Review Article
LncRNAs and miRNAs: potential biomarkers and therapeutic targets for prostate cancer

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Abstract: Prostate cancer (PCa) is the second lethal disease for men in western countries. Although androgen receptor (AR) signaling has been widely investigated, noncoding RNAs (ncRNAs), deficient of open reading frame, have also received considerable attention. Growing studies showed that the aberrant ncRNAs expression contributed to cell proliferation, metastasis and drug resistance in PCa. Therefore, therapeutically targeting ncRNAs may synergize androgen deprivation therapy (ADT) to have a better effect to fight against PCa, especially castration-resistant prostate cancer (CRPC). This review would systematically summarize the multicellular events controlled by ncRNAs and give a snapshot of future scientific activities and clinical applications.

Keywords: Prostate cancer, lncRNA, miRNA, diagnosis, therapeutic target

Introduction

PCa, the second lethal disease among male in western countries [1], is responsible for approximate 300,000 deaths annually worldwide [2]. ADT shows promising effect, castration resistance invariably occurs due to multiple reasons. Therefore, it is a need to find novel therapeutic targets for advanced PCa.

NcRNAs, originally viewed as "junk RNAs", bear no translational potential and account for approximate 90% RNAs in human [3]. Accumulating evidences suggest that ncRNAs should be implicated into numerous biological processes: alternative RNA splicing, epigenetic modification, mRNA titration, regulation of protein stability and so on [4-7]. Of note, the spatial and temporal deregulation of ncRNA in different types of cancer strongly highlights its crucial role in cancer pathogenesis. Indeed, a variety of ncRNAs have been experimentally and clinically reported to participate into cancer progression including PCa. Overall, the importance of ncRNAs has been recognized even though they cannot be translated into protein. This mini review would summarize the roles of several well-known ncRNAs in the pathogenesis of PCa and also give some insights for further studies or clinical applications.

Long non-coding RNAs in PCa

Long non-coding RNAs (lncRNAs) are RNA species with a length of more than 200 nucleotides. After being transcribed by Polymerase II, lncRNAs are 5' capped and 3' adenylated [8]. Generally speaking, lncRNA functions to regulate gene expression at the transcriptional level via recruiting chromatin modifying complexes or other transcription factors (**Figure 1**). LncRNA can also stabilize protein via direct binding (**Figure 1**). Recent reports demonstrated that some lncRNAs act as sponge to titrate miRNAs, leading their targeting proteins to be post-transcriptionally altered (**Figure 1**). Additionally, lncRNAs have been well acknowledged as prognostic factor in many cancers. Here, we would review some cellular consequences triggered by the deregulation of several well-known lncRNAs in PCa.

