J Biosci (2019) 44:102
DOI: 10.1007/s12038-019-9920-4
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Review

miR-425-5p suppresses tumorigenesis and DDP resistance in human-prostate cancer by targeting GSK3β and inactivating the Wnt/β-catenin signaling pathway

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MS received 27 February 2019; accepted 22 June 2019; published online 9 August 2019

Prostate cancer (PCa) represents the most frequently diagnosed cancer in men. Cisplatin, also known as *cis*-diamminedichloroplatinum (DDP), is a standard chemotherapeutic agent used to treat PCa, and DDP resistance remains one important obstacle in DDP-based chemotherapy. In our research, we found miR-425-5p was down-regulated in PCa and even lower in DDP-resistant PCa determined by quantitative polymerase chain reaction; in contrast, GSK3β mRNA expression was upregulated in PCa and even higher in DDP-resistant PCa. Moreover, there was a modest but significant inverse correlation between the expression of GSK3β mRNA and miR-425-5p. Functional experiments showed that miR-425-5p mimic inhibited DDP resistance as evidenced by a promoted apoptosis rate (flow cytometry) and suppressed cell viability (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay) and expressions of MDR1 and MRP1 (western blotting) in DU145/DDP and PC3/DDP cells. Luciferase reporter assay and RNA immunoprecipitation identified GSK3β was a potential target of miR-425-5p. The effect of miR-425-5p mimic on DDP resistance was partially reversed by pcDNA-GSK3β. Mechanically, miR-425-5p mimic reduced expression of β-catenin, cyclin D1 and C-myc, which was further blocked when GSK3β overexpressed. *In vivo* experiments, recovery of GSK3β prevented xenograft tumor growth and DDP resistance in the presence of miR-425-5p mimic. To sum up, miR-425-5p upregulation might sensitize human PCa to DDP by targeting GSK3β and inactivating the Wnt/β-catenin signaling pathway.

Keywords. β-Catenin; DDP resistance; GSK3β; miR-425-5p; prostate cancer (PCa)

1. Introduction

Prostate cancer (PCa), the leading male cancer worldwide, is a hormone-driven tumor, and a common therapy is androgen-deprivation therapy and/or chemotherapy/radiotherapy (Felgueiras *et al.* 2014). Cisplatin, also known as *cis*-diamminedichloroplatinum (DDP), is a standard chemotherapeutic agent used to treat PCa, and DDP resistance remains one important obstacle in DDP-based chemotherapy (Gasparian *et al.* 2017). When PCa evolves into a stage of hormone resistance or metastasis, the fatality rate is very high (Damodaran *et al.* 2017; Polotti *et al.* 2017). PCa detection is currently based on the serum prostate-specific antigen biomarker and digital rectal examination (Dell'Atti 2015; Druskin *et al.* 2018). However, these methods are limited and have adverse consequences. New biomarkers that could be used for PCa diagnosis and treatment are still needed.

Recent studies have demonstrated that aberrant expressions of microRNAs (miRNAs) are associated with the underlying mechanisms of PCa (Filella and Foj 2017), and miRNAs as biomarkers have opened the potential of the diagnosis of PCa, especially early diagnosis (Razdan *et al.* 2018). miRNA-425-5p (miR-425-5p) has previously been reported to be abnormally expressed in a number of different types of human cancers (Sun *et al.* 2017; Zhang *et al.* 2017; Zhu *et al.* 2017), including gastric, cervical, colorectal cancer, etc. Several research studies indicated the association of miR-425-5p with malignancy and the inverse regulation of miR-425-5p on tumor cell viability and invasion (Al-Eryani *et al.* 2018; Zhu *et al.* 2018). In addition, miR-425-5p was involved in the role of metformin in PCa cells (Avci *et al.* 2013). Hence, studies on the role of miR-425-5p in DDP-resistant PCa can be instrumental in developing new and broader therapeutic strategies.

