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MiR-185 attenuates androgen receptor function in prostate cancer indirectly by targeting bromodomain containing 8 isoform 2, an androgen receptor co-activator

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ABSTRACT

Objectives: Aberrant androgen receptor (AR) signaling functions are implicated in prostate cancer (PCa) pathogenesis. Here, we studied interactions between miR-185 and the bromodomain containing 8 isoform 2 (BRD8 ISO2) to investigate indirect mechanisms of miR-185 with respect to AR function through BRD8 ISO2 in PCa.

Methods: Putative miRNA response element (MRE) of miR-185 in 3′-untranslated region (3′-UTR) of BRD8 ISO2 mRNA was predicted by software and confirmed using dual-luciferase assays and Ago2 immunoprecipitation. BRD8 and AR expression were determined by qRT-PCR and Western blot in PCa cells and tissues. MMTV-Fluc reporter plasmids and dual-luciferase assays were used to evaluate AR activity.

Results: MRE prediction, dual-luciferase assays and Ago2 immunoprecipitation confirmed that miR-185 is capable of binding the 3′-UTR of BRD8 ISO2 mRNA. QRT-PCR and Western blot indicated that BRD8 ISO2 expression is decreased by miR-185 mimic transfection while increased by miR-185 inhibitor transfection. MMTV-Fluc reporter assays revealed that miR-185 can attenuate AR function by suppressing BRD8 ISO2. Additionally, Pearson's correlation analyses confirmed that BRD8 ISO2 mRNA expression is inversely correlated with miR-185 expression in clinical specimens.

Conclusion: In addition to suppression of AR expression, miR-185 can attenuate AR function indirectly by suppressing BRD8 ISO2. MiR-185 and BRD8 ISO2 may be possible therapeutic targets for PCa treatment.

1. Introduction

Prostate cancer (PCa) is an endocrine-related disease and one of the most frequent reasons for male cancer-related mortality worldwide. Localized PCa can be resected by radical prostatectomy. However, because it is highly metastatic, late-stage PCa may be untreatable and lethal. Until now, the pathogenesis of PCa remains unclear and further studies for understanding the underlying molecular and cellular mechanisms of PCa progression are in demanded. Prostate gland growth, differentiation, and development are regulated by androgen – androgen receptor (AR) signaling, and PCa progression is also influenced by this essential interaction. Previous works indicated that, compared with normal prostate luminal cells, PCa cells possessed a higher AR expression levels as well as enhanced and abnormal AR functions (Kuo et al.,

Abbreviations: 3′-UTR, 3'-untranslated region; Ago2, argonaute 2; AR, Androgen receptor; BRD8, bromodomain containing 8; CDS, coding sequence; DHT, dihydrotestosterone; Fluc, firefly luciferase; GS, Gleason score; MMTV, murine mammary tumor virus; MRE, microRNA response element; Mut, mutant; Nkx-3.1, NK3 homeobox 1; PCa, prostate cancer; PSA, prostate specific antigen; qRT-PCR, real-time quantitative polymerase chain reaction; RIP, RNA immunoprecipitation; Rluc, *renilla* luciferase; SD, standard deviation; siRNA, short interfering RNA; SV40, Simian virus 40; WT, wild type.

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2015; Vander Griend et al., 2014). In addition, studies indicated that aberrant AR functions are involved in the transformation from androgen naïve PCa to castration-resistant PCa which may bring failure to androgen deprivation therapy (Chuu et al., 2011; Kong et al., 2015). As a whole, functions of AR protein and its underlying mechanisms in PCa are critical.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNA with 20–22 nucleotides that can interfere with protein expression by inducing post-transcriptional repression of mRNA (Iorio and Croce, 2009). A mature miRNA and assisted proteins assemble to form the RNA-induced silencing complex (RISC) which is capable of binding the miRNA response element (MRE) in 3′-untranslated region (3′-UTR) of its targeted mRNA and then induces mRNA degradation. Expression alterations of certain miRNAs are involved in many malignancies, including PCa (Iorio and Croce, 2009; Yu and Xia, 2013). Among these miRNAs, a large number of them are decreased in PCa which act as tumor suppressors. Introduction of the tumor suppressor miRNAs exogenously may carry out for either inhibiting proliferation or promoting apoptosis in PCa. Previous studies have revealed that miR-185 can inhibit PCa cell proliferation and invasiveness by binding the 3′-UTR of AR mRNA and suppressing AR protein level in PCa (Liu et al., 2015; Qu et al., 2013). Here, we report another mechanism by which miR-185 is capable of attenuating AR function indirectly by suppressing bromodomain containing protein 8 isoform 2 (BRD8 ISO2), an AR co-activator.

2. Materials and methods

2.1. Cell culture

The human PCa LNCaP cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA). The PC-3(AR)9 human PCa cell line which stably and moderately expressed functional AR (Altuwaijri et al., 2007) was a generous gift from Dr. Chawnshang Chang (University of Rochester medical center). LNCaP and PC-3(AR)9 cells were maintained in RPMI-1640 medium (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (FBS) (Gibco, Foster City, CA), and 1% penicillin-streptomycin (HyClone). All cells were cultured at 37 ℃ in 5% CO_2 environment.