PCAT-1

Prostate Cancer Associated Transcript 1 (PCAT-1), located on chromosome 8, encodes an lncRNA that is PCa specific. Through high
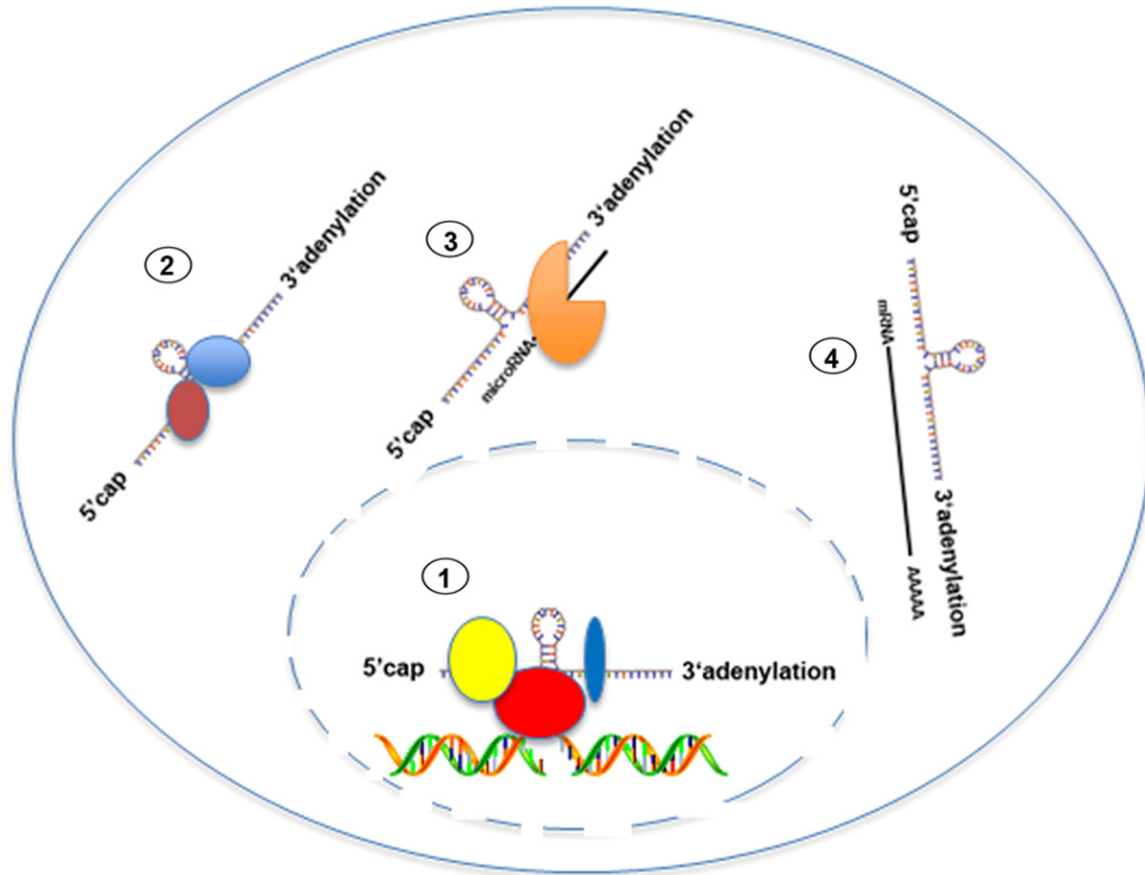
Figure 1. Schematic map of the functions of lncRNAs. LncRNAs regulate gene expression via recruiting chromatin modifying complex, stabilize protein and mRNA via direct binding. LncRNAs still can titrate miRNAs levels.

throughout RNA-seq, Prensner found that about 112 lncRNAs were differentially expressed in benign, localized and metastatic PCa [9]. PCAT-1 was further investigated because it was highly upregulated in subset of metastatic and high-grade localized PCa. Indeed, forced expression of PCAT-1 in normal prostate cell line RWPE triggered the cells to transform into cancerous cells. In parallel, knockdown of PCAT-1 in LNCaP cells with independent small interfering RNAs (siRNAs) could cause a decreased cell proliferation, supporting the clinical relevance of PCAT-1 with PCa malignant status.

Unexpectedly, the expression of PCAT-1 is mutually exclusive to that of enhancer of zeste homolog 2 (EZH2), another oncogene that is also enriched in advanced PCa, indicating that PCAT-1 and EZH2 may define two different subsets of advanced PCa. In fact, inhibition of EZH2 by short hairpin RNA (shRNA) or pharmacologic inhibitor (3-deazaneplanocin) in VCaP cells induced PCAT-1 expression level

dramatically with a release expression of its repressed genes (i.e. breast cancer 1, BRCA1, centromere protein E, CENP E and centromere protein F, CENP F).

As EZH2 is a component of polycomb repressive complex 2 (PRC2), there is a rational to hypothesize that PCAT-1 may act as one of the downstream genes repressed by PRC2. Indeed, ChIP assay with SUZ12 (another component of PRC2) antibody showed that PRC2 was recruited to the promoter of PCAT-1. Meanwhile, PCAT-1 was also reported to impair double-stranded DNA repair (DSBs) via down-regulating the expression of breast cancer 2 (BRCA2) that was considered as a tumor suppressor by repairing damaged DNA [10, 11]. Importantly, loss of BRCA2 or BRCA2 mutation frequently occurs in a variety of cancers including PCa [12]. PCAT-1 mediated reduction of BRCA2 expectedly involved in PCa progression. Intriguingly, PCAT-1 suppressed BRCA2 expression via binding to the 3'UTR of its messenger

RNA, which extends our knowledge of lncRNAs and sets an example for future lncRNA exploration.

