis and delineate tumors with a high risk for progression to metastasis the preferred approach would be to use a set of lncRNAs and protein-coding biomarkers. In this respect, Prensner et al. developed an expression array for the screening of the PCAT family of PCa-specific lncRNAs (patent WO 2012068383 A2). The PCAT-1 transcript was selectively elevated in high-grade localized PCa and metastatic samples (Prensner et al., 2011). Likewise, the expression of SChLAP1 was restricted to malignant PCa samples (Prensner et al., 2013). In contrast, PCAT-29 was recently found to be lower expressed in patient with poor prognosis (Malik et al., 2014).

Lavorgna and colleagues identified the lncRNA TMPR2-AS (transient receptor potential cation channel, subfamily M, member 2, antisense RNA) as a novel risk marker for biochemical recurrence. High expression of TMPR2-AS was associated with a signature of 496 genes, including the oncogene ERG (v-ets avian erythroblastosis virus E26 oncogene homolog), that predicted disease outcome independent of Gleason score. TMPR2-AS silencing increased the expression of the sense transcript TRPM2 (transient receptor potential cation channel, subfamily M, member 2) and reduced tumor growth and mortality rate in a PC3 xenograft model. The authors suggest that the detection of TMPR2-AS and its co-regulated set of transcripts in biopsies could be useful for PCa patient stratification to identify patients with poor prognosis for more aggressive treatment strategies (Orfanelli et al., 2014).

Hence, the simultaneous detection of several lncRNAs, among them PCAT-1, SChLAP1 and TMPR2-AS along with its related gene signature could be used as biomarker combination to delineate high-risk patient. However, the potential to detect these transcripts in biofluids is unknown so far (Chinnaiyan et al., 2012)

Therapeutic potential of lncRNAs

LncRNAs are characterized by a tissue- and physiology-specific expression pattern. This characteristic might enable their use as a specific therapeutic target for one tumor type or subtype.

The silencing of RNAs is an efficient approach to correct aberrant expression levels of lncRNAs, for example by RNA interference or antisense oligonucleotides (ASOs). In addition to enhanced potency and specificity, ASO-mediated downregulation compared to RNA interference has the advantage that ASOs can be easily delivered to the cell by gymnosis (a term coined for 'naked delivery'). Unassisted ASO uptake circumvents the pathogenic effects of transfection reagents. An ASO-mediated approach was successfully employed to knockdown MALAT1 in a mouse xenograft derived from human lung tumors and reduced the metastatic spreading of cancerous cells (Gutschner et al., 2013). A similar result was obtained with siRNA (small interfering RNA) in a CRPC mouse model, where the intratumor injection of siRNAs significantly reduced the development of metastases (Ren et al., 2013a).

Even though ASOs are transiently expressed in cells without being incorporated in the genome, they show a surprising long-term potency and stability. Indeed, in an animal model of myotonic dystrophy, the silencing of specific RNAs was persistent for 1 year after the last ASO administration, rescuing the wild-type phenotype (Wheeler et al., 2012). These data highlight ASOs as a long-term and effective way to silence RNAs *in vivo* and represent a promising therapeutic tool for cancer therapy.

The breakthrough of genome editing tools, especially the CRISPR (clustered, regularly interspaced, palindromic repeats) technology enables the stable and specific editing of genes. The therapeutic potential of this tool was recently highlighted in a mouse model with a specific mutation giving rise to tyrosinemia. Correcting the mutation in adult mice rescued the wild-type phenotype (Yin et al., 2014). Genome editing could also be applied to permanently disable the expression of onco-lncRNAs exclusively expressed in carcinogenic cells. Nevertheless, genome engineering methods are still linked to several drawbacks. Improvement of targeting efficiency and specificity as well as their delivery method is essential for future *in vivo* and therapeutic applications.

The functional role of many lncRNAs arises from their interaction with chromatin modifiers. Therefore, the use of small molecules to inhibit the catalytic activity of epigenetic regulators represents an alternative to silencing of lncRNAs. The PCR2 complex is a common cofactor for thousands of RNAs (Zhao et al., 2010), with EZH2 as its catalytic subunit. Several EZH2 inhibitors have been developed, among them EPZ-6438 (or E7438) as a highly potent and specific S-adenosyl-methionine competitor of EZH2. This compound hindered tumor growth in xenograft mouse models with *EZH2* mutant lymphoma cells or *SMARCB1* (SWI/SNF related, matrix associated, actin

dependent regulator of chromatin, subfamily B, member 1)-deleted rhabdoid tumor cells (Knutson et al., 2013, 2014). Given its potent anti-tumorigenic activity, EPZ-6438 has recently entered clinical trials for the treatment of advanced solid tumors and B cell lymphoma (ClinicalTrials.gov identifier: NCT01897571) (Simo-Riudalbas and Esteller, 2014).

