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The role of MicroRNA in castration resistant prostate cancer

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Abstract

Introduction—Castration resistant prostate cancer (CRPC) has a historically low median survival rate but recent advances and discoveries in micro RNAs (miRNAs) have opened the potential for new prognostication modalities to enhance therapeutic success. As new chemotherapies and immunotherapies are developed there is an increasing need for precision and stratification of CRPC to allow for optimization and personalization of therapy.

Methods—A systematic literature review was conducted via electronic database resulting in the selection of forty-two articles based on title, abstract, study format, and content by a consensus of all participating authors. The majority of selected articles were published between 2002 and 2013. In this review, we will discuss the robustness of miRNAs as a biomarker platform, miRNAs associated with prostate cancer, and recent discoveries of miRNA associations with CRPC.

Results—The associations discovered have been of interest due to the ability to differentiate between CRPC and localized prostate cancer. With evaluation of multiple miRNAs, it is possible to provide a profile in regards to tumor characteristics. Furthermore, actions of miRNAs on CRPC tumor cells have the ability to suppress metastatic phenotypes.

Conclusion—miRNAs may have a growing role in CRPC prognostication and potentially transform into a therapeutic potential.

Keywords

Castration Resistant Prostate Cancer; micro RNA; Review

Introduction

Prostate Cancer is the leading cancer of men in the developed world and is quickly rising in incidence in the developing world.[1] In 2013, prostate cancer is estimated to be responsible for 239,000 new cases with 29,700 deaths in the United States alone.[2] Localized prostate cancer can be treated and is potentially curable when detected early. A variety of therapy modalities are available including radical prostatectomy, hormone therapy, chemotherapy, radiation therapy, and cryotherapy. Unfortunately, despite recent advances in detection and localized curative treatment, 23 – 40% of those patients will go on to develop metastatic

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disease.[3] Commonly, metastatic disease is treated with androgen deprivation therapy to induce apoptosis of tumor cells or arrest growth.[4]

Castration-Resistant Prostate Cancer (CRPC) arises when hormone-refractory growth occurs in a castrate androgen level environment. Although the mechanism is not fully understood, the androgen receptor (AR) has emerged as important target for therapy for metastatic prostate cancer.[5] As the pathogenesis of CRPC is further elucidated, newer diagnostic modalities may provide early detection of CRPC enabling survival benefit and prevention via targeted pathway specific therapies.[6]

MicroRNAs (miRNAs) have recently demonstrated its versatility in assisting in diagnosis of recurrence, monitoring progression, and predicting treatment response. miRNAs are small non-coding 21 to 23 nucleotide base pair RNA molecules that serve as transcriptional and post-transcriptional regulators of gene expression. These regulation effects are exerted over multiple cell types targeting approximately 60% of human genes. miRNAs regulate via basepairing with messenger RNA (mRNA) thereby exerting effect on gene expression. Recently miRNAs have been at the forefront of urological oncology attention with more than 40 miRNAs implicated in urologic cancers that target common carcinogenic pathways providing novel opportunities to develop strategies for prognostication and therapy.[7] This review will comprehensively discuss the development of miRNAs related to CRPC in their evolution into a future diagnostic and prognostic biomarker. Furthermore, we will outline a path to their potential development as a novel therapeutic agent.

Methods

A systematic literature review was conducted via electronic database searches of PubMed/Medline. Searches were conducted with the following combinations and iteration of the following terms: castration resistant prostate cancer, CRPC, prostate cancer, micro RNA, miRNA, biomarker, and metastasis. A total of 1304 references were obtained. Forty-two articles were selected based on title, abstract, study format, and content by a consensus of all participating authors. The majority of selected articles were published between 2002 and 2013. The literature regarding miRNA and castration resistant prostate cancer is generally based on basic science experiments performed on obtained human samples or cancer cell lines. Published conclusions from these studies are subject to the limitation of small sample size, selection bias, and clinical applicability.

What are miRNAs?

miRNAs are small non-coding RNA molecules which have been found in plants and animals. They are well conserved in eukaryotic organisms and found to be a crucial component in genetic regulation. miRNA genes are transcribed and regulated similarly to those of protein-coding genes. Transcription occurs in the nucleolus and after subsequent processing and transport to the cytoplasm for further processing.[8,9]

Over 2019 unique mature human miRNAs have been identified, with some having ability to interact with multiple mRNAs.[10] This provides a glimpse at the immense role that miRNAs play in gene regulation. Current epigenetic estimates place the potential targeting

of miRNAs to 60% of genes over a diverse array of human cell types.[11,12] As the involvement of miRNA is further elucidated, a growing image of miRNA critical involvement with normal physiology begins to form. Several experiments affecting biogenesis of miRNA with mutations in Dicer enzymes have revealed development abnormalities including germ-line defects, abnormal embryogenesis, developmental arrest, and depletion of stem cells.[13]

How miRNAs are connected with Cancer?

