Critical Review

Uncovering the Roles of miRNAs and Their Relationship with Androgen Receptor in Prostate Cancer

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Abstract

Prostate cancer (PCa) is the second most commonly occurring malignant tumor in Europe and America. Normal and neoplastic growth of prostate gland are dependent on androgen receptor (AR) expression and function. PCa is driven by androgen and its receptor, and they continue to be the key drivers of castration-resistant prostate cancer (CRPC). CRPC is the terminal stage of PCa and seriously jeopardizes the patient’s quality of life and lifespan. miRNAs are small noncoding RNAs, 18–25 nt in length that destabilize mRNA or repress protein synthesis by interacting with the 3'-untranslated regions (3'-UTR) of target mRNAs. miRNAs can regulate AR or be regulated by AR and then affect various signaling pathways related to cellular functions and tumor processes. In this review, we focus on the relationship between miRNAs and AR in PCa and elucidate their roles in the induction of malignant changes in PCa. © 2014 The Authors IUBMB Life published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology, 66(6):379–386, 2014

Keywords: miRNAs; prostate cancer; androgen receptor; target genes; castration-resistant prostate cancer

Introduction

miRNAs are a group of small noncoding RNAs that are 18–25 nt in length. miRNAs were originally discovered in Caenorhabditis elegans. Lee et al. (1) discovered the first developmental regulatory factor in 1994 and named it Lin-4. miRNAs act as post-transcriptional regulators that bind to 3'-untranslated regions (3'-UTR) of target mRNAs by base pairing. In June 2013, 2,582 human miRNAs were reported in the miRBase v20 database. Over 60% of human protein-coding genes have been predicted to be targeted and regulated by miRNAs, and their mRNAs could be inhibited or degraded depending on the degree of complementation between miRNAs and mRNAs. Increase or decrease of miRNAs in human castration-resistant prostate cancer (CRPC) could be important for progression of prostate cancer (PCa) from castration sensitive to castration resistant. miRNAs could either be the driving factors or the outcome during the process of malignant transformation. Some of them could act as oncomirs or antioncomirs by regulating androgen receptor (AR) expression and function. The interactions of these miRNAs with AR play important roles in the progress of prostate carcinogenesis and CRPC evolution.

Growth of prostate gland is initially dependent on androgen. Under constant stimulation of androgen, prostate gland gradually develops into PCa and then becomes CRPC. CRPC is the terminal stage of PCa, and seriously jeopardizes the patient’s quality of life and lifespan, is an incurable highly aggressive disease after exclusive surgical castration therapy. In CRPC, AR remains a key growth factor to promote malignant change. AR is one of the most important nuclear transcription factors from the steroid hormone receptor superfamily of genes. Normal prostate growth and development, prostate carcinogenesis, and castration-resistant progression of PCa are dependent on AR expression and function. Alterations in AR structure, expression

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and signaling could have a defining role in PCa progression toward an incurable castration-resistant state. AR is translocated to the nucleus in a dimerized form and regulates gene expression by binding to specific hormone response elements. AR plays an essential role in the carcinogenesis of PCa, and 10–80% of CRPC cases show elevated levels of AR protein (2). We hypothesize that high expression or gene amplification of AR is a driving force for castration-resistant progression of PCa.

In recent studies, some miRNAs were reported to target AR, or be regulated by AR, and subsequently affect several pathways involved in cell proliferation, cell cycle regulation, apoptosis, angiogenesis, castration-resistance, invasion, metastasis and so on. Herein, we review the relationship between miRNAs and AR and their roles in the developmental processes of PCa.

