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Aryl hydrocarbon receptor enhances the expression of miR-150-5p to suppress in prostate cancer progression by regulating MAP3K12

Jingsong Yu, Yue Feng, Yan Wang, Ruihua An*

Department of Urology, The First Affiliated Hospital of Harbin Medical University, Harbin, 150001, China

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ABSTRACT

It has been reported that mircoRNAs (miRNAs) can act as tumor inhibitors in multiple malignant tumors. As a tumor suppressor, miR-150-5p has been reported in some cancers. However, the biological impacts of miR-150-5p in prostate cancer is not fully elaborated. This study aims to explore the biological role and mechanism of miR-150-5p in prostate cancer. The expression level of miR-150-5p was examined with Quantitative real time polymerase chain reaction (qRT-PCR). Moreover, Kaplan Meier analysis revealed that downregulation of miR-150-5p predicted unfavorable prognosis for patients with prostate cancer. To identify the inhibitory effects of miR-150-5p on the cellular processes of prostate cancer, gain-of function assay was conducted. Next, the inhibitory effects of Tetrachlorodibenzo-p-dioxin (TCDD) and 3,3′-Diindolylmethane (DIM) on the proliferation and invasion of prostate cancer cells were demonstrated. Knockdown of Ahr reversed the TCDD/DIM-mediated proliferation and invasion. The expression level of CYP1A1 also was measured to confirm that Ahr was activated by TCDD or DIM in prostate cancer cells. Mechanism experiments revealed that MAP3K12 is a target mRNA of miR-150-5p in prostate cancer cells. In conclusion, Aryl hydrocarbon receptor enhances the expression of miR-150-5p to suppress cell proliferation and invasion in prostate cancer by regulating MAP3K12.

1. Introduction

As a kind of urological tumor, prostate cancer (PC) is one of the leading cause of cancer death in male all over the world [1]. It is commonly acknowledged Aryl hydrocarbon receptor (Ahr) is a ligand-activated transcription factor. It has been recorded that Ahr could modulate multiple signaling pathways in the development of prostate cancer [2]. Ahr can bind to some specific compounds with procarcinogenic properties (such as PAHs, heterocyclic aromatic amines and dioxin-like compounds), therefore to induce the expression changes of some special xenobiotic metabolizing genes (CYP1A1 and CYP1B1) [3]. Over the several decades, Ahr has been defined as a modulator by interacting with environmental Ahr xenobiotic Tetrachlorodibenzo-p-dioxin (TCDD) [4] and 3,3′-Diindolylmethane (DIM) [5]. These ligands are commonly derived from the environmental contaminants. The biological function of Ahr has been verified in various cancers [6–8], including prostate cancer [9–11]. TCDD and DIM are two well-known ligands of Ahr which have been reported to exert its functions in human cancers through increasing the expression of Ahr [12–14]. It has been demonstrated that activation of Ahr by TCDD and DIM represses the proliferation and metastasis of cancer cells [15–17]. This study uncovered that Ahr could be activated by TCDD in prostate cancer cells. The activated Ahr suppressed proliferation and invasion of PC cells. As small non-coding RNAs, miRNAs are shorter than 22 nt. They can negatively modulate gene expression at the posttranscriptional level to affect some significant biological processes, such as cell proliferation, metastasis and apoptosis, etc [18–20]. As a member of miRNA family, miR-150-5p can act as a tumor suppressor in various human cancers. For instance, miR-150-5p can suppress hepatoma cell metastasis via targeting MMP14 [21]; miR-150-5p inhibits cell metastasis in colorectal cancer via targeting MUC4 [22]. Moreover, it has been reported in pancreatic cancer for its anti-oncogenic function [23]. Moreover, Ahr can induce upregulation of miRNA to inhibit carcinogenesis [24]. In this study, we supposed that Ahr-induced upregulation of miR-150-5p inhibits prostate cancer progression. At first, the expression pattern of miR-150-5p was determined in PC tissues and cell lines. To analyze the prognostic value of miR-150-5p for the patients with prostate cancer, Kaplan Meier analysis was conducted. Due to the low expression of miR-150-5p in PC cell lines, gain-of-function assays were conducted in PC cell lines. Subsequently, the interaction between miR-150-5p and Ahr was analyzed in PC cells. The anti-oncogenic effects of TCDD and DIM were determined by applying functional assays. Furthermore, the

* Corresponding author. Department of Urology, The First Affiliated Hospital of Harbin Medical University, No.23 Youzheng Street, Nangang District, Harbin, Heilongjiang, 150001, China.
E-mail address: an_ruihua@126.com (R. An).

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target mRNA of miR-150-5p was uncovered by performing mechanism experiments. Taken together, this study revealed the biological effects of Ahr-miR-150-5p-MAP3K12 axis on the cell proliferation and invasion in prostate cancer.

2. Materials and methods

2.1. Clinical samples

All tissues used in this study were acquired and collected from patients diagnosed with PC in the Department of Urology, The First Affiliated Hospital of Harbin Medical University from 2012 to 2016. All patients participated in this study had signed the informed consents before all experiments were conducted. In addition, this study had acquired the approval of the ethic committee of The First Affiliated Hospital of Harbin Medical University. All tissue specimens were stored at −80 °C for use.