Unfortunately, most PCa tumors progress to an aggressive state, known as castration-resistant prostate cancer, and two mechanisms are involved: androgen receptor (AR)-dependent way and AR-independent way, in which the acquisition of castration resistance was implicated in the Wnt-signaling pathway (Pakula *et al.* 2017; Wang *et al.* 2018). During the canonical Wnt-signaling pathway, Wnt ligands bind to frizzled and/or LRP5/6 resulting in the phosphorylation of the latter by CK1 and GSK3 and recruitment of axin and Dsh. A hallmark of Wnt/β-catenin signaling is the stabilization and nuclear translocation of β-catenin, and the accumulation of β-catenin subsequently either activates other transcription factors or cooperates with TCF/LEF to activate target genes. In the absence of WNT ligands, β-catenin is recruited and degraded by the destruction complex, whose components include axin, GSK3, CK1 and APC.

In consideration of the results in the YM500v3 database, we aimed to figure out the expressions of miR-425-5p in DDP-sensitive and -resistant PCa tissues and cells (PC3 and DU415) and to explore the precise mechanism of miR-425-5p dysregulation in drug resistance. Promisingly, we observed that miR-425-5p was lower expression and exhibited an anti-tumor role, indicating miR-425-5p as one emerging biomarker of PCa, especially DDP-resistant PCa.

2. Materials and methods

2.1 *Acquirement of tissue samples*

With the approval of Research Ethics Committee of Chongqing Three Gorges Central Hospital and written informed consent from PCa patients, a number of 26 tissue samples were obtained from 2013 to 2017 and 12 adjacent normal tissues were collected at the time of operation simultaneously. All tissue samples were immediately stored in liquid nitrogen.

2.2 *Cells and cell culture*

PCa cell lines (DU145 and PC3) and a human normal prostate epithelial cell line (RWPE-1) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). These cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin at 37°C.

DDP-resistant PCa cells (DU145/DDP and PC3/DDP) were developed from DU145 and PC3 cells with incubation in medium containing gradually increased concentrations of DDP (Sigma-Aldrich, St. Louis, MO, USA) from 1 to 5 μg/mL for 10 days. All cells were stimulated with 5 μg/mL DDP for 24 h following pretreatment with serum-free DMEM (Gibco) for 16 h.

2.3 *Cell transfection*

For overexpression, GSK3β coding-domain sequences were amplified and cloned into the multiple cloning site of the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). miR-425-5p/NC mimic and miR-425-5p/NC inhibitor were purchased from Ribobio (Guangzhou, China).

Cell transfection with oligonucleotides or plasmids into cells was performed by using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Cells were subsequently cultured for further study, including cell viability assay and western blotting.

2.4 *Total RNA isolation and real-time quantitative PCR (qPCR) and $2^{-\Delta\Delta CT}$ method*

Total RNA from tissue samples and cultured cells was extracted using the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. The concentration and purity of total RNA was examined by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). The cDNAs depended on a reverse transcription kit (Abcam, Cambridge, UK). The amplification of cDNAs was performed by using SYBR Premix Ex Taq Master Mix (2 ×) (Takara, Shiga, Japan) on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific). The expression levels of mature miR-425-5p and GSK3β were calculated by $2^{-\Delta\Delta CT}$ methods with normalization to U6 small-nuclear RNA (U6) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. In brief, $\Delta CT_{gene} = CT_{experimental\ group} - CT_{control\ group}$, $\Delta\Delta CT = \Delta CT_{experimental\ gene} - \Delta CT_{internal\ reference}$. Relative expression of experimental gene = $2^{-\Delta\Delta CT}$. Primers used are as follows: miR-425-5p: 5′-TGCGGAATGA CACGATCACTCCCG-3′ (forward) and 5′-CCAGTG CAGGGTCCGAGGT-3′ (reserve); GSK3β: 5′-GGA ACTCCAACAAGGGAGCA-3′ (forward) and 5′-TTCGG GGTCGGAAGACCTTA-3′ (reserve); GAPDH: 5′-GTC AACGGATTTGGTCTGTATT-3′ (forward) and 5′-AGTC TTCTGGGTGGCAGTGAT-3′ (reserve); U6: 5′-GCTTC GGCAGCACATATACTAAAAT-3′ (forward) and 5′-CGCT TCACGAATTTGCGTGTCAT-3′ (reserve). The experiments were conducted three times and each group was in quadruplicate.