With its established influence over normal physiological development and growth, it can be carried over that miRNAs also have an important role in the abnormal growth and development of cancer. It has been observed that within tumorigenesis, miRNA expression is altered.[14] With a regulatory role, miRNAs can serve in tumor promoting (oncomir) or inhibiting depending on its targets. Tumorigenic roles of miRNAs have been observed in targets to tumor-suppression genes such as PTEN by miR-21.[15,16] miRNA-125b has been attributed as a oncogene in the pathogenesis of prostate cancer through down regulation of Bak1.[17] Furthermore, the contrast of silencing tumorigenesis genes has been observed with let-7 targeting KRAS.[18,19]

Conversely, miRNAs are affected in the mechanism of cancer development that can be observed quantitatively and qualitatively.[20] Changes in quantitative expression arise with genetic mechanisms. Qualitative effects manifest with changes in recognition sequences or mutations to its target. Recently polymorphisms in miRNA sequences and binding sites have been correlated to increased cancer risks in multiple case-control studies. Specifically polymorphisms of the miRNA-let-7c binding site on the KRAS 3'-untranslated region (UTR) have been implicated in increased risks of gastric cancer, ovarian cancer, colorectal cancer, and non-small cell lung cancer (NSCLC).[21–24]

Using multiple miRNA in a expression profile Lu et al. demonstrated the utility in acquiring history of human cancers regarding their developmental origin and malignancy.[25] In addition, miRNA profiles were able to partition tumors of a single lineage to further illuminate their development. The signatures provided by the miRNA profile allowed determining diagnosis for tumors of uncertain cellular origin. These characteristics have enabled use of miRNA profiles to successfully distinguish and define pathological environments for normal versus malignant samples of unknown origin.[26–28]

Supporting the miRNA profile ability to successfully profile tumors, it possesses characteristics that allow for practical application in a clinical testing setting. The stability of miRNAs have allowed its extraction from multiple types of pathological samples ranging from fresh, frozen, and formalin fixed paraffin embedded (FFPE).[29] Due to is smaller sequence length, miRNAs have shown superior analysis performance via RTPCR from FFPE samples and analysis.[30] Furthermore, RT-PCR analysis of miRNA profiles has been successful in scant and poorly preserved samples lending its ability for analysis from limited cell supply samples such as peripheral blood. With a combined ability to analyze profiles compared to single analytes, the resolution of diagnostic detection is greatly enhanced. Development of new miRNA based diagnostic tests may exploit the use of animal models

owing to the highly conserved nature of miRNAs between different species. Lastly, discovery of miRNAs linked to disease can be profiled in a reproducible and profile manner with the employment of microarray assays such as those used to discover the miR-99 family and its connection to PSA and prostate cancer cell proliferation suppression.[31]

Potential Roles of miRNAs in CRPC

As these developments advance, great excitement is generated in how they can be applied to enhancing diagnosis and prognostication of CRPC. miRNAs offer a new hope in changing the landscape of how CRPC is managed. Attard and colleagues have reviewed the potential benefits to the management of CRPC with robust biomarker strategies that miRNAs currently show promise in fulfilling. [32] A recent study has demonstrated the utility of miR-141 as a biomarker in castration-resistant and hormone-sensitive metastatic prostate cancer that holds promise in ability to aid in selecting patients for therapy and monitoring treatment efficacy.[33] Several recent miRNA discoveries based on human CRPC patient serum, tumor, and tissue-derived samples demonstrate potential utility as biomarkers in CRPC (Table 1).[34–39] Furthermore, our developing understanding of how miRNAs influence the relationship of AR and CRPC as oncomirs and suppressors is generating traction towards miRNA-based therapy. [3,4,17,57–63]

miRNAs as Potential Biomarkers in CRPC

Zhang and colleagues demonstrated the first correlation of elevated serum miRNA levels within CRPC patients.[34] Using qRT-PCR, serum levels of miR-21 were quantified in CRPC, androgen dependent prostate cancer (ADPC), localized prostate cancer, and benign prostate hypertrophy (BPH) patients. Chemotherapy resistant CRPC patients had the highest miR-21 levels compared with ADPC and localized prostate cancer. Low PSA ADPC had similar levels of miR-21 to localized prostate cancer and BPH. The authors inferred that miR-21 could be used to differentiate CRPC and add prognostic information to PSA testing. This opened the potential for miRNA as a gateway to diagnosis and prognostication.

