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MicroRNA-195 regulates docetaxel resistance by targeting clusterin in prostate cancer

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ABSTRACT

MicroRNAs (miRNAs) have been implicated in neoplasm growth, metastasis, vasculogenesis, and drug resistance. It has been validated that abnormal miR-195 expression was related with poor survival of prostate cancer (PC); however, its role in the resistance to chemotherapeutic drugs docetaxel (DOC) in PC is still acquainted scarcely. In our study, the lower expression of miR-195 was appeared in DOC-resistant PC cells (DU145/DOC) rather than DOC-sensitive DU145 cells. The up-regulation of miR-195 lowered the IC50 of DOC, facilitated the apoptosis and inhibited the colony formation ability in DU145/DOC cells. Moreover, we also found that miR-195 had the binding site with clusterin (CLU) by the online TargetScan database mining. Luciferase tests revealed that miR-195 binds to the 3′-UTR of CLU. MiR-195 overexpression decreased the amassment of CLU in DU145/DOC cells. Knockdown of CLU diminished the IC50 of DOC and enhanced the apoptosis of DU145/DOC cells, which was consistent with the influence of miR-195 on DOC-induced cell apoptosis. Taken together, our results illuminated that miR-195 improved the sensitivity of resistant PC cells to DOC by suppressing CLU. Hence, miR-195 may be a potentially promising molecular target for drug resistance of PC.

1. Introduction

Prostate cancer (PC) is the most frequently malignancy in male genitourinary system and strongly threatened their health. According to an estimate in the United States, new cases of PC account for 19% of all cancer cases in men (161,360 of 836,150 patients) and approximately 8% of all cancer deaths (26,730 of 318,420 patients) among males in 2017 [1]. The morbidity of PC in developing countries is significantly less than that in developed countries [2]. In China, the trend of PC prevalence increased rapidly in the past ten years that the averagannual growth rate during 2000–2005 and 2005–2011 was respectively 12.6% and 4.7% [3]. Currently, it is sensitive to androgen resistance therapy at the early PC and hence androgen deprivation therapy (ADT) is the common treatment of PC. However, almost all patients eventually evolve into castration-resistant prostate cancer (CRPC) which will ineluctably lead to cancer metastasis and patients' death [4]. Docetaxel (DOC) is the preferred chemotherapy drug for CRPC which could extend the survival time of patients and improve the living quality [5,6]. However, the resistance is still a major problem in limiting the clinical use of DOC. It is well known that chemotherapy resistance is an extremely complex process involving various factors. However, the potential mechanisms of resistance to chemotherapy drugs still know little.

MicroRNAs (miRNAs) are a kind of small noncoding RNA molecules (containing 19–25 nucleotides) which have been considered as crucial modifiers of gene expression on the level after transcription [7,8]. MiRNAs have has been proven to be pivotal in neoplasm growth, tumor cell differentiation, metastasis, vasculogenesis, apoptosis and drug resistance [7,9,10]. In the recent literatures, multiple miRNAs have been recognized to involve in drug resistance of PC such as miR-375, miR-4638, miR-17-92, miR-205, miR-31, and so on [11–14]. The characterization of miRNA probably promotes seeking the novel tumor markers and drug targets, which makes it a good tool for tumor diagnosis and treatment. Furthermore, miR-195 expression in metastatic PC tissues was low and was closely associated with poor survival of PC patients, which would act as an underlying and promising predictor [15,16]. However, the biological functions of miR-195 in chemotherapy response of PC are still acquainted scarcely. In cervical cancer, miR-195 has been shown to suppress taxol induced epithelial to mesenchymal transition (EMT) and also sensitize cervical cancer cells to taxol [17]. Thus, we guess that miR-195 may be participated in the resistance to chemotherapeutic drugs taxol analogues DOC in PC.

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In this study, miR-195 expression in DOC-resistant PC cells was found to be increased. MiR-195 overexpression obviously enhanced the sensitivity of DOC-resistant PC cells, increased the resistant cell apoptosis and inhibited the cell colony formation. Additionally, our data indicated that miR-195 may increase the sensitivity of resistant PC cells to DOC through diminishing the expression of its target gene clusterin (CLU).

2. Material and methods

2.1. Cell culture and transfection

Prostate cancer cells DU145 and DOC-resistant cells DU145/DOC were obtained from American Type Culture Collection (Manassas, VA, USA). Both DU145 and DU145/DOC cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and then maintained at 37 °C in a humidified atmosphere of 5% CO_2. MiR-control (NC), miR-195 mimics, and small interfering RNA targeting CLU (siCLU) were all purchased from Vipotion Co., Ltd. (Guangzhou, China). DU145 or DU145/DOC cells were seeded in a 96-well plate at a density of 2×10^4/well. After attached to the wall, cells were transfected with miR-195 mimics, or siCLU using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the directions and then cells were further analyzed after 48 h.