2.2. Human PCa tissue specimens

Human PCa tissue specimens were obtained from patients who underwent radical prostatectomies at Shanghai General Hospital between January 2011 and July 2015. Patients' ages and PCa clinical data including PSA values and Gleason scores (GS) were recorded (Supplementary Table S1.). All tissues were confirmed by pathology and preserved in liquid nitrogen. The Ethical Review Board of Shanghai General Hospital approved all experimental procedures, and informed consent was obtained from each patient before surgery.

2.3. Real-time quantitative polymerase chain reaction (qRT-PCR)

Total RNA of PCa cells or tissue specimens were extracted using TRIzol reagent (Life Technologies, Foster City, CA, USA). Reverse transcription of mRNA was carried out using PrimeScript RT Master Mix (Takara, Otsu, Shiga, Japan) according to the manufacturer's instructions. QRT-PCR was carried out using SYBR Premix Ex Taq (Takara). GAPDH was used as internal control for mRNAs expression. MiRNAs purification and reverse transcription was carried out using SanPrep Column microRNA Mini-Preps Kit (Sangon, Shanghai, China) and miRNA First Strand cDNA Synthesis Kit (Sangon) respectively; MicroRNAs Quantification PCR Kit (Sangon) was then used for qRT-PCR. U6 small nuclear RNA was used as internal control for miRNA expression. Expression fold-change of mRNAs and miRNA was calculated on the basis of the $2^{-\Delta\Delta Ct}$ method. Primers detailed in Supplementary Table S2. All experiments were performed in triplicate.

2.4. Western blot

Western blot was performed as described previously (Yu et al., 2014). Primary antibodies against BRD8 (GTX115500, 1:1000 dilution, GeneTex, Irvine, CA), AR (#5153, 1:2000 dilution, Cell Signaling Technology, Danvers, MA), PSA (ab76113, 1:10,000 dilution, Abcam, Cambridge, UK), Nkx-3.1 (ab196020, 1:1000 dilution, Abcam), β-Actin (#3700, 1:1000 dilution, Cell Signaling Technology) were used in this study.

2.5. MiRNA mimics, inhibitors and short interfering RNAs (siRNAs)

MiR-185 mimics/inhibitors (RiboBio, Guangzhou, China) were used for overexpression and knockdown of miR-185 in PCa cells respectively. NC mimics/inhibitors (RiboBio) were used as transfection control. As for knockdown of BRD8 expression, siISO1/2 was used to inhibit both BRD8 ISO1 and ISO2 expression; siISO1 was used to inhibit BRD8 ISO1 expression; and siISO2 was used to inhibit BRD8 ISO2 expression. SiNC was used as transfection control. MiRNA mimics, miRNA inhibitors, and siRNAs were transfected into PCa cells using Lipofectamine 2000 transfection reagent (Life Technologies). Sequences of siRNAs detailed in Supplementary Table S2.

2.6. Plasmids and dual-luciferase assays

The putative binding site of miR-185 in the 3′-UTR of BRD8 ISO2 mRNA was predicted using open-source software miRanda and Targetscan. The wild type (WT) sequence of 3′-UTR of BRD8 ISO2 or its mutant (Mut) sequence (Fig. 4D) were amplified by PCR and cloned into the 3′-end of the firefly luciferase (Fluc) gene in pmirGLO vector plasmids (E1330, Promega, Madison, WI). MiRNA mimics with WT or Mut pmirGLO dual-luciferase reporter plasmids were co-transfected into LNCaP cells for analysis of interaction between miR-185 and BRD8 ISO2 mRNA. All transfections were carried out using Lipofectamine 2000 reagent (Life Technologies). Fluc and *Renilla* luciferase (Rluc) activities were measured as described previously (Zhang et al., 2014) and all experiments were performed in triplicate.

2.7. Lentivirus construction and infection

The coding sequence (CDS) of BRD8 ISO2 mRNA was synthesized and cloned into pLV5 (GenePharma, Shanghai, China) lentivirus overexpression vector. After packaging, the lentivirus supernatant were used to infect LNCaP and PC-3(AR)9 cells for construction of BRD8 ISO2 overexpression stable cell line.

2.8. RNA immunoprecipitation (RIP)

LNCaP and PC-3(AR)9 cells were transfected with miR-185 mimics or NC mimics, incubated for one day and washed in cold PBS before cell lysis. The antibody against human Ago2 (ab32381, Abcam) was used for protein immunoprecipitation. RIP experiments were carried out using Magna RIP Kit (Millipore, Billerica, MA) according to the manufacturer's instructions. Following cell Lysis, Ago2 immunoprecipitation, and protein–RNA complexes digestion, the RNA was isolated using TRIzol reagent (Life Technologies). Then BRD8 ISO2 mRNA levels were examined by qRT-