Given the expression profile of PCAT-1 and EZH2 in metastatic PCa, we thought that anti-EHZ2 drug would ignore another subset of PCa patients who have higher expression levels of PCAT-1. Therefore, a combinational therapy with PCAT-1 and EZH2 would be highly recommended for clinical trials in the future. Furthermore, the importance of PCAT-1 in advanced PCa prompts our expectation of the cross talk between PCAT-1 and androgen receptor signaling.

PCGEM1

As one of the earliest identified lncRNAs, prostate cancer gene expression marker 1 (PCGEM1) located on chromosome 2q32, is over-expressed in 84% PCa patients by *in situ* hybridization. This might imply that PCGEM1 should act as an onco-lncRNA which is involved in the progression of PCa [13, 14]. In a previous study, PCGEM1 was reported to show no protein coding capacity based on the *in vitro* translation assay and online analysis of TESTCODE program [13]. Further analysis of PCGEM1 level in various PCa cell lines found that such a lncRNA was undetectable in AR-null cell lines such as DU145 and PC3 [13, 15]. Accordingly, it could be substantially induced by 10nM synthetic androgen R1881 [15]. All these datas suggest that PCGEM1 should be androgen/androgen receptor dependent. The function of PCGEM1 has not been determined before Petrovics's group shared their data in oncogene. According to their report [16], forced overexpression of PCGEM1 into LNCaP and NIH3T3 evidently enhanced cell proliferation and their colony-forming capacity. By detecting cell cycle related genes, they demonstrated that the biological function of PCGEM1 was resulted from its ability to stimulate Rb phosphorylation at serine 801 residue, which prevented Rb from binding to E2F and allowed cell to enter into S phase. Nevertheless, the detailed mechanism by which PCGEM1 impairs the inhibitory effect of Rb on cell cycle progression still remains unknown. It is possible that PCGEM1 functions as a scaffold lncRNA which might bring Rb together to let the phosphorylation reaction happen.

Furthermore, Rosenfeld and colleagues in 2013 found that both of PCGEM1 and prostate cancer associated non-coding RNA 1 (PRNCR1) (also named PCAT8) are responsible for AR activation [17]. The result from native RNA immunoprecipitation revealed that both PCGEM1 and PRNCR1 bind to AR, and such an interaction could be further enhanced by dihydrotestosterone propionate (DHT) treatment. Knockdown of PCGEM or PRNCR1 abolished DHT-induced gene expressions and inhibited cell proliferation *in vitro.* In xenograft model, silenced expression of PCGEM1 or PRNCR1 by doxycycline-induced shRNA also diminished tumor size compared to the control cohorts (scramble shRNA) [17]. The PRNCR1-AR interaction mediated the methylation of AR at K349 by disruptor of telomeric silencing 1-like (DOT1L) at the molecular levels, which was indispensable for the binding of PCGEM1 to the N terminal of AR [17]. ChIP-3C assay confirmed that the recruitment of AR to the enhancer region of its downstream genes required the binding of PRNCR1 and PCGEM1, supporting the idea that PRNCR1 and PCGEM1 make the distant regulation of AR possible by exerting as scaffold lncRNA to connect chromatin structure together. Interestingly, PCGEM1 and PRNCR1 were highly induced in castration resistant PCa. Thereby, whether PCGEM1/PRNCR1 participates in the development of castration resistance especially enzalutamide resistance deserves more efforts in the future scientific exploration. In addition, it is also prompting to block the interaction of PCGEM1/PRNCR1 with AR by synthesis of oligoes in clinical trials.

HOTAIR

HOX transcript antisense RNA (HOTAIR), located within HOX gene cluster, encodes 2.2 kb RNA transcript to influence chromatin state via binding PRC2 at its 5' termini [18, 19]. More importantly, the function of Lysine Specific Demethylase (LSD1) on the epigenetic modification was also coordinated with PRC2 with the assistance of HOTAIR. In clinical practice, HOTAIR has been recognized to be strongly associated with metastasis in various cancers, including breast cancer, renal cell carcinoma, colorectal cancer and PCa [19-25].