2.5 *Total protein extraction and western blotting*

Treated cells were extracted for total protein using RIPA lysis buffer (Beyotime, Shanghai, China) to measure protein expressions, including GSK3β, β-catenin, cyclin D1, C-myc, MDR1 and MRP1. Western blotting was performed according to standard procedures, and GAPDH on the same membrane was used as a loading control. The primary antibodies were purchased from Abcam and are as follows: GSK3β (#93926,

1:1000); β-catenin (#6302, 1:4000); C-myc (#39688, 1:1000); cyclin D1 (#226977, 1:5000); MDR1 (#129450, 1:5000) and MRP1 (#32574, 1:500). The proteins were visualized using the electrochemiluminescence (ECL) procedure, and ImageJ was used to analyze the gray intensity of the bands.

2.6 *MTT assay*

Cell viability was assessed by 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining. About 5 mg/L of MTT was added to DMEM and transfected cells were incubated for 4 h, followed by incubation of 150 μL of dimethyl sulfoxide. The spectrophotometric absorbance of each sample was measured at 570 nm. The experiments were conducted three times and each group was in quadruplicate.

2.7 *Flow cytometry*

Cells with different processing methods were analyzed by using an annexin V-FITC/PI kit (Beyotime) on flow cytometry. Apoptotic cells were labeled complying with the protocol. Fluorescence was analyzed on a cytoFLEX LX flow cytometer (Beckman-Counter Electronics, Jiangsu, China) using CytExpert software. The experiments were conducted three times and each group was in triplicate.

2.8 *Luciferase reporter assay and RNA immunoprecipitation (RIP)*

Plasmids of pGL3-GSK3β wild type (WT) or pGL3-GSK3β mutant type were co-transfected with the miR-425-5p/NC mimic into DU145/DDP and PC3/DDP cells. All transfection procedures were performed using Lipofectamine 2000 (Invitrogen). After 48 h of transfection, the luciferase activity was measured using a dual-luciferase reporter system (Promega, Madison, WI, USA). The ratio of Firefly to Renilla luciferase activity was used as the relative luciferase activity. Each group was repeated three times.

RIP was performed with the DU145/DDP and PC3/DDP cell extracts after transfection of miR-425-5p/NC mimic. A Magna RIP^{TM} RNA-binding protein immunoprecipitation kit (Millipore, Bradford, MA, USA) was chosen to detect the expression of miR-425-5p or GSK3β from the samples bound to the AGO2 or immunoglobulin G (IgG) antibody. After washing, total RNAs were extracted with TRIzol and subjected to real-time quantitative polymerase chain reaction (RT-qPCR) assay. All operation obeyed the standard instructions.

2.9 *Xenograft mouse models*

Four-week-old nude mice were obtained from the Model Animal Research Center of Nanjing University. The animal experiments were approved by the Animal Research Committee of Chongqing Three Gorges Central Hospital and were taken in accordance with the National Institutions of Health Guide for Care and Use of Laboratory Animals. Equal numbers (10^6) of DU145/DDP cells in 0.2 mL of phosphate-buffered saline with forced expressed miR-425-5p/NC mimic and pc-GSK3β/NC or not were injected into the subcutaneous area of a nude mouse (10 mice per group) for 15 days. The tumors were measured using a caliper once 2 days, and the mice were practiced with euthanasia on day 15. The tumor volume was calculated using the formula: V (mm^3) = 1/2 ab^2 (a is the longest tumor axis and b is the shortest tumor axis). After xenograft for 15 days, tumor images were taken and tumor weight was evaluated using an electronic balance. Immediately, the xenograft tumors were stored at −80 °C for further isolation of total RNA and protein.

