MicroRNAs in androgen-dependent PCa

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1. ABSTRACT

Prostate cancer (PCa) is the most commonly diagnosed cancer among men in Western countries and is one of the leading causes of cancer deaths. The growth and progression of PCa is related to androgen levels. In cancer, nicroRNAs (miRs) function as either oncogenes or tumor suppressor genes. In androgen-dependent PCa, miRs play a role in the growth, development, progression, and metastasis of the disease, and are also involved in the response to therapy and therefore affect the prognosis. In this review, we focus on the role played by miRs concerning the mechanisms of androgen-dependent PCa.

2. INTRODUCTION

Prostate cancer (PCa) is considered to be the most common malignant tumor in males (1) and the second leading cause of cancer mortality in men in the USA (2). According to Cancer Research UK, an estimated 913,000 men worldwide were diagnosed with PCa in developed countries in 2008. Although over 70% of diagnosed PCa patients now survive beyond 5 years, this cancer is still associated with significant mortality and morbidity, and metastatic prostate tumors are responsible for the majority of deaths associated with this cancer.

Almost all PCa cells, as well as normal prostate tissues, require androgens for growth and survival. Currently, localized PCa is usually treated with radical prostatectomy or radiation. For more advanced cancers that have recurred or metastasized, the gold standard treatment is androgen ablation therapy (3). Even though hormone therapy is no longer effective in advanced-stage PCa, aggressive forms of PCa still rely on androgen signaling (4). Thus, we will discuss the relevant mechanisms of androgen-dependent PCa in this review.

MicroRNAs (miRs) are small non-coding, single-stranded RNAs, typically 19–25 nucleotides in length, that regulate gene expression by affecting the stability or translation efficiency of target mRNAs (5). By binding to partially complementary target sites within the 3′-untranslated regions (3′-UTRs) of selected messenger RNAs, they function as translational repressors; perfect complementarity induces degradation of the target mRNA (6,7). One miR can target hundreds of mRNAs; thus, alterations in a single miR can have dramatic effects on cell biology. Recent studies have found that aberrations in miR expression are associated with various human cancers, including PCa. Approximately half of the known miR genes are located at cancer-related genomic regions (8), and in cancer, miRs can function as either oncogenes or tumor suppressor genes (9,10).

The precise molecular mechanisms underlying the development, progression, and therapeutic response of PCa remain poorly understood; however, miRs have been demonstrated to play a role. At present it is recognized that the process of PCa development is a consequence of genetic and epigenetic alterations that transform normal glandular epithelium to pre-neoplastic lesions that lead to invasive carcinoma (11,12). Genomic profiling of miR and messenger RNA has revealed deregulated miR expression in PCa (13). For example, prostate tumors express the miR-106b-25 cluster, which maps to intron 13 of MCM7, and miR-32, which maps to intron 14 of C9orf5, at significantly higher levels than nontumor prostate tissue. Tumor miRNAs influence transcript abundance of protein-coding target genes in the cancerous prostate, some of which are androgen-regulated (13). Global gene expression analysis reveals that miR targets exhibit significantly reduced transcript abundance in PCa compared with the combined pool of all mRNAs (14). Furthermore, the transcript levels of miR targets were higher in androgen-dependent PCa compared to androgen-independent PCa (14). Regarding the differential expression of miRs in cancerous prostate, miR expression of tumor samples can accurately separate cancerous from benign prostatic hyperplasia (BPH) samples and further classify the carcinoma tumors according to androgen dependence (hormone naive versus hormone refractory) (15). Thus, miRs have the potential to serve as a novel diagnostic and prognostic tool for PCa. In this review, we focus on the role of miRs and the mechanism of androgen-dependent PCa.