Particularly in PCa, Zhang found that HOTAIR expression was indistinguishable between be-

nign prostate and localized PCa, which was attributable to the transcriptional suppression of HOTAIR expression by androgen/AR in androgen dependent PCa [25]. However, upon castration resistance, the dominance of androgen independent pathway in PCa allows cells to release HOTAIR expression from androgen/AR inhibition. Based on these previous clinical findings, Zhang implemented a series of experiments to uncover HOTAIR's function in PCa. Targeted expression of HOTAIR in androgen-dependent cell line LNCaP could promote cell growth and cell invasion even in the absence of androgen stimulation. Meanwhile, knockdown of HOTAIR in androgen independent cell line C4-2B dramatically suppressed cell proliferation and cell invasion, indicating HOTAIR was tumor-progression driver in castration resistant PCa [25]. Mechanistically, HOTAIR binds to the N terminal of AR with its 5' end, preventing its interaction with E3 ubiquitination ligase MDM2 [26]. As a result, AR is more stable and more active even without androgen stimulation.

Given the interaction between the HOTAIR and the N terminal of AR, it is possible that HOTAIR may also bind to another AR isoform AR-v7, which has been widely accepted as one of essential driving forces to confer enzalutamide resistance to PCa patients, to mediate castration resistance. Consistently, Zhang found that enzalutamide resistant cells were noticed by their higher induction of HOTAIR level compared to parental cells. In the future, much attention should be paid on how HOTAIR synergizes AR-v7 to control enzalutamide resistance.

MALAT1

MALAT1, also known as metastasis associated lung adenocarcinoma transcript 1, was documented as critical regulator in metastasis, alternative RNA splicing, nuclear organization and epigenetic modification [27, 28]. It is worth noting that MALAT1 has been highlighted for its crucial role in the progression of multiple cancers such as lung cancer, PCa, bladder cancer and breast cancer [29-32].

In 2013, Sun and colleagues demonstrated that MALAT1 was significantly elevated in human prostate cancer compared to the adjacent normal prostate tissues, and its expression was highly associated with Gleason stage,

tumor stage, prostate specific antigen (PSA) level and castration resistance. All these strongly imply that MALAT1 may function as an onco-lncRNA in PCa [29]. To test MALAT1 function in PCa, they knocked down MALAT1 expression by using siRNA oligoes and observed a reduced cell growth and metastasis in MALAT1 deficient PCa cells. In vivo xenograft animal model, delivery of siRNA against MALAT1 contributed to the delay of tumor growth and inhibition of metastasis. However, the detailed mechanism by which MALAT1 controls cell growth and cell invasion is not clear at that moment.

Recently, Wang discovered that MALAT1 could bind the N terminal of zeste homolog 2 (EZH2), a component of PRC2 complex that is frequently over-expressed in castration resistant prostate cancer, to regulate its methylating activity. SiRNA against MALAT1 could impair EZH2 recruitment to its favorable chromatin locus, leading to the transcriptional suppression of EZH2 downstream genes [33]. Now it is considerably clear that MALAT1 promotes PCa progression by partially modulating EZH2 activity.

Since MALAT1 is up-regulated in castration resistant PCa, scientists have compelling rational to explore MALAT1 status/MALAT1 function in enzalutamide resistant PCa. The fact that MALAT1 is documented as a splicing regulator and AR splice variant 7 (AR-v7) is produced by alternative splicing gives us much scientific imagination. Therefore, more efforts are suggested to put into the exploration of the relationship between MALAT1 and enzalutamide resistance or AR-v7 production.