Current development of miRNA based biomarkers have expanded the investigation to include miR-21, 141, and 221 levels of prostate cancer patients to explore using multiple markers as a diagnostic profile.[35] miRNA levels were measured from serum samples using qRT-PCR of metastatic prostate cancer, localized prostate cancer, and control patients. Their results showed similar significant elevation of miR-21 levels in prostate cancer patients as discovered previously. Investigation of miR-141 levels demonstrated a significant elevation in metastatic patients compared to localized prostate cancer patients. Using multiple miRNA levels allows for the comparison and analysis in a pattern profile potentially allowing for a synergistic effect in diagnosis and prognosis.

Generating new hits for potential biomarkers, the search included a new avenue in discovery of miRNAs with a mouse model that shared commonality in human samples.[37] Initial discovery found a set of 46 miRNAs in serum of Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice with advanced prostate cancer compared to healthy controls. A subset with homologues to human miRNAs was measured in men with metastatic CRPC and healthy controls. miR-141, miR-298 and miR-375 were found to be elevated in the serum of

metastatic CRPC men. Furthermore intra-tumor samples revealed elevation of miR-141 and miR-375 with association of disease outcome. This lends to the involvement of miRNAs with deregulation for development and progression of castration resistant prostate cancer. This study highlights the utility of mouse models in discovery of future miRNAs and reinforces ability of miRNAs in diagnosis and prognosis.

Further potential miRNA CRPC biomarkers are summarized in Table 1 representing the diverse manner of their discovery and the growing interest in miRNA based diagnostic and prognostic assays.

miRNA Functional Role in CRPC

As the search for biomarkers continues, it has been found that miRNA expression alterations occur during the development of CRPC providing a potential picture of their functional roles in CRPC.[38] Using miRNAs derived from prostatic tissues and bone marrow of metastatic CRPC, miR-221/-222 were found to be upregulated while miR-23b/-27b were down regulated in tissues of metastatic CRPC compared to normal and primary prostate cancer. The development and/or maintenance of CRPC may be connected to the increase of miR-221/-222 due to its involvement in essential components for androgen receptor functional integrity. miR-221/-222 unregulated expression is involved in c-kit, PTEN, and TIMP3 maintenance of TRAIL-resistant non-small cell lung cancer and liver cancer.[40,41] Furthermore, miR-221/-222 is involved in conference of resistance to tamoxifen, fulvestrant, and cisplatin in the setting of breast cancer.[42–45] Via miR-23b repression by MYC protein transcription, upregulation of mitochondrial glutaminase and glutamine metabolism occurs thus providing the energy for cancer growth. MYC transcription has been found significantly elevated in metastatic prostate cancer.[46,47] Potentially repressed levels of miR-23b in metastatic CRPC maybe due to the effects of MYC overexpression and glutaminase activity required for maintained growth.

Further supporting the use of miR-23b/-27b as a biomarker for CRPC, Ishteiwy et al. were able to confirm the repression in metastatic CRPC and demonstrate the functionality of miR-23b/-27b to suppress metastatic phenotype of CRPC.[36] miR-23b/-27b administration to CRPC cell lines was able to suppress motility, anchorage-independent growth. Metastatic phenotype was determined with the observation of increased E-cadherin expression and reduced Rac1 activity. The adhesion molecule E-cadherin has been demonstrated to suppress invasive and migratory phenotype of cancer cells and loss has been demonstrated to confer metastatic activity to transformed breast epithelial cells.[48–51]. The Rho GTPase Rac1 regulation of cytoskeleton rearrangements is involved in cell migration and associated aggressive prostate cancer demonstrated by its requirement in the invasive behavior prostate cancer cell line PC3.[52–56]. miR-23b/27b down regulation in CRPC and its ectopic suppressive effects lends to its ability to function as a specific and valuable biomarker in diagnosis and prognosis of CRPC.

miRNA role in the relationship of AR and CRPC

Our group has laid a foundation towards miRNA-based therapy through understanding the influence that miRNAs have on the relationship of AR and CRPC and exposing potential targets for future therapy (Figure 1).[3,4,17,57–63]

Initially we demonstrated a facet of this relationship through the miR-124-AR-mirR-125b pathway.[61] Using a miRNA array we revealed a dramatic reduction of miR-124 in prostate cancer tissues compared with BPH tissues. Hyper-methylation of all three miR-124 loci resulted in reduced expression in prostate cancer tumors. Furthermore, miR-124 was characterized to have a direct targeting of AR thereby inducing downregulation of miR-125b and upregulation of p53. Contrasting with that effect, miRNA was found to be downregulated in clinical prostate cancer samples with high levels of AR. With the application of miR-124 on prostate cancer cells, apoptosis and growth inhibition was incurred thereby leading to potential novel targets in the miR-124-AR-miR-125b pathway.