The Roles of miRNAs and their Relationship with AR in PCa

Several researchers have found abundant differential expression of miRNAs in CRPC cells or tissues as compared to castration-sensitive cells or tissues based on microarray analyses or deep-sequencing data. Ozen et al. (3) analyzed 480 types of miRNAs in 10 cases of prostate gland hyperplasia and 16 cases of PCa and found abnormal expression of 85 miRNAs in these PCa samples, of which 76 were significantly downregulated, while only nine miRNAs were elevated. Ma et al. reported a functional impact of miR-616 overexpression in PCa cells, which occurred consistently in castration-resistant cells versus castration-sensitive cells. miR-616 overexpression was confirmed in malignant prostate tissues as opposed to benign prostate specimens (4). miR-296-5p expression was upregulated by 2.22-fold in the CL-1 cells, which did not express significant AR as compared to LNCaP cells (5). Lin et al. (6) also compared the expression of miRNAs and found that miR-184, miR-361 and miR-424 were elevated, while miR-19b, miR-128b, miR-146a/b, miR-221/222 and miR-663 were decreased in castration-sensitive cells as compared to castration-resistant cell lines.

miRNAs could be regulated by AR. The AR binding sites could be located in the miRNA flanking regions. To obtain a complete profile of miRNA expression response to androgen, miRNA array analysis of castration-sensitive cells treated with synthetic androgen R1881 identified 16 miRNAs that were subsequently upregulated, including miR-594, miR-16, miR-21, miR-29b, miR-148b, miR-148b, miR-156a, miR-17-5p, miR-20a, miR-20b, miR-29a, miR-19b, miR-93, let-7d, let-7g and miR-15b (7). Serum concentrations of miR-214 and miR-125a are increased by androgen in PCs (8). Seven androgen-regulated miRNAs, including miR-21, miR-32, miR-99a, miR-99b, miR-148a, miR-221 and miR-590-5p, were found to be differentially expressed in CRPC as compared to benign prostate hyperplasia based on microarray analyses. Among them, miR-32 was shown to reduce apoptosis, while miR-148a enhanced proliferation (9). Moreover, some findings indicated that the negative correlation between AR and DNMT activity is one of mechanisms that influence the methylation status of miRNA promoters, which in turn regulates their expression. Chu et al. found that AR-positive PCs showed high expression and hypomethylation of miR-375. In contrast, AR-negative PCs displayed low expression and hypermethylation of miR-375 (10).

Several miRNAs are associated with AR. Some of them are regulated by AR, while others regulate AR mRNA or protein levels. The 3′-UTR of AR bound by miRNAs, which is 436 nt in length, was analyzed by TargetScan program (Fig. 1). Ostling et al. performed a gain-of-function screening to systematically analyze 1,129 miRNAs and identified 71 unique miRNAs that influenced the level of AR in human PCa cells. Their sequencing data revealed an extended 6 kb 3′-UTR of AR that was much longer than the sequence used in miRNAs-Target prediction software (Fig. 1). Luciferase reporter assays validated 13 miRNAs that regulate this long AR 3′-UTR (9). miR-488/488* downregulated the transcriptional activity of AR and inhibited endogenous AR protein production in human PCa cells (Fig. 1), thereby regulating the proliferation and apoptosis of these cells (7,13). In addition, miR-147, miR-298, miR-491-5p and miR-876-3p are efficient in downregulating AR mRNA, whereas miR-30d and miR-644 increase AR mRNA level (11).
### TABLE 1  
**Summary of the roles of miRNAs and their relationship with AR**