PCAT3

Development of diagnostic and prognostic biomarkers for PCa is important to reduce overtreatment and prolong overall survival. Serum PSA has been used to predicate the prognosis of PCa since 1994 [34]. Nevertheless, such a marker is organ specific rather than cancer specific, of which the indiscriminate utilization would result in over-diagnosis and over-treatment in many patients. Therefore, it is necessary to develop an efficient marker for the diagnosis of PCa. Of note, prostate cancer antigen 3 PCA3 (also referred as DD3), a prostate tissue specific lncRNA, is developed as an advanced PCa biomarker [35-37]. Compared to
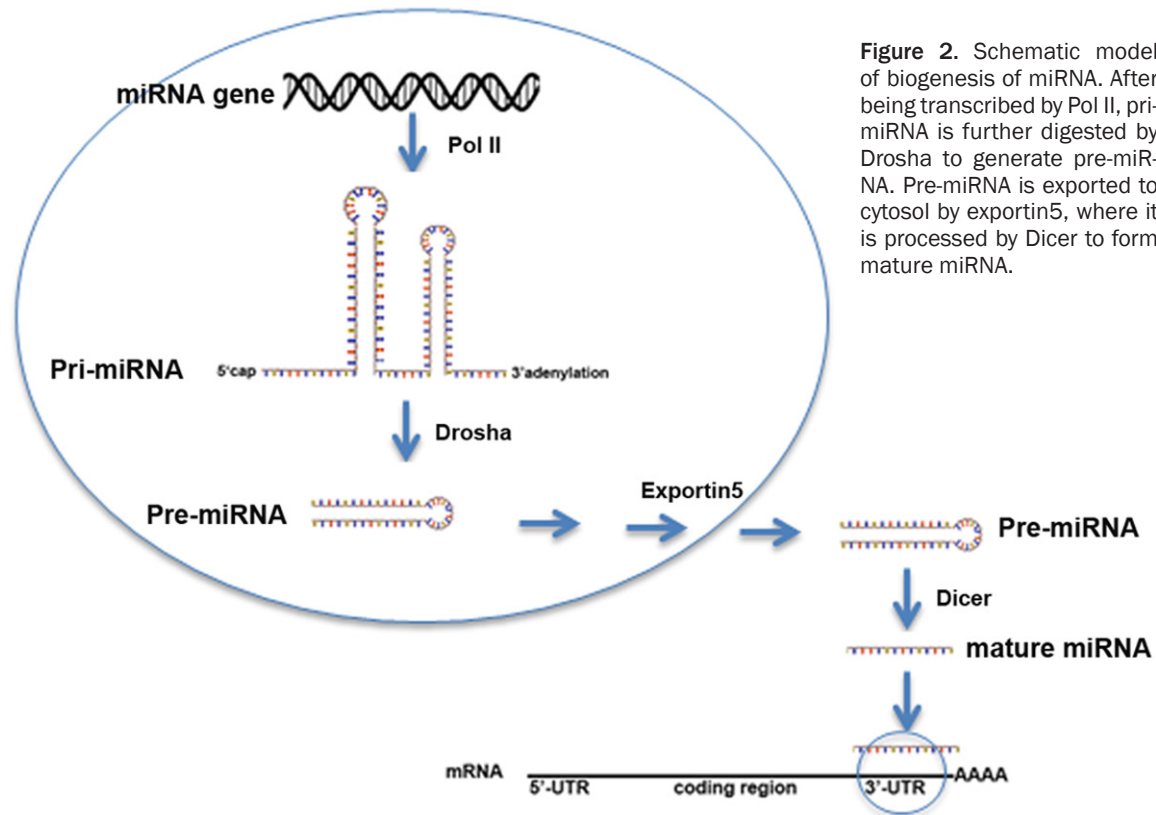
Figure 2. Schematic model of biogenesis of miRNA. After being transcribed by Pol II, pri-miRNA is further digested by Drosha to generate pre-miRNA. Pre-miRNA is exported to cytosol by exportin5, where it is processed by Dicer to form mature miRNA.

PSA, PCA3 is of a lower sensitivity but a higher specificity. Nowadays, urinary detection of PCA3 by Progensa PCA3 has been proved by Food and Drug Administration (FDA) to predict the malignancy of PCa.

However, there is a conflicting evidence when PCAT3 is used to correlate with prognostic factors such as Gleason score and tumor stage, indicating only PCAT3 alone is not adequate and enough to predict PCa status. To improve its prognostic value, the combination of PCAT3 with one fused gene TMPRSS22-ERG, which is induced in advanced PCa, is highly recommended in clinical practice [38]. This combined detection had surprisingly increased the predictive sensitivity by about 20% and significantly reduced the rate of unnecessary biopsies.