Nevertheless, inhibiting the enzymatic activity of a protein such as EZH2 might affect the regulation of thousands of targets, resulting in undesired side-effects. A locus-specific therapeutic approach disrupting the interaction of a lncRNA with a protein or with DNA could therefore more selectively hinder the lncRNA-dependent regulation of specific target genes. In this respect, small molecule inhibitors could be designed to bind structural domains of the lncRNAs critical for its folding capacity or interaction with protein partners or DNA. This approach seems feasible, as such type of inhibitors have been developed to block the binding of HIV TAR (trans activation responsive region) RNA to Tat protein (transactivator of transcription) with the aim to treat HIV-1 infections (Stevens et al., 2006).

More recently, locked nucleic acids were used to block the recruitment of Xist RNA to the X chromosome by interfering with the binding of the lncRNA to PRC2 (Sarma et al., 2010). Based on this strategy and on the previous identification of 9000 non-coding RNAs interacting with PRC2 (Zhao et al., 2010), Lee and colleagues envisioned the design of antagonist oligonucleotides targeting specific lncRNA-PRC2 interactions, allowing for de-repression of the respective target gene (patent WO 2012087983 A1) (Borowsky et al., 2012). Use of a combination of oligonucleotides blocker to simultaneously disrupt the interaction of several onco-lncRNAs with the PRC2 complex, such as PCAT-1 and ANRASSF1 could represent an attractive strategy to limit the pro-cancerous potential of lncRNAs in cancer. Nevertheless, the design of small molecule inhibitors or antagonist oligonucleotides requires downstream characterization of the RNA structural domains relevant for its binding properties to proteins or DNA for example by RIP experiments. Moreover, the potency of these methods still needs to be tested *in vivo*.

Another potential approach is to take advantage of the miRNA sequestration ability of ceRNAs such as PTENpg1 (Poliseno et al., 2010b). Ebert et al. described the development of a synthetic miRNA sponge binding to a defined set of miRNAs and leading to their inhibition (Ebert et al., 2007). Administration of an oligonucleotide mimicking the binding sites present on PTENpg1 transcript could hypothetically derepress the PTEN transcript in PCa. Using this strategy could derepress the expression of TSG silenced by miRNAs. But the potential of synthetic sponges and their effectiveness needs to be tested *in vivo*.

Summary and outlook

Here we give an overview on lncRNAs that have been identified in PCa and summarize current knowledge on pathways that are affected by dysregulated lncRNAs in PCa. Several profiling projects have identified hundreds of lncRNAs with altered expression in PCa. However, only a limited number has been functionally characterized so far. Prominent mechanisms targeted by lncRNAs in PCa include the PTEN/AKT pathway, AR signaling, as well as chromatin modifications *via* interaction with components of polycomb repressive complexes, thus targeting these complexes to specific genomic loci. Future research will certainly reveal additional targets and pathways regulated by the function of lncRNAs.

Most of the studies focused on the mechanism of one particular lncRNA. However, the regulation of PCa driver genes might be more complex, involving the simultaneous interaction of several lncRNAs recruiting chromatin modifiers or specific lncRNAs responsible for AR activation to specific loci.

Independent of their function, based on their selective expression patterns lncRNAs might be very useful for the development of selective and specific biomarkers for the diagnosis of aggressive forms of PCa or prediction of PCa progression. First examples for non-invasive detection of lncRNAs in serum of PCa patients have been described. Again, future studies will have to prove the usefulness of such approaches and validate whether single lncRNAs or lncRNA profiles might have predictive potential.

Finally, with the emergence of powerful tools to modulate lncRNA expression such as ASOs and CRISPR/Cas technology, it might become possible to correct aberrant lncRNA expression patterns and thus revert a cancerous to a normal phenotype. These approaches will not only transform biological research, but are expected to contribute to the development of molecular therapeutics for human diseases such as PCa (Sander and Joung, 2014).

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