3. MicroRNAs IN THE DEVELOPMENT AND PROGRESSION OF ANDROGEN-DEPENDENT PCA

3.1. MicroRNAs regulate androgen-dependent PCa

The androgen receptor (AR), a protein that binds to androgens, acts as a transcription factor and regulates a wide array of genes involved in various processes, including cell proliferation and growth (16–18). It is activated by binding of either of the androgenic hormones, testosterone or dihydrotestosterone (19), in the cytoplasm and then translocating into the nucleus. The role played by miRs in androgen-dependent PCa has been determined to be associated with AR in numerous reports. Currently, 71 unique miRNAs have been identified that influence the level of AR in androgen-dependent LNCaP cells, among which 13 miRs were found to regulate the 6-kb long AR 3′-UTR; namely, miR-135b, miR-185, miR-297, miR-299-3p, miR-34a, miR-34c, miR-371-3p, miR-421, miR-449a, miR-449b, miR-634, miR-654-5p, and miR-9, whereas miR-421, miR-449a, miR-449b, and miR-9 were found to also be able to decrease the levels of the exogenous AR. The AR down-regulating miRs decreased the androgen-induced proliferation of PCa cells (20). These findings suggest that the miRs interacting with the long 3′-UTR of the AR gene are important regulators of AR protein levels in androgen-dependent PCa cells. In addition, overexpression of miR 488 downregulates the transcriptional activity of AR and inhibits endogenous AR protein production in androgen-dependent PCa cells (21). These miRs that target AR are potential modulators of AR-mediated signaling. Furthermore, miR-141 has been found to modulate AR transcriptional activity in LNCaP cells by targeting the small heterodimer partner protein (22). MiR-141 potentially targets the 3′-UTR of the orphan receptor small heterodimer partner (Shp) mRNA, which is a co-repressor to AR and represses AR-regulated transcriptional activity, resulting in translational suppression and RNA degradation. Moreover, enhanced expression of Shp or inhibition of miR-141 function by anti-miR-141 has been shown to attenuate AR-regulated transcriptional activity in AR-responsive LNCaP cells (22).

MiRs also regulate AR signaling during the development and progression of PCa. The ERBB-2 tyrosine kinase receptor is frequently overexpressed in PCa and is associated with disease progression and poor survival. Transfection of LNCaP cells with miR-331-3p has been reported to reduce ERBB-2 expression and block downstream phosphatidylinositol 3-kinase/AKT signaling. MiR-331-3p transfection blocked the androgen receptor signaling pathway in PCa cells, resulting in reduced activity of an androgen-stimulated prostate-specific antigen promoter and block of prostate-specific antigen expression (23). These findings suggest that miR-331-3p exhibits the capacity to regulate signaling pathways critical to the development and progression of PCa cells.

MiRs regulate the apoptosis of PCa cells. Simultaneous inhibition or forced overexpression of both miR-34a and 34c result in modulation of AR-dependent p53-induced apoptosis (24). By binding to the 3′-UTR of cullin-associated and neddylation-dissociated 1 (CAND1)

mRNA, miR-148a reduces the expression of CAND1, a negative regulator of SKP1-Cullin1-F-box (SCF) ubiquitin ligases. CAND1 knockdown by small interfering RNA promotes the proliferation of androgen-dependent LNCaP cells; this indicates the potential contribution of miR-148a to the growth of human PCa (25). Thus, miRs regulate the development and progression of androgen-dependent PCa in several ways.

3.2. Androgen regulates microRNAs in androgen-dependent PCa

During the development and progression of androgen-dependent PCa, miRs are regulated by androgen. The miR-125b-2 cluster on chromosome 21 has been identified as a cluster of androgen-inducible miRNA (26). The expression of miR-125b is up-regulated by androgen signaling (27). MiR-125b can suppress translation in LNCaP cells and an anti-sense miR-125b up-regulates expression of MUC1 protein (28). The MUC1 heterodimeric oncoprotein is aberrantly overexpressed in human PCa, and is associated with more aggressive pathologic and clinical features. AR occupies a consensus AR element on the MUC1 promoter in androgen-dependent LNCaP (28). Thus, miR-125b acts as a bridge between androgen and its effect on protein expression in tumor cells.