Small ncRNAs in PCa

Given the evidence that miRNAs play regulatory roles in the development of various cancers and deregulation of miRNAs could cause adverse consequences, we therefore only focus on the discussion of miRNAs in this mini review. MiRNAs were 20-22 nucleotides RNA mole-

cules derived from so-called "pri-miRNA". After being transcribed by Pol II, pri-miRNA is cleaved by nuclear RNase III endonuclease Drosha to produce pre-miRNA, which is exported from the nucleus and further processed by another endonuclease Dicer to generate mature miRNA (**Figure 2**) [39, 40]. Functionally, miRNA directly binds to the 3' UTR of mRNA via incomplete base pairing and inhibits its translation. It is estimated that 60% mRNAs can be regulated by miRNAs [41]. Extensive studies have demonstrated that aberrant miRNA expression could contribute to cancer carcinogenesis including PCa [42, 43]. Meanwhile, several miRNAs have been highlighted for their crucial roles in determining PCa survival and metastasis.

MIR-205

One of the well-documented miRNAs in PCa is miR-205, which is substantially decreased in prostate cancer compared to the matched benign PCa. Its level was further decreased in advanced or metastatic PCa [44]. In cancerous cells, the folded chromosomal structure led by hyper-methylation on miR-205 locus contributed to its silenced expression. Indeed,

addition of 5-Aza, one de-methylating reagent, into LNCaP caused dramatic induction of miR-205, which is associated with a reduced cell proliferation. Therefore, the methylation status in miR-205 promoter has been considered as a predictive biomarker for advanced or recurrent PCa.

Based on online software prediction and experimental results, miR-205 targeting genes are involved in signaling pathways which are related to MAPK (mitogen activated protein kinase), androgen receptor, EMT (epithelial to mesenchymal transition), migration and invasion [45]. For instance, zinc finger E-box binding homeobox 1 (ZEB1) and zinc finger E-box binding homeobox 2 (ZEB2), two crucial molecules in governing EMT transition, were directly suppressed by miR-205 [46]. Androgen receptor, one of the most importantly regulatory factors in PCa, was also post-transcriptionally inhibited by miR-205 [47]. Since up-regulation of miR-205 provides beneficial effects to PCa cells, the study of its upstream pathways looks attractive for scientists. Recent findings suggested that P63 showed anti-tumor activity via transcriptional activation of miR-205 in PC3 cells [48]. More efforts should be taken to dissect how miR-205 is regulated in PCa.

On this basis, development of new drugs that could induce miR-205 expression is appealing for the treatment of PCa. The 5-Aza may be one candidate but is not the perfect one due to its off-targeting effect. Therefore, identification of the specific methylating enzyme responsible for the hyper-methylation of miR-205 promoter is of necessity to design matched drug to alter miR-205 status without attendant toxicity.

MIR-34a

Just like the other cancers, PCa is also heterogeneous, composing of various types of cells including epithelial cells, fibroblasts, immune cells and cancer stem cells (CSCs) [49]. Prostate cancer stem cells (PCSCs) are potently tumorigenic because of their strong self-renewal capability and potential differentiation ability [49, 50]. More importantly, PCSCs are relatively resistant to ADT and chemotherapy treatment due to their AR-negative characteristics [51].

To explore the function of miRNAs in PCSCs, Liu found that miR-34a was under-expressed

in isolated CD44 molecule (CD44$^+$) cancer stem cells derived from either xenografts or primary tumors compared to their relative CD44$^-$ parental cells [52]. Enforced expression of miR-34a in CD44$^+$ cells could suppress their sphere-forming ability, tumor regeneration and cell invasion. Consistently, introduction of miR-34a inhibitor into CD44$^-$ cells could redirect cells to be stem cell like [53]. In xenograft model, systematic delivery of miR-34a delayed tumor growth and inhibited metastasis. The mechanistic dissection found that CD44 was a direct target of miR-34a. A decreased level of miR-34a in CD44$^+$ cells guaranteed CD44 expression level and maintained their tumorigenic status. All these data suggest that miR-34a could be served as a therapeutic target for PCa, PCSCs in particular.

MiR-34a is one of the downstream targets mediated by p53 [54, 55]. Therefore, researchers should have compelling rational to investigate the role of p53 in balancing the homeostasis of PCSCs. Also, prostate cancer stem cells were also considered as AR-negative cells. Is there any cross-talk between p53 and AR signaling in PCSCs to regulate miR-34a expression level? All these questions deserve further studies.