2.10 *Statistical analyses*

Data given were the means ± standard deviation (SD) from three independent experiments. Statistical significance was determined by two-tailed Student's t test. The correlation between the expression of miR-425-5p and GSK3β was examined by Spearman's rank analysis using SPSS 16.0 software (Chicago, IL, USA). $P<0.05$ was considered as significant difference.

3. Results

3.1 *Expressions of miR-425-5p and GSK3β in DDP-resistant/sensitive PCa tissues and cells*

First, we detected the expression of miR-425-5p and GSK3β in both DDP-resistant and -sensitive PCa tissues and cells using RT-qPCR. The $2^{-\Delta\Delta CT}$ methods suggested that miR-425-5p was down-regulated (figure 1A) and GSK3β (figure 1B) was up-regulated in PCa tissues compared with adjacent tissues; relative expression levels of miR-425-5p were lower and those of GSK3β were higher in PCa cells (DU145 and PC3) than human normal prostate epithelial cells RWPE-1 (figure 1C and D). What's more, compared with DDP-sensitive PCa groups, expression of miR-425-5p was even lower and that of GSK3β was even higher in DDP-resistant PCa tissues and cells (figure 1A–D). Additionally, a modest but significant inverse correlation between miR-425-5p and GSK3β expression was also observed in PCa-tissue samples (figure 1E). Taken together, dysregulation of miR-425-5p and GSK3β in PCa tissues and cells was probably related to the pathogenesis and development of PCa, especially with DDP resistance.