2.2. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA and then corresponding complementary DNA (cDNA) was synthesized using M-MuLV Reverse Transcriptase (Sangon Biotech, Shanghai, China). Subsequently, real-time PCR reactions were executed to analyze the expression of miR-195 and CLU mRNA as formerly described [18].

2.3. Western blot analysis

Western blot analysis was carried out using the process described previously [19]. Briefly, the equivalent proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad, CA, USA). After blocked in 5% sealing solution, the membranes were hatched with anti-Clusterin antibody (1:1000; Abcam, Cambridge, UK), anti-GAPDH antibody (1:1000; Abcam, Cambridge, UK), probed with corresponding secondary antibody (Abcam, Cambridge, UK) and visualized by Pierce™ ECL Western Blotting Substrate (Invitrogen, Carlsbad, CA, USA).

2.4. Cell counting kit-8 (CCK8) assays

Briefly, 2000 cells per well were implanted into the 96-well plates and then different concentrations of docetaxel (Selleck, Houston, TX, USA) was added in each well. After 24 h, cellular proliferative activity was evaluated via CCK8 assay as described previously [11]. Finally, SPSS software was used to calculate the half maximal inhibitory concentration (IC50) value.

2.5. Colony formation assay

Briefly, 500 cells per well were implanted into 6-well plates and then treated with 5 μM DOC when cells were attached to the wall. After cultured for 10 days, cells were successively immobilized and dyed with 4% paraformaldehyde and crystal violet (Sangon Biotech, Shanghai, China). Finally, the number of colonies was counted manually.

2.6. Apoptosis assays

Cell apoptosis was analyzed using Annexin V-Fluorescein Isothiocyanate (FITC)/ Propidium Iodide (PI) double staining Kit (KeyGEN Biotech, Nanjing, China) according to the instruction as described previously [20].

2.7. Luciferase reporter analysis

The supposed complementary site of miR-195 in the 3′-UTR of CLU mRNA and the mutational sequence was severally inserted into the psiCHECK™-2 vector (Promega, Madison, Wisconsin, USA). Then DU145/DOC cells were incubated in 24-well plates and co-transfected with luciferase reporter vectors (psiCHECK™-2-CLU–3′UTR-WT or psiCHECK™-2-CLU–3′UTR-MUT) with miR-195 mimic or NC using Lipofectamine 2000 according to the directions. After 48 h of transfection, the Dual-Luciferase Reporter Assay System (Promega, CA, USA) was used to analyze luciferase activity.

2.8. Statistical analysis

SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA) was applied to perform the statistical analyses. The results are presented as means ± SD and analyzed using Student's t test. $P < 0.05$ was identified as the standard for significance.

3. Results

3.1. MiR-195 was up-regulated in DOC-resistant PC cells

In this study, we firstly performed CCK8 assay to measure the IC50 values via treating DU145 and DU145/DOC cells with DOC of different concentrations for 24 h and found the IC50 values for DU145 and

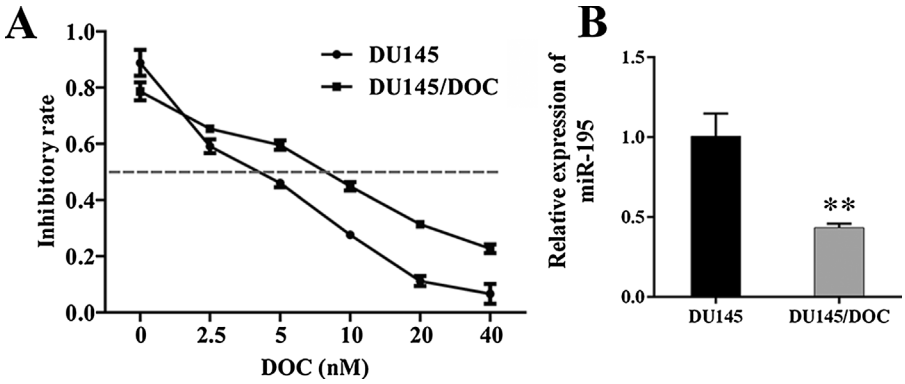

Fig. 1. MiR-195 was over-expressed in DOC-resistant PC cells. (A) Different concentrations of DOC of were added into parental DU145 cells and DOC-resistant DU145/DOC cells for 24 h and cell activity was recorded using CCK8 assay. (B) miR-195 expression was examined in DU145 and DU145/DOC cells using qRT-PCR. U6 served as an internal control. $^{**}P < 0.01$.