| Name                  | Target gene                  | Function                                                                 | Reference |
|-----------------------|------------------------------|--------------------------------------------------------------------------|-----------|
| **miRNAs that are upregulated by androgen** |                              |                                                                          |           |
| miR-21                | TGFBR2, PTEN, Pdcd4          | Growth, cell migration, apoptosis, castration resistant                   | (8,12,15,16) |
| miR-99a/125b-2        | NCOR2, IGF1R, BAK1, BBC3, p53| Apoptosis, growth, Her2-AR pathway                                       | (20–22)   |
| miR-141               | PTEN, PDK2, p21, CDKN1B, PDE3B, Shp | AR pathway, growth                                                      | (7,17–19) |
| miR-23a/27a/24-2      | ABCA1, PDS5B, Prohibitin, FAF1| Invasion and migration, survival in soft agar. Apoptosis                 | (23–26)   |
| miR-19a               | SUZ12, RAB13, SC4MOL, PSAP, ABCA1 | Apoptosis                                                               | (23)      |
| miR-133b              | CDC2L5, PTPRK, RB1CC1, CPNE3  |                                                                          | (23)      |
| **miRNAs that are downregulated by androgen** |                              |                                                                          |           |
| miR-221/222           | DVL2, HECTD2, RAB1A          | Castration-resistant, growth, migration, NE differentiation             | (27–29)   |
| miR-34a/b/c           | HuR, SIRT1, Bcl2, PSA, Notch-1| Apoptosis, growth, self-renewal, paclitaxel resistance                  | (30–33)   |
| miR-375               |                              |                                                                          | (10)      |
| **miRNAs that upregulate AR** |                              |                                                                          |           |
| miR-30d, miR-644      |                              |                                                                          | (11)      |
| **miRNAs that downregulate AR** |                              |                                                                          |           |
| miR-185               | AR, CDC6, SREBP-1/2, FASN, HMGCR | Growth, cell cycle, invasive, migration, tumorigenicity, apoptosis     | (34,35)   |
| miR-31                | AR, MCM2, EXO1, E2F1, E2F2, FOXM1 | AR pathway, cell cycle, growth                                          | (36)      |
| miR-203/205           | AR, MAPK, IL-6, HRAS, AGO2   | Castration-resistant                                                    | (37,38)   |
| Let-7b/c/d/g          | AR, c-Myc, PBX3              | AR pathway, radioresistance, growth                                      | (39,40)   |
| miR-488/488*          | AR                            | Growth, apoptosis                                                       | (7,13)    |
| miR-654-5p            | AR                            | Growth, decrease PSA                                                    | (12)      |
| **miRNAs that are upregulated in CRPC** |                              |                                                                          |           |
| miR-616               |                              |                                                                          | (4)       |
| miR-296-5p            |                              |                                                                          | (5)       |
| miR-184, miR-361, miR-424 |                              |                                                                          | (6)       |
| miR-21                |                              |                                                                          | (7,14)    |
| miR-141               |                              |                                                                          | (17)      |
| miR-221, miR-222      |                              |                                                                          | (27)      |
miRNAs that are Upregulated by Androgen

miR-21
miR-21 has an androgen-response element (ARE) within its promoter. miR-21 and AR regulate each other in a positive feedback loop. Serum concentration and tissue expression level of miR-21 are increased in CRPC as compared to localized PCa and are especially high in drug-resistant PCa. miR-21 can mediate cell growth, cell migration, apoptosis and androgen insensitivity through various pathways (7,40) (Table 1).

Studies by Ribas et al. showed that androgen-induced AR bound to miR-21 promoter and subsequently led to overexpression of miR-21, which was assumed to be associated with castration-sensitive cell growth and castration-resistance in PCa (8,12). miR-21 can also inhibit transforming growth factor beta receptor II (TGFBR2) expression by binding to its 3'-UTR. The AR and miR-21 axis exerts its oncogenic effects in PCa by downregulating TGFBR2 and inhibiting the tumor-suppression activity of TGFβ pathway. In contrast, targeting miR-21 alone or in combination with AR could restore the tumor-inhibitory activity of TGFβ in PCa. Furthermore, miR-21/AR executes tumor-promoting function by attenuating TGFβ-mediated Smad2/3 activation (15) (Table 1). miR-21 can also inhibit transforming growth factor beta receptor II (TGFBR2) expression by binding to its 3'-UTR.