As mentioned previously, miR-141 acts as a regulator in PCa while it is also regulated by androgen. It is up-regulated by androgen in both LNCaP cells and xenograft models. miR-141 expression was also found to be increased in PCa compared to BPH (29). Additionally, the overexpression of miR-141 enhanced the growth of parental LNCaP cells whereas inhibition of miR-141 by anti-miR-141 suppressed the growth of the LNCaP subline that overexpressed AR (29). The ectopic overexpression of miR-141 increased the growth of LNCaP cells, suggesting that the regulation of miR-141 by androgen potentially contributes to the progression of androgen-dependent PCa. In addition to miR-141, miR-10a, miR-150, and miR-1225-5p also exhibited androgen regulation in both LNCaP cells and xenografts (29). The expression of miR-101 in LNCaP cells is modulated at different physiological conditions, such as androgen stimulation, which differentially regulates prostate cell proliferation (30). In LNCaP cells, by microarray analysis, fifteen miRNAs have been shown to be differentially regulated by 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) and testosterone (T). Among these miRs, miR-22, miR-29ab, miR-134, miR-1207-5p, and miR-371-5p are up-regulated, whereas miR-17 and miR-20a, members of the miR-17/92 cluster, are down-regulated. Moreover, 1,25(OH)2D3 and T modulate miRNA-mediated mRNA degradation, resulting in regulation of steady state mRNA levels and generation of attenuation feedback loops that result in global changes in mRNA and protein levels (31).

MiR-21 has been demonstrated to act as an androgen receptor-regulated microRNA that promotes hormone-dependent PCa growth. The miR-21 promoter, miPPR-21, binds to androgen-induced AR, suggesting direct transcriptional regulation. Elevated expression of miR-21 has been shown to enhance PCa growth in vivo, and is sufficient for androgen-dependent tumors to overcome castration-mediated growth arrest (32). Let-7d is an androgen-regulated microRNA; one target of Let-7d is pre-leukemia transcription factor 3 (PBX), which is known to regulate genes involved in differentiation of urogenital organs and steroidogenesis. This association is of interest because regulation of steroidogenesis is one of the mechanisms underlying PCa progression. Through Let-7d, PBX3 is up-regulated in PCa and post-transcriptionally regulated by androgen (33). MiR-27a expression is regulated by androgens both transcriptionally (via AR binding to the cluster promoter) and post-transcriptionally (accelerating primiR processing to the mature form). MiR-27a acts by targeting the tumor suppressor and AR co-repressor, Prohibitin (PHB). This involves a novel mechanism for androgen-mediated miR regulation, whereby AR induces a transient increase in miR-23a27a24-2 transcription, but more significantly accelerates processing of the primiR-23a27a24-2 cluster (34).

The novel mechanism for androgen-mediated miR regulation that involves several miRs potentially provides insight into the mechanism of androgen-dependent PCa. The androgen may participate in the initiation and progression of PCa via miRs. Thus, the regulation of selected miRs could potentially serve as a therapeutic target of androgen-dependent PCa in the future.

4. MicroRNAs IN METASTASIS OF ANDROGEN-DEPENDENT PCA

Although numerous miRNAs have been identified with abnormal expression in PCa, leading to the corresponding metastasis of this disease, only several miRNAs have been demonstrated to be involved in the metastasis of androgen-dependent PCa. A set of miRNAs have been shown to be differentially expressed in androgen-dependent metastatic PCa cells compared to androgen-independent metastatic PCa cells. Among them, the differential expression of miR-205 and miR-200c were further validated by Northern blot analysis in these two types of PCa cells (35). MiR-205 was found to exert a tumor-suppressive effect in human prostate tissue, including in LNCaP cells, by counteracting epithelial-to-mesenchymal transition and reducing cell migration/invasion, at least in part via down-regulation of protein kinase C (36) (Figure 1).

At present, there are reports regarding the expression of selected miRs in metastasis of androgen-dependent PCa; however, the underlying mechanism remains unclear. This may be due to androgen-insensitivity in advanced-stage PCa when the tumor becomes metastatic. However, studies on miRs involved in the metastasis of androgen-dependent PCa could potentially elucidate the mechanism through which androgen-dependent PCa becomes androgen-independent PCa during metastasis, and provide additional information helpful for the development of novel PCa therapies.

