Fundamental Role of microRNAs in Androgen-Dependent Male Reproductive Biology and Prostate Cancerogenesis

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Keywords
Androgen receptor, microRNA, prostate cancer

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Submission February 29, 2012;
accepted March 13, 2012.

Citation
Todorova K, Mincheff M, Hayrabedyan S, Mincheva J, Zasheva D, Kuzmanov A, Fernández N. Fundamental role of microRNAs in androgen-dependent male reproductive biology and prostate cancerogenesis. Am J Reprod Immunol 2012

doi:10.1111/j.1600-0897.2012.01139.x

Male reproductive failure has been linked to successive development of various urologic diseases including prostate cancer. There is strong epidemiologic data in support of this association, it is important therefore to identify the fundamental grounds that lay beneath such a connection. Male reproductive biology, as sex determined, is significantly dependent upon the hormonal regulation of androgens. With the advancement of knowledge on androgen receptivity and epigenetic regulation, the role of new regulatory factors such as microRNAs becomes essential. This review focuses on unraveling the role of microRNA tight incorporation in androgen-dependent male reproductive biology in the context of recent prostate cancer data.

Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules about 21–25 nucleotides in length. They are partially complementary to one or more messenger RNAs, and their main function is related to downregulation of gene expression by binding post-transcriptionally to the 3′ untranslated region (UTR) of the target mRNAs. They negatively regulate gene expression in a variety of manners, including translational repression, mRNA cleavage, and deadenylation. MiRNAs were discovered in 1993 by Victor Ambros, Rosalind Lee, and Rhonda Feinbaum during a study of the gene lin-14 in *C. elegans* development;[1] the term microRNA was adopted in 2001.[2] It is only recently that researchers have begun to understand their scope and diversity. Growing evidence shows that miRNAs exhibit a variety of crucial regulatory functions related to cell growth, development, and differentiation[3,4] and are associated with numerous human diseases, including cancer[4] and heart ailments[5]. Bioinformatics prediction indicates that mammalian miRNAs may regulate approximately 30% of all protein-coding genes.[6] So far, approximately 1527 precursors and 1921 mature human miRNAs have been identified (miRBase release 18).

Prostate cancerogenesis in the context of pre-existing male infertility

Recently, male reproductive failure has been linked to the development of urologic diseases, including prostate cancer (PCa), and epidemiologic data, exploring the association between male reproductive health and PCa, have already been published. TJ Walsh suggests a link between man's fourth decade reproductive health (30s) and the development of aggressive PCa in his sixth decade (50s),[7] and studies on the impact of inherited or acquired male infertility on normal or malignant prostate tissue development seem very important. There is little information regarding microRNAs expression in prostate tissue and their biological effect on normal prostate development. Some information has come from prostate cancer differential expression studies. Several microRNAs like miR-106b-25 cluster and miR-32[8] exert regulatory functions over tumor suppressors like E2F1, p21/WAF1, and Bim. MiR-106b-25 cluster upregulation significantly affects caspase-3 and caspase-7, suggesting an anti-apoptotic role in prostate cells.[8] Bonci et al.[9] demonstrate the essential control of miR-15a–miR-16-1 cluster on human prostate cell survival, proliferation, and invasion. MiR-15a and miR-16 cluster also stimulate cell growth and invasiveness, inducing hyperplasia in the RWPE-1 normal human prostate cell model[9] after antagomirs (artificial miRNAs) induction. Growth modulation of prostate cell lines has also been attributed to miR-23b, miR-145, and miR-100.[10] In addition, prostate cancer-related miRNAs have been described as tumor suppressors. Presently, miR-205 is considered to represent one of the most relevant miRNAs in prostate cancer.[11] The antagomir-mediated restoration of miR-205 to normal levels in aggressive prostate cancer cells induces marked morphological changes and cytoskeleton rearrangements, followed by a reverse transition from a mesenchymal to an epithelial state.[11] This finding has led to the understanding that miR-205 has a crucial role for maintaining the epithelial organization of human prostatic tissue. It upregulates the level of E-cadherin expression (and other cell adhesion molecules) and downregulates several factors known to be involved in the acquisition of a motile and invasive behavior (i.e., interleukin-6, enhancer of zeste homolog 2, caveolin-1, and metalloproteinase-2). These broad effects are driven possibly by the concurrent suppression of specific putative miR-205 targets, such as N-chimerin, ErbB3, E2F1, E2F5, ZEB2, and protein kinase Cε, the latter having a direct role in regulating epithelial-to-mesenchymal transition, which is important for metastasis.[11]