AR/AR-v7 associated miRNAs

Enormous efforts have been paid to develop methods for inhibiting AR/AR signaling given the roles of AR/AR signaling in determining the pathology of PCa. ADT, anti-androgen and anti-AR are all well-defined methods to block AR/AR signaling in PCa. However, further studies are still needed to develop miRNA-based therapeutic approaches to target AR in order to fight against PCa.

To systematically analyze AR-based miRNAs, Ostling applied LMA screening using 2 miRNA libraries with 1129 miRNAs to identify the miRNA candidates which have potential ability to target AR via binding to its 3' UTR [56]. Data showed that about 77 miRNAs were reported to negatively affect AR protein level in 5 cell lines. After validation by 3'UTR based assay, 14 out 77 miRNAs (miR-135b, miR-185, miR-297, miR-299-3p, miR-34a, miR-9, miR-34c, miR-654-5p, miR-634, miR-449b, miR-371-3p, miR-421, miR-449a, miR-449b) were reported to bind 3'UTR of AR directly and inhibited cell prolifera-

tion of LNCaP and CWR22Rv1. Since AR-v7 had its unique 3'UTR, its regulatory miRNAs were supposed to be different from these against full length AR. In fact, miR-124 was the only reported one that directly targeted AR-v7 [57]. *In vitro* study showed that miR-124 evidently suppressed cell proliferation of CWR22Rv1 cells via degrading AR-v7 protein level. As AR-v7 is a critical molecule that confers enzalutamide resistance to PCa, miR-124 could re-sensitize enzalutamide resistance by probably altering AR-v7 level. *In vivo* intravenous delivery of miR-124 could increase tumor apoptosis in combination with enzalutamide administration. Taken together, these findings would build up strong rational to develop miRNAs as therapeutic approach to fight against PCa via reducing AR/AR-v7 level.

MIR-15A-MIR-16-1

Allelic loss of miR-15a-miR-16-1 on chromosome 13 was viewed as predictive signature of metastatic PCa. Previous investigation showed that introduction of miR-15a-miR-16-1 inhibitor into non transformed prostate cells promoted cell carcinogenesis [58]. Delivery of mR-15a-miR-16-1 antagomirs into normal mouse prostate could induce hyperplasia. Conversely, over-expression of miR-15a-miR-16-1 in prostate cancer cells resulted in remarkable growth arrest, cell apoptosis and tumor shrinkage of tumor bearing xenografts. MiR-15a-miR-16-1 cluster could reduce CCND1, WNT3A and BCL2 via directly binding to their 3'UTRs, as well as preventing their translation. Furthermore, miR-15a level was considered to be highly associated with cMYB function in PCa. MiR-15a reconstitution induced a significant down-regulation of cMYB expression and an evident up-regulation of AR in androgen dependent LNCaP cell lines, suggesting that the deficiency of miR-15a in PCa cells contributed to cMYB-induced cell progression. However, two questions are raised from this report and deserves further investigation: does miR-15a directly regulate the cMYB via base pairing binding; what's the underlying mechanism by which miR-15a up-regulates AR.

Summary

Novel target-based drugs are urgently needed for better improving the treatment of PCa.

NcRNAs, including long noncoding RNAs and miRNAs have been extensively investigated in recent years. The aberrant expression of ncRNA caused by either genomic modification or transcriptional regulation was highly related to PCa progression. Even though targeting ncRNAs are effective in both the cell line experiment and animal model study, the application of ncRNAs into clinical trial is still far away from satisfaction. The off-target effect and the low efficiency of small RNA delivery are currently two major obstacles to prevent ncRNAs from further clinical application.

The exploration on ncRNAs is still in its infancy. On one hand, the biological processes ncRNAs involved are largely uncovered. Earlier reports have mentioned that some ncRNAs could be translocated to certain organelle such as mitochondria or endoplasmic reticulum [59, 60], whereas they possibly control certain biological events that requires our exploration. On the other hand, for those lncRNAs functioning to control gene regulation, their regulatory codes remain largely unknown. The map of lncRNA-regulated downstream genes and the DNA binding code are still mysterious to us. More investigations are required to solve these problems if we want to translate lncRNA functions into clinical use.

Acknowledgements

Disclosure of conflict of interest

None.

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