5. MicroRNAs IN CASTRATE-RESISTANT PCA

Because the relationship between androgen levels and PCa growth has been previously demonstrated,
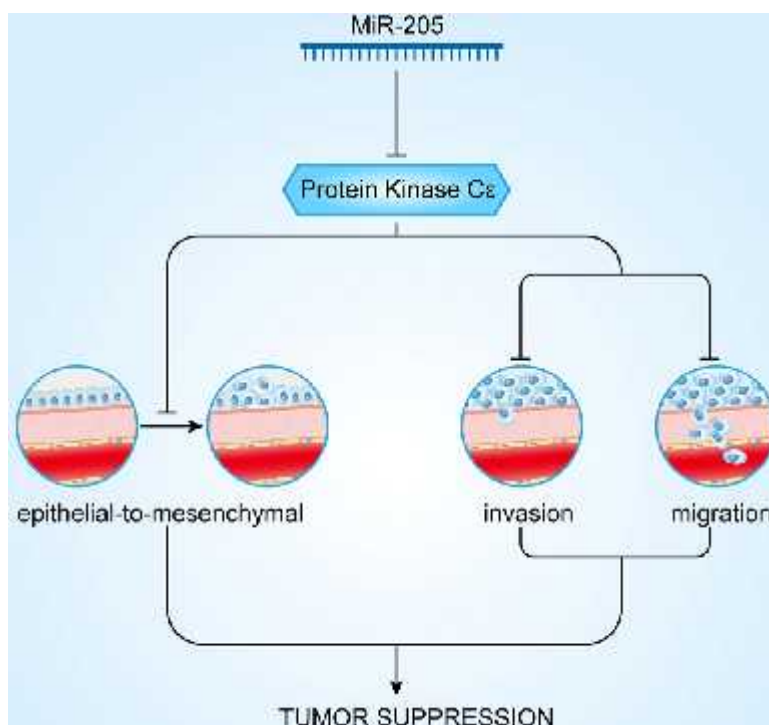
Figure 1. The tumor-suppressive effect of miR-205 in LNCaP cells. MiR-205 exerts a tumor-suppressive effect in LNCaP cells by counteracting epithelial-to-mesenchymal transition and reducing cell migration/invasion, at least in part through the down-regulation of protein kinase C .

androgen-deprivation therapy (castration) has been a key treatment option for PCa in monotherapy or in combination with other methods (37–39). Despite a good initial response, remissions last on average 2–3 years, with eventual progression occurring despite castration (38–40). In these cases, PCa progresses to a castration-resistant form. Elucidation of how androgen-dependent PCa transforms into a castration-resistant form would be helpful to allow for arresting or even reversing the process. During this process, miRs have been shown to be involved. MiR-125b has been demonstrated to have the ability to render LNCaP cells resistant to androgen withdrawal; furthermore, it has been shown to be androgen regulated, and one of its targets, BAK1, has been identified as being involved in the functional apoptosis of LNCaP cells (41). Thus, the ability of miR-125b is associated with apoptosis of PCa cells. In addition, miR-130a, miR-203, and miR-205 act jointly as tumor suppressors in PCa and potentially interfere with castration resistance (42). These miRs directly target several components of the mitogen-activated protein kinase (MAPK) and AR signaling pathways (42). Both pathways are central for the development of the primary tumor and, in particular, the progression to its incurable castration-resistant form.

6. TUMOR SUPPRESSOR MicroRNAs IN ANDROGEN-DEPENDENT PCA

In androgen-dependent PCa, miRs act as tumor suppressors. MiR-146a was found to function as a tumor-suppressor gene in modulating hyaluronan (HA)/ROCK1-mediated tumorigenecity in androgen-dependent PCa (43). In tumors of mice treated with epigallocatechin-3-gallate (EGCG), miR-330 was found to be a tumor suppressor, and was up-regulated (44). Furthermore, this study demonstrated that EGCG functionally antagonizes androgen action at multiple levels, resulting in inhibition of androgen-dependent LNCaP growth (44). These findings suggest that miRs act as tumor suppressors could potentially play an important role in anti-tumor therapies.

Let-7 encodes an evolutionarily conserved family of 13 homologous miRs located in genomic locations frequently deleted in human cancers (45). Let-7 expression was found to be down-regulated in localized PCa tissues compared with benign peripheral zone tissues (46,47). Let-7 members have been shown to regulate expression levels of oncogenes such as HMGA2 (48), RAS (49), and Myc (50) along with genes involved in cell cycle and cell division regulation. A member of the let-7 family, let-7c, has been demonstrated to act as a tumor suppressor. In PCa, miR-let-7c suppresses AR expression and activity by targeting its transcription via c-Myc, resulting in decreased cell proliferation of human PCa cells (51). Nadiminty and colleagues confirmed the role of miR-let-7c as a tumor suppressor, and further demonstrated that suppression of let-7c expression enhanced the ability of androgen-sensitive PCa cells to grow in androgen-deprived conditions in vitro (52). This suggests that miRs acting as tumor suppressors exhibit the potential to improve androgen-deprivation therapies, and could act as potential therapeutic targets for PCa.