To understand the mechanisms of miRNA action as oncogenes or tumor suppressors, miRNAs targets are constantly characterized. Oncogenic miRNAs downregulate the expression of apoptosis-related genes, and tumor suppressor miRNAs target the proliferation-related genes. Importantly, there is evidence that PCa-related miRNAs are regulated through androgen signaling, and this regulation may contribute to the development of androgen independence. Because of the oncogenic or tumor-suppressive properties of PCa-related miRNAs, they are likely candidates as biomarkers and more importantly as therapeutic targets for prostate cancer treatment.

Recent advancement in therapeutic approaches in PCa have incorporated a second generation A10 aptamer-conjugated miR15a and miR-16 targeted to PSMA-positive PCa cells.[12] We have studied the effect of miR-15a and miR-204 on master transcriptional factors (TFs) regulation in PCa and on androgen receptor (AR) receptivity and PCa cells metastasis (unpublished data). It has been very recently reported that the new generation of highly prostate-specific markers – AMACR[13,14] and PSMA[15,16] – along with other PCa-associated markers correlates with several miRNAs – miR-138, miR-224, and miR-186 significant downregulation, data backed up by a new 2011, gene expression profiling study identifying AMACR, EZH2, TMPRSS2-ERG, miR-221, and miR-141[17] as promising markers.

Lots of master regulator TFs are affected by many miRNAs, thus modulating cell and lineage faith, determination and differentiation. Similarly, TF like c-Myc exert a reciprocal miRNA depression effect, as observed in many cancers.[18]

Androgen receptor signaling is directly mediated and dependent upon miRNA

Androgen receptor function is critical for the development of male reproductive organs, muscle, bone, and other tissues. Only two miRNAs, miR-214 and miR-125a, positively correlate with androgen actions in prostate.[18] miRNA research is predominantly focused on oncology, and little research has been performed to understand the importance of miRNAs in normal tissue development. The results presented

by Narayanan et al.[19] are the first to demonstrate that miRNAs are also mediators of androgen action. Although the work clearly demonstrates that androgens increase the expression of a large set of miRNAs, it is possible that only a few miRNAs may mediate androgen action in prostate. MiR-21 and miR-125, which have been shown to be androgen responsive and to play a role in prostate carcinogenesis,[20] are also upregulated in normal prostate.[21,22] More importantly only miR-125b was found highly expressed in both androgen-dependent and androgen-independent prostate cancer cells,[20] suggesting that miR-125b has a role in androgen signaling beyond the receptor itself.

Recent studies in mammalian tissues and drosophila indicate the involvement of miRNAs in nuclear hormone receptor functions.[21,22] Studies *in vitro on* prostate cancer cells identified androgen responsive elements (AREs) in the promoter of miR-21 and miR-125b.[20,23] MicroRNAs are synthesized as primary miRs (pri-miRs) by RNA Pol II and are later converted to mature miRNAs by the RNAse enzymes Drosha and Dicer[24]. Narayanan et al.[19] found that tissue-specific knockout of Dicer in mice completely impairs AR function leading to an androgen-insensitivity syndrome. Their work clearly demonstrates that miRNAs are mediators of AR function and suggest the existence of a possible feedback loop between miRNAs, AR, and AR corepressors, contributing significantly to the emerging research field of the role of miRNAs in normal tissue development, hormone action, and steroid receptor function. Classical hormone action theory postulates that the ligand-bound AR is recruited to the promoter of target genes mediating their transcription and translation. Surprisingly, miRNAs are also mediators of androgen action. Instead of the classical one-step model of gene activation, AR also appears to regulate gene expression through a three-step pathway including miRNA activation, corepressor suppression, and DNA interaction to elicit its action. Even though androgens increase the expression of a large set of miRNAs, clinically only a few miRNAs mediate androgen action in prostate.[19] The fundamental finding that AR interacts with Dicer only ligand-dependently during its nuclear translocation process, and ultimately, the lack of interaction between unliganded AR and Dicer, despite their co-existence in the cytoplasm, suggests that conformational changes in AR upon ligand binding lead to its interaction with Dicer.[19] The implications of this phenomenon are enormous because it proves the existence of tight interaction between miRNAs and hormone signal-specific stimuli. The causal relationship between cellular proliferation and miRNA expression, whether androgens activate miRNAs to proliferate the tissues or *vice versa*, is clarified by the results obtained with dihydrotestosterone (DHT) action in Dicer$^{-/-}$ knockout (KO) mice.[19] Dihydrotestosterone is not capable to sustain its effects in prostate or muscle tissue in Dicer KO mice, therefore proving miRNAs to mediate androgen actions as miRNAs restoration results in subsequently increase in DHT-induced cellular proliferation. Experiments with seminal vesicles prove that tissue selective regulation of androgen action is mediated by miRNAs. MiRNAs have sexually differentiated mode of action, as the major difference between estrogen and androgen action is that estrogens work at Drosha level, whereas androgens appear to work at the Dicer level, indicating that even the presence of pre-miRNAs is insufficient for androgen action.[25]