miR-141
Waltering et al. profiled androgen-responsive miRNAs in cells and xenografts. Of the 55 candidates identified, miR-141 was found to be most strongly regulated by androgen and was also overexpressed in PCa. AR amplification increased miR-141 expression by 8–10-fold via direct transcription (17). The orphan receptor, small heterodimer partner (Shp), is a corepressor of AR and represses AR-regulated transcriptional activity. miR-141 can target 3'-UTR of Shp mRNA resulting in its translational suppression and mRNA degradation. Forced expression of Shp or inhibition of miR-141 by anti-miR-141 attenuated AR-regulated transcriptional activity in AR-responsive LNCaP cells (18). miR-141 is predicted to target five genes: PTEN, PDK2, cyclin-dependent kinase inhibitor 1A (p21), CDKN1B and PDE3B (7) (Table 1). Additionally, overexpression of miR-141 enhanced growth of LNCaP cells, while inhibition of miR-141 by anti-miR-141 suppressed growth of the LNCaP subline (19).

Taken together, these results suggest that miR-141 acts as an oncomir not only in response to androgen but also by regulating expression of many downstream target genes to promote the progression of PCa.

miR-99a/125b-2 Cluster
miR-125b and AR form a complex feedback loop. miR-125b is regulated by androgen via ARE within the promoter of miR-125b gene. NCOR2 is a repressor of AR, and an additional target of miR-125b that was confirmed by luciferase-binding assay. Androgen regulates the miR-99a/125b-2 cluster expression through AR, which directly binds to the cluster genes region, and then recruits chromatin remodelers EZH2 or JMJD3 in the presence or absence of androgen, respectively.
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expression of CRPC cells and LNCaP-Abl relative to castration-sensitive cells, LNCaP and LAPC-4. Functional studies of miR-221 and/or miR-222 reveal that overexpression of miR-221/222 in LNCaP and LAPC-4 triggers castration-resistant growth, and their inhibition converts LNCaP-Abl into castration-sensitive phenotype (27). Thrombin downregulates p27 (Kip1) in TRAMP mice and PCa cell lines, and miR-222 expression is increased by thrombin. miR-221 could control migration of castration-resistant cells through DVL2, working as a key regulator in advanced PCa. miR-221 could contribute to NE differentiation, thereby causing CRPC (28). miR-221/222 cluster is highly expressed in bone metastatic CRPC specimens. Stably overexpressing miR-221 confers castration-resistant cell growth by rescuing LNCaP cells from growth arrest at G1 phase due to lack of androgen. Overexpressing miR-221 in LNCaP reduces the transcription of a subgroup of androgen-responsive genes without affecting AR or AR-androgen integrity. Using systematic biochemical and bioinformatic analyses, HECTD2 and RAB1A were identified as miR-221 targets, which could mediate the development of CRPC phenotype in multiple PCa cell lines. Downregulation of HECTD2 significantly affects androgen-induced and AR-mediated transcription, and downregulation of HECTD2 or RAB1A enhances castration-resistant cell growth (29) (Table 1). We hypothesize that a major biological consequence of miR-221 upregulation is reprogramming of AR signaling, which in turn could mediate transition to CRPC phenotype.

miR-34a/b/c Cluster

miR-34a/b/c cluster expression is reduced in PC3PR cells as compared to PC3 cells and has a negative correlation with AR level in PCa (30,31). miR-34a acts on 3'-UTR of SIRT1 and Bcl2 mRNAs directly as well as indirectly via regulation of HUtr expression. Upregulation of SIRT1 and Bcl2 in PCa cells confers paclitaxel resistance (31). Rokhlin et al. (32) reported that p53 induced overexpression of miR-34 in AR positive PCa cells only, while miR-34a/b/c induced apoptosis only in the presence of AR and p53 activated by agents that induce DNA double-strand breaks. Several genes are direct targets of miR-34a, PSA and Notch-1. Overexpression of miR-34a significantly inhibits growth of PCa cells and decreases their self-renewal capacity (33) (Table 1).