After establishing that AR regulates miR-125b that directly targets Bak1[17], we focused our attention to new miR-125b targets to further exploit the miR-124-AR-miR-125b pathway. Using gene expression profiling, eight miR-125b targets were discovered that had activity as pro-apoptotic factors or tumor suppressors. Apoptosis assays were conducted with presence of anti-miR-125b to demonstrate the repression of miR-125b and its effect on these factors (p52, Puma, Bak1, and $p14^{ARF}$).[60,64] Both androgen dependent and independent prostate cancer cells underwent apoptosis under the influence of anti-miR-125b. In addition, anti-miR-125b promoted release of mitochondrial CytC, SMAC, and activated Cas3 indicating further apoptotic response. With the foundational work of establishing the miR-124-AR-miR125b pathway, the next step was taken to distinguish their effects on tumor growth in an *in vivo* mouse model. Mice were injected subcutaneously with lenti-miR-125b-PC346C tumors with a 19-fold greater miR-125b level over controls.[60] Tumors grew significantly faster than controls and only exhibited temporary growth regression after castration. miR-124 was evaluated with lenti-miR-124 vectors infected 22Rv1 AI prostate cancer cells.[61] With a 23-fold higher expression of miR-124 than controls, growth of tumors was inhibited and AR expression was significantly downregulated. These results establish the exciting prospect of miRNA contribution in androgen dependent and independent pathogenesis of prostate cancer.

In efforts to explore different pathways, recent developments with miR-let-7c have led to the discernment of the connection of its expression with the downregulation of AR expression and potential CRPC development.[3] Prostate tumor xenografts in a mouse model demonstrated reduced tumor cell proliferation in presence of miR-let-7c. As androgen receptor upregulation has been implicated in the conversion of prostate cancer to CRPC, miR-let-7c may be involved in this potential pathway.[6] Further studies supported this role of let-7c by revealing its down regulation in CRPC cells.[63] Let-7c suppressed prostate xenografts demonstrated growth in androgen-deprived environments with reduction of tumor burden when expression was activated. Moreover, it was discovered that let-7c and its repressor Lin28 shared a inverse relationship expression in clinical prostate cancer specimens compared to benign samples with the former down regulated and latter up

regulated. Lin28 is upregulated by NF-kappaB2/p52 that has been previously implicated in its role of development of CRPC via aberrant activation of AR.[62] Let-7c may provide a novel approach as a therapeutic target in suppressing prostate cancer and development of CRPC.

Therapeutic Roles of miRNA in CRPC

As we continue to further understand the functional roles of miRNAs in CRPC, they can be exploited to develop novel therapeutic modalities. Most excitingly, anti-miR-125b sensitized prostate cancer cells to cisplatin and genistein combined polysaccharide. miR-125b inhibition may play a role in increasing efficacy of current therapy as p53 functionality is required for docetaxel sensitivity in prostate cancer.[65]. This opens a novel treatment strategy of inducing apoptosis and increasing efficacy of anti-prostate cancer drugs via manipulation of miRNAs.

Recently miR-30 has been a focus of interest in CRPC due to its involvement with the Src tyrosine kinase pathway and potential to direct Src inhibitor therapy.[4] As miR-30 family is downregulated in prostate cancer cells by Src tyrosine kinase[66], the opposing effect is noted in this study with the presence of Src inhibitors in a castration-resistant VCap xenograft model. This upregulation in the miR-30 profile was correlated to inhibition of CRPC malignancy via inhibition of growth, invasion, and migration. Overexpression of miR-30 inhibited growth, invasion, and migration of CRPC cells. It was demonstrated that miR-30 binds to oncogene Ets-related gene (ERG) at the 3'UTR. miR-30 may exert its effect on CRPC via ERG down stream targets such as C-MYC.[67] miR-30 maybe part of a broader array of miRNAs that can be used as viable biomarker for targeting of Src inhibitor therapy for ERG-positive CRPC patients and tumor suppression therapies for CRPC.