Epigenetic regulation of androgen receptivity is also modulated by miRNA

While in some cases, AR is not functional because of genetic mutations, it has been found in almost 40% of PCa patients (like in DU145 cell line) to be silenced by promoter hypermethylation.[19,26,27] In PCa, AR can function in two opposite directions:[28] on one side, AR signaling is crucial for prostate and PCa cell survival;[29] however, its activation can also limit cell proliferation and mediate apoptotic induction under specific circumstances,[28,29] like in conditions of genotoxic stress, when AR is fundamental for p53 activation and for the subsequent induction of apoptosis.[30,31]

A new study has found at least 71 miRNAs affecting the AR expression in PCa cell lines, 13 of them directly binding to and regulating its 6-kb extended 3′-UTR. Among them, miR-34a and miR-34c are particularly interesting, as they are epigenetically regulated[18] and might possibly link the epigenetics with miRNAs functions in AR regulation in PCa. Rokhlin et al.[31] (2008) have demonstrated that miR-34a and miR-34c are involved in the apoptotic induction after p53 activation in PCa cells and that pro-apoptotic function of DNA damage-induced p53 and miR-34s is dependent upon AR activation, suggesting a central role of the epigenetically regulated

miR-34 family members in the physiology of AR in PCa.

In addition to miR-34s, other miRNAs have been reported to be controlled by AR, as mentioned earlier. Also miR-21 has been demonstrated to be directly transactivated by AR, through an AR binding site on the miRNA promoter,[23] raising the need for a deeper investigation on the role of miR-21 in the acquisition of a hormone-resistant PCa phenotype.[28] Finally, it has been demonstrated that a group of five miRNAs (namely miR-141, miR-494, miR-29a, miR-29b, and miR-29c), which directly target effectors of the epigenetic machinery, are also induced by DHT stimulation in VCaP and AR-expressing LNCaP cells.[32]

To our knowledge, another miR and miR-204 are also implicated in prostate cancerogenesis, being differentially regulated in prostate cancer $AR^{+/+}$ and $AR^{-/-}$ cell lines. miR-204 inhibition results in AR transcript level upregulation in LNCaP ($AR^{+/+}$, $p53^{+/+}$), while in PC3 ($AR^{-/-}$, $p53^{-/-}$), it becomes depressed (unpublished data). Stemming from the regulation interactions between p53 and AR, it becomes evident that its role is tightly interconnected with the p53 pathway signaling, the lack of p53 might be directly related to lack of AR or *vise versa*, and this is probably mediated by miRNAs disregulation.

Summary

The new data implicating miRNAs in direct mediation of androgen receptivity and its integration to DNA damage, cell death, survival, and signaling p53-mediated pathway illustrate the cellular complexity of the male reproductive system. In this context, miRNA disregulation may have a profound impact and highlights its importance in diagnostic, prognostic marker selection and therapy.

Acknowledgements

Dr. Todorova research is supported by a Young Scientist Competition Grant (Contract DMU 03/27 12.12.2011) sponsored by the Bulgarian National Science Fund.

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