miRNAs that are Downregulated by Androgen

miR-221/222 Cluster

Sun et al. found that androgen downregulated oncomirs, miR-221 and miR-222, which transcribe from a cluster of chromosome X, have identical seed sequences and possibly common target genes. miR-221 and miR-222 have increased levels of expression in CRPC cells and LNCaP-Abl relative to castration-sensitive cells, LNCaP and LAPC-4. Functional studies of miR-221 and/or miR-222 reveal that overexpression of miR-221/222 in LNCaP and LAPC-4 triggers castration-resistant growth, and their inhibition converts LNCaP-Abl into castration-sensitive phenotype (27). Thrombin downregulates p27 (Kip1) in TRAMP mice and PCa cell lines, and miR-222 expression is increased by thrombin. miR-221 could control migration of castration-resistant cells through DVL2, working as a key regulator in advanced PCa. miR-221 could contribute to NE differentiation, thereby causing CRPC (28). miR-221/222 cluster is highly expressed in bone metastatic CRPC specimens. Stably overexpressing miR-221 confers castration-resistant cell growth by rescuing LNCaP cells from growth arrest at G1 phase due to lack of androgen. Overexpressing miR-221 in LNCaP reduces the transcription of a subgroup of androgen-responsive genes without affecting AR or AR-androgen integrity. Using systematic biochemical and bioinformatic analyses, HECTD2 and RAB1A were identified as miR-221 targets, which could mediate the development of CRPC phenotype in multiple PCa cell lines. Downregulation of HECTD2 significantly affects androgen-induced and AR-mediated transcription, and downregulation of HECTD2 or RAB1A enhances castration-resistant cell growth (29) (Table 1). We hypothesize that a major biological consequence of miR-221 upregulation is reprogramming of AR signaling, which in turn could mediate transition to CRPC phenotype.

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Roles of miRNAs and their Relationship with AR in PCa

Cholesterol metabolism is associated with PCa development and progression to end-stage disease. SREBP-1, a key transcription factor for lipogenesis, induces fatty acid and lipid accumulation and AR transcription, and also promotes PCa cell growth and castration resistance. SREBP-1 is overexpressed in CRPC specimens. miR-185 inhibits SREBP-1/2 expression and downregulates their target genes, FASN and HMGCR, which control lipogenesis and cholesterogenesis (35). In addition, restoration of miR-185 leads to caspase-dependent apoptotic death in PCa cells (35) (Table 1). Therefore, miR-185 represents a novel target for PCa therapy.

**miR-31**

A complex interaction exists between expression of the tumor suppressor miRNA, miR-31, and AR signaling. miR-31 expression is reduced as a result of promoter hypermethylolation in PCa. Importantly, expression of miR-31 is inversely correlated with aggressiveness of the disease (36). miR-31 and AR are suggested to mutually repress each other through multiple mechanisms. Among them, miR-31 directly targets the coding region of AR, which is commonly mutated in PCa. Up-regulation of miR-31 effectively suppresses AR expression, and inhibits PCa growth in vivo. Additionally, miR-31 suppresses cell-cycle regulators, including MCM2, EXO1, E2F1, E2F2 and FOXM1 (36) (Table 1).

Taken together, these findings suggest a novel AR regulatory mechanism mediated by miR-31 expression. Downregulation of miR-31 could disrupt cellular homeostasis and contribute to evolution and progression of PCa. These findings have implications for epigenetic and clinical treatments and support the detection of miR-31 promoter methylation as a novel biomarker.

**miR-203/205 Cluster**

miR-203 and miR-205 are underexpressed in CRPC. miR-205 binds to AR 3'-UTR, as confirmed by luciferase reporter assay. Using transcriptomics, miRNAs were found to repress several gene products that are known to be overexpressed in PCa. Using communoprecipitation assays and Western blot analysis, miR-203/205 was demonstrated to directly target several components of mitogen-activated protein kinase (MAPK), IL-6 and AR signaling pathways, several AR coregulators, Harvey rat sarcoma viral oncogene homolog (HRAS) and Argonaute 2 (AGO2). Both pathways are crucial for the development of primary tumor, and in particular, the progression to incurable castration-resistant form (37,38) (Table 1). We therefore, propose that these miRNAs jointly act as tumor suppressors in PCa, and could interfere with progression to castration resistance.