7. MicrorNAS AS THERAPY FOR ANDROGEN-DEPENDENT PCA

The growth of PCa is androgen dependent, and metastatic tumors are generally treated with androgen ablation therapy, with or without antiandrogen supplementation (37,53,54). MiRs have been found to play a role in the treatment of PCa chemotherapy. In the chemotherapy regimens of PCa, panobinostat/everolimus combination can enhance anti-tumor activity mediated by decreased tumor growth concurrent with augmentation of p21 and p27 expression and the attenuation of angiogenesis and tumor proliferation via AR, c-Myc, and HIF-1 signaling. These three transcription factors have been found to be associated with altered expression of miRs (55). This suggests that the response of selected miRs could be utilized to monitor panobinostat/everolimus activity in vivo. An additional study has found that chemotherapeutic agents goserelin and bicalutamide can affect the expression of different miRs with an in silico Bayesian modeling tool (56).

MiR-210 is the miR most frequently associated with tumor hypoxia, and it has been shown in several studies that it was a target of HIF-1 (57-59). Although miR-210 has been found to be an interesting marker of chronic hypoxia irrespective of androgen dependancy, it has not yet been demonstrated to be a therapeutic target in PCa. Pretreatment with miR-210 inhibitors did not sensitize cells to irradiation under either normoxia or anoxia. Downstream miR-210 targets are potentially more likely to be beneficial therapeutic targets in PCa, such as RAD52, which is involved in homologous recombination and has a direct effect on radiation efficacy (60).

After androgen-deprivation therapy in univariate analysis, single nucleotide polymorphisms (SNPs) of miRs were significantly associated with PCa progression, PCa-specific mortality, and all-cause mortality as following: KIF3C rs6728684, CDON rs3737336, and IFI30 rs1045747 genotypes remained as significant predictors for disease progression; KIF3C rs6728684, PALLD rs1071738, GABRA1 rs998754, and SYT9 rs4351800 remained as significant predictors for PCa-specific mortality (PCSM); and SYT9 rs4351800 remained as a significant predictor for all-cause mortality (ACM) in multivariate models that included clinicopathologic predictors. Moreover, strong combined genotype effects on disease progression and PCSM were also observed. Polymorphisms inside miRs enable prediction of clinical outcomes in PCa patients receiving androgen-deprivation therapy. Patients with a greater number of unfavorable genotypes exhibited a shorter time to progression and worse PCa-specific survival during androgen-deprivation therapy (61).

In summary, miRs act as mediators in PCa therapy and could potentially influence the effects of chemotherapy or prognosis in PCa patients. Further studies on the miRs in the androgen-deprivation therapy of PCa are warranted and could be valuable, particularly in castrate-resistant PCa therapy.

8. CONCLUSIONS

The genetics of androgen-dependent PCa are complex and only partially understood. In recent years, reports on the miRs in the pathogenesis of this cancer have shown that the molecular mechanism of androgen-dependent PCa is more complex than initially thought. Despite recent studies that have found that miRs are involved in the development, progression, metastasis, therapy, and even prognosis of this disease, the precise mechanism underlying miRs in androgen-dependent PCa remains unclear.

9. ACKNOWLEDGMENTS

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Abbreviations: PCa: Prostate cancer; miRs: MicroRNAs; 3′ UTRs: 3′ untranslated regions; BPH: benign prostatic hyperplasia; AR: androgen receptor; Shp: small heterodimer partner; CAND1: cullin-associated and neddylation-dissociated 1; 1,25(OH)2D3: 1,25-dihydroxyvitamin D3; PHB: Prohibitin; MAPK: mitogen-activated protein kinase; EGCG: epigallocatechin-3-gallate; HA: hyaluronan; SNPs: single nucleotide polymorphisms; ACM: all-cause mortality

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