Conclusion

The future use of miRNAs as a diagnostic and prognostic biomarker for CRPC has been developing upon a growing body of research for the past few years. Currently, there has been an active search in identifying miRNAs with valuable prognostic properties from animal models, prostate caner cell lines, patient samples, and markers from other cancers. These discoveries have been demonstrated to have prognostic properties when used in singularity and multiplicity to create a diagnostic profile. Furthermore, the stability and robustness of miRNAs as a clinical biomarker platform has been supported by a large body of evidence in regards to its stability and ease of extraction. We anticipate the prognostic value of miRNAs to grow as larger studies further elucidate the validity of the current miRNAs discovered overcoming the inherent inconsistencies of small sample size studies. Moreover, lager sample sizes will allow for more precise stratification of tumor activity, prediction of metastatic qualities, and estimation of mortality. The excitement behind the discoveries of the repressive effects of miRNAs on CRPC tumors opens a potential avenue for future therapeutics from the current search for a novel biomarker. As we better understand the influence of miRNAs on AR and CRPC, newer treatment modalities can be developed to exploit the interplay of these factors and serve to identify patients who potentially have an advantage with current therapies.

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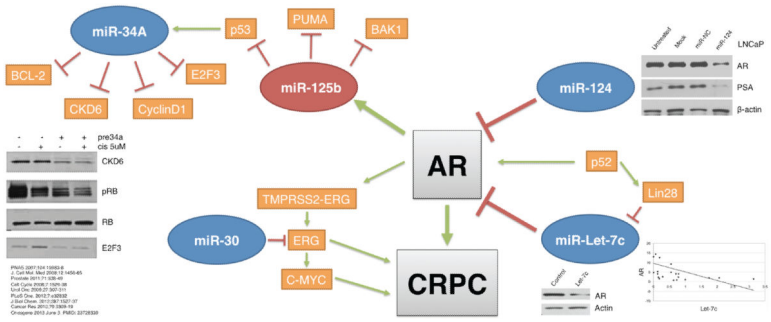

Figure 1.
Interactions of miRNAs with AR and CRPC.

Table 1

Potential miRNA biomarkers of CRPC (Castration Resistant Prostate Cancer)

miRNA Identified	Source	Sample Size	Method	Major Findings	References
miR-21	Plasma	56 Patients (20 Localized, 20 Androgen Dependent, 10 CRPC)	qRT-PCR	miR-21 elevated in CRPC patients, especially in docetaxel-based resistance	Zhang et al., 2011
miR-21, miR-141, miR-221	Serum	51 Patients (18 Localized, 8 Local Advanced, 25 Metastatic)	qRT-PCR	miR-21 and miR-221 levels elevated prostate cancer. miR-21, miR- 141, and miR-221 levels higher in metastatic compared to localized disease	Yaman Agaoglu et al., 2011
miR-23b/-27b	Cell Lines	ALVA31, PC3-ML, LNCaP Cell Lines	Migration, Proliferation, Invasion Assays, Immunoblotting	miR-23b/-27b suppresses metastatic phenotype, decreases Rac1 activity, and increases of E-cadherin levels	Ishteiwy, Ward, Dykxhoorn, & Burnstein, 2012
miR-141, miR-298 and miR-375	Serum and Tumor	25 metastatic CRPC Patients	qRT-PCR	miR-141, miR-298 and miR-375 elevated in metastatic CRPC serum. miR-141 and miR-375 intra-tumor elevation in CRPC	Selth et al., 2012
miR-221/-222, miR-23b/-27b	Tumor	51 Patients (34 Localized, 17 metastatic CRPC)	qRT-PCR	miR-221/-222 increased in CRPC, miR-23b/27b repressed in CRPC	Sun et al., 2012
Group A	Tumor	4 CRPC	qRT-PCR	Group A: Exclusive down regulation in hormone-refractory prostate cancer. Group B: Exclusive up regulation in hormone-refractory prostate cancer.	Porkka et al., 2007
let-7f	Samples	Patients			
miR-19b					
miR-22					
miR-26b					
miR-27a					
miR-27b					
miR-29a					
miR-29b					
miR-30a_5p					
miR-30b					
miR-30c					
miR-100					
miR-141					
miR-148a					
miR-205					
Group B					
miR-184					
miR-198					

miRNA Identified	Source	Sample Size	Method	Major Findings	References
miR-302c					
miR-345					
miR-491					
miR-513					