**Let-7 Family**

The members of the Let-7 family are sequentially distinguished by letter (Let-7a, Let-7b, etc). Let-7c suppresses AR expression and activity in PCa cells by targeting c-Myc. Suppression of AR by Let-7c leads to decreased proliferation of human PCa cells. Downregulation of Let-7c in PCa specimens is inversely correlated with AR expression, whereas the expression of Lin28 (a repressor of Let-7) is positively correlated with AR expression (39). PBX3 is post-transcriptionally regulated by androgen in PCa cells, and this effect could be independent of AR. Furthermore, PBX3 is identified as a target of Let-7d, an androgen regulated miRNA. Let-7d is downregulated in malignant PCa as compared to benign prostate tissue (40). Overexpression of Let-7b results in radiosensitization, while inhibition of Let-7g causes radioresistance. Let-7a is moderately decreased in both LNCaP and C4-2 cells in response to radiation. Let-7f is decreased in LNCaP and increased in C4-2 cells. Let-7d is increased in LNCaP cells (41) (Table 1).

The above findings demonstrate that Let-7 family members play important roles in regulation of androgen signaling in PCa by downregulating AR expression. Therefore, reconstituting these miRNAs as potential therapeutic targets could help to target AR in advanced PCa.

**Other miRNAs**

miR-19a is directly upregulated by AR and represses SUZ12, RAB13, SC4MOL, PSAP and ABCA1 (23) (Table 1). miR-133b is directly upregulated by AR and represses CDC2L5, PTPRK, RB1CC1 and CPNE3 (23) (Table 1). miR-101 inhibits expression of Ezh2, as confirmed by a reporter construct containing Ezh2 3'-UTR. In addition, expression of miR-101 is regulated by AR and hypoxia-inducible factor (HIF)-1α/β (42) (Table 1).

miR-331-3p blocks AR signaling pathway in PCa cells (30) (Table 1). miR-17-5p was shown to target P300/CBP associated factor (PCAF) by a luciferase reporter assay. PCAF is upregulated in several PCa cell lines and promotes AR transcriptional activation and cell growth (43) (Table 1).

**Conclusion and Future Perspectives**

One of the hallmarks of CRPC is overexpression of AR. AR plays a central role in the development of PCa and progression to castration resistance during and after androgen deprivation therapy (ADT), which comprises of medical or surgical castration. Almost all PCa initially respond to ADT, it is usually not permanently effective or curative, and most tumors eventually regrow after a certain period. Several reports have shown that expression of AR is increased in human CRPC tissues, and a significant association exists between high AR expression and clinical outcomes. As a consequence of overexpression, increased AR sensitizes PCa cells to low levels of androgen, leading to progression of castration resistance. These findings indicate that AR persistently stays at the core of therapeutic targets even after castration-resistant progression of PCa.

miRNAs are small noncoding RNAs that modulate translation of mRNAs by base-pairing interactions with the 3'-UTR, thereby destabilizing mRNAs or repressing protein synthesis. The miRNAs described in this review regulate AR or are regulated by AR. Among them, oncomirs are highly expressed in CRPC, while anti-oncomirs are underexpressed. In CRPC, these special miRNAs result in cell proliferation, cell cycle transition, apoptosis, angiogenesis, castration resistance, invasion, metastasis and so on. They could be diagnostic markers and potential therapeutic
targets. Moreover, if miRNA drugs attenuate overexpression of AR in PCa patients, Malignant phenotype could be reversed by a way close to natural process in vivo. It will be the goal of that researchers and doctors are willing to see and strive for.

This precise and complex network needs further study. This review outlines recent advances in PCa research and experimental evidence on the relationship between miRNAs and AR and their roles in PCa.

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