2.2. Cell culture and reagents

Two normal prostate epithelial cells (RWPE-1 and PWR-1E) and four prostate cancer cells (PC-3, VCaP, LNCaP, DU145) were all bought from The American Type Culture Collection (ATCC, Manassas, Virginia). All cells were cultivated in Dulbecco modified Eagle medium (DMED; Thermo Fisher Scientific, Waltham, Massachusetts) by adding a mixture [(10% FBS (HyClone, Logan, Utah), penicillin (100 U/mL), and streptomycin (100 mg/mL)]. Cells were preserved in a moist air of 37 °C and 5% CO_2. DMSO was bought from Sigma-Aldrich (St. Louis, MO, USA). TCDD and DIM were purchased from AccuStandard (New Haven, CT, USA). Ahr antibody (ARP32243) was purchased from Aviva Systems Biology (San Diego, CA, USA). The concentration of Ahr agonists used in this study was shown as follows: TCDD (10 nmol/L) and DIM (25 nmol/L).

2.3. Transfection

To overexpress and knock down miR-150-5p, cells were separately transfected with miR-150-5p mimic and miR-150-5p inhibitor. All the above plasmids were synthesized and purified by Molbase Co Ltd, Shanghai, China. Based on the user guide, Lipofectamine 2000 (Invitrogen) was used to finish all transfections.

2.4. Colony formation assay

After PC-3 and DU145 cells were transfected by the indicated vector, they were plated in six-well plates at a density of 500 cells per well. Then, they were incubated at 37 °C. Cells were then incubated again in charcoal-stripped medium in which DMSO or Ahr agonists were added for 48 h. Two weeks later, using 0.1% of crystal violet to stain after the cells were fixed. Finally, we calculated the number of visible colonies by our own hand.

2.5. Caspase-3 activity assays

According to the instruction for users (Merck Millipore), caspase-3 assays were conducted. Cells were centrifuged at a speed of 1200 rpm for about 10 min and re-suspended in 50 μl of lysis buffer. After incubation, the samples were further centrifuged at 10000 × g for 5 min. Next, the pellet was discarded, the supernatant was removed into a microcentrifuge tube on ice. In order to measure the concentration of the supernatant's protein, a BCA™ assay was performed. Equal amount of protein was diluted before they were added into 96-well plate. Next, each sample was added into 20 μl of 5X assay buffer. 10 μl of peptide substrate was added after incubation (37 °C + 2 h). At last, the absorbance was evaluated at 405 nm.

2.6. Transwell invasion assay

In short, transwell insert chamber coated with Matrigel (BD Biosciences, NJ, USA) was used to detect the invasive ability. After incubation of cells for about 24 h, the migrating cells were fixed on the bottom side with 4% polyoxymethylene. At last, cells stayed on the bottom was stained by 0.1% crystal violet. The number of migratory cells was counted with a microscope at 100 × magnification.

2.7. qRT-PCR analysis

Trizol kit (Invitrogen) was used to extract total RNA from tissues or cells. According to the user guide, anOmniscript RT kit (Qiagen, Valencia, CA, USA) was subsequently utilized to synthesize cDNA from total RNA. Reverse transcription was conducted in a thermal cycler with RT enzyme.

The real-time PCR was performed in a ViiA 7 system by utilizing the TaqMan® gene expression assays. The cycling conditions were shown as follows: 50 °C for 2 min and 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The $2^{-\Delta\Delta Ct}$ method was applied to count the fold change of target genes. U6 snoRNA (RiBoBio, Guangzhou, China) was used as endogenous control for miRNA expression. All reagents and Kits used for RT-PCR were bought from Applied Biosystems (Grand Island, NY, USA). The primers for PCR were shown as follows: miR-150-5p-F: 5′-TCGGCGTCTCCCAACCCTTGTAC-3′, miR-150-5p-R: 5′-GTCGTATCC AGTGCAGGGTCCGAGGT-3'; U6-F: 5′-CTCGCTTCGGCAGCACATAT ACT-3′, U6-R: 5′-ACGCTTCACGAATTTGCGTGTC-3'.

2.8. Luciferase reporter assay

The PC-3 and DU145 cells were co-transfected with wild-type or mutant 3′-UTR sequence of MAP3K12 which containing the complementary sequence of miR-150-5p and NC mimic (miR-NC) or miR-150-5p mimics by Lipofectamine (2000) (Invitrogen). The luciferase activity was measured with Luc-PairmiR Luciferase Assay (GeneCopoeia, Rockville, Maryland).

2.9. Western blot analysis

For western blot, PC-3 or DU145 cells were lysed by a RIPA lysis buffer system (Santa Cruz Biotechnology). Next, the lysate was fractionated by the SDS-PAGE system (Bio-Rad, Richmond, CA). The Ahr, CYP1A1, and MAP3K12 were detected using their corresponding rabbit polyclonal antibodies (Santa Cruz Biotechnology). The protein bands were quantified with ImageJ v.1.48 [25].

2.10. Statistical analysis

Data were displayed as the means ± SD of more than two independent experiments. The SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was utilized for statistical analysis. Comparisons between groups were made with Student's t-test or one-way ANOVA. Kaplan-Meier method was used to generate the overall survival curves via the log-rank test. $P < 0.05$ indicted a statistically significant difference.

3. Results

3.1. Downregulation of miR-150-5p predicts poor prognosis for patients with PC

The expression levels of miR-150-5p was firstly detected in PC tissues and the adjacent non-tumor tissues (Fig. 1A). The results manifested that miR-150-5p was expressed lower in PC tissues than that in the non-tumorous tissues. Next, the mean value of the expression levels of miR-150-5p in all tissues samples were taken as the threshold. We divided the PC tissues into two groups in accordance with the mean