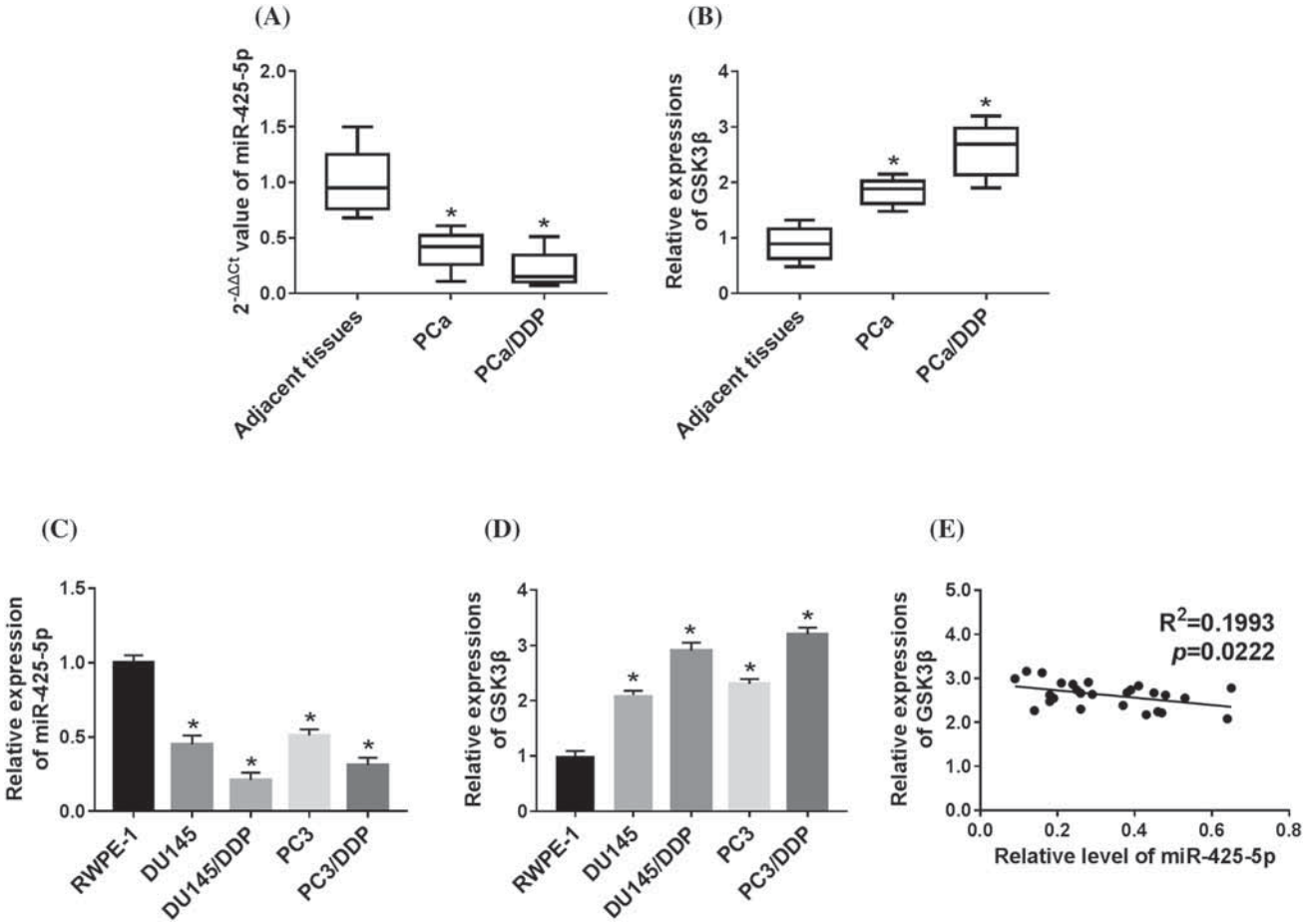

Figure 1. Expressions of miR-425-5p and GSK3β in PCa tissues and cells. (**A** and **B**) Expression of miR-425-5p and GSK3β in PCa tissues, compared with normal adjacent tissues. Levels of miR-425-5p and GSK3β were detected using qPCR and $2^{-\Delta\Delta CT}$ method. (**C** and **D**) Expression of miR-425-5p and GSK3β in PCa cells (DU145 and PC3) and DDP-resistant PCa cells (DU145/DDP and PC3/DDP), compared with human normal prostate epithelial cells RWPE-1. Levels of miR-425-5p and GSK3β were detected using qPCR. (**E**) Spearman's correlation analysis detected the relationship between miR-425-5p and GSK3β expressions in 26 PCa-tissue samples. Data were from three independent experiments and $*P<0.05$.

3.2 *Overexpression of miR-425-5p decreased DDP resistance in PCa cells*

Either miR-425-5p mimic or NC mimic was transiently transfected into DU145/DDP and PC3/DDP cells. Above all, miR-425-5p was forced high-expressed after 30 h of transfection, as shown in figure 2A. Then, we detected the cytotoxicity of miR-425-5p mimic. Lower-cell viability was observed in miR-425-5p-overexpressed DU145/DDP and PC3/DDP cells (figure 2B and C), and the difference became more and more remarkable over time; apoptotic cells were identified by flow cytometry, and the apoptosis rate was elevated from about 7 to over 22% (figure 2D and E). Next, we monitored the expression of drug-resistant genes, and reduced protein levels of MDR1 and MRP1 were recorded in miR-425-5p overexpressed cells (figure 2F–H). These results showed that miR-425-5p overexpression inhibited DDP resistance through decreasing cell viability and expression of drug-resistant genes and promoting cell apoptosis in PCa.

3.3 *GSK3β was negatively regulated by miR-425-5p via target binding*

Searching for target binding in GSK3β in miRcode software, we noticed a potential target site of miR-425-5p in GSK3β 3′-UTR. As shown in figure 3A, –GUGUCAU– in GSK3β 3′-UTR was mutated into –CACAGUA–. The full length of GSK3β 3′-UTR WT and the corresponding mutant (GSK3β WT/MUT) was cloned into the pGL3 vector. Luciferase reporter assay (figure 3B and C) showed a relative declined luciferase activity after co-transfected with the miR-425-5p mimic and GSK3β WT in DU145/DDP and PC3/DDP cells, compared with co-transfected with the miR-NC mimic and GSK3β WT; whereas, there was no significant difference in the GSK3β MUT group. Figure 3D and E depicts that the levels of miR-425-5p and GSK-3β immunoprecipitated were respectively higher than that of the anti-IgG group, disclosing that they co-existed in an AGO2-related RNA-induced silencing complex. Next, expression of GSK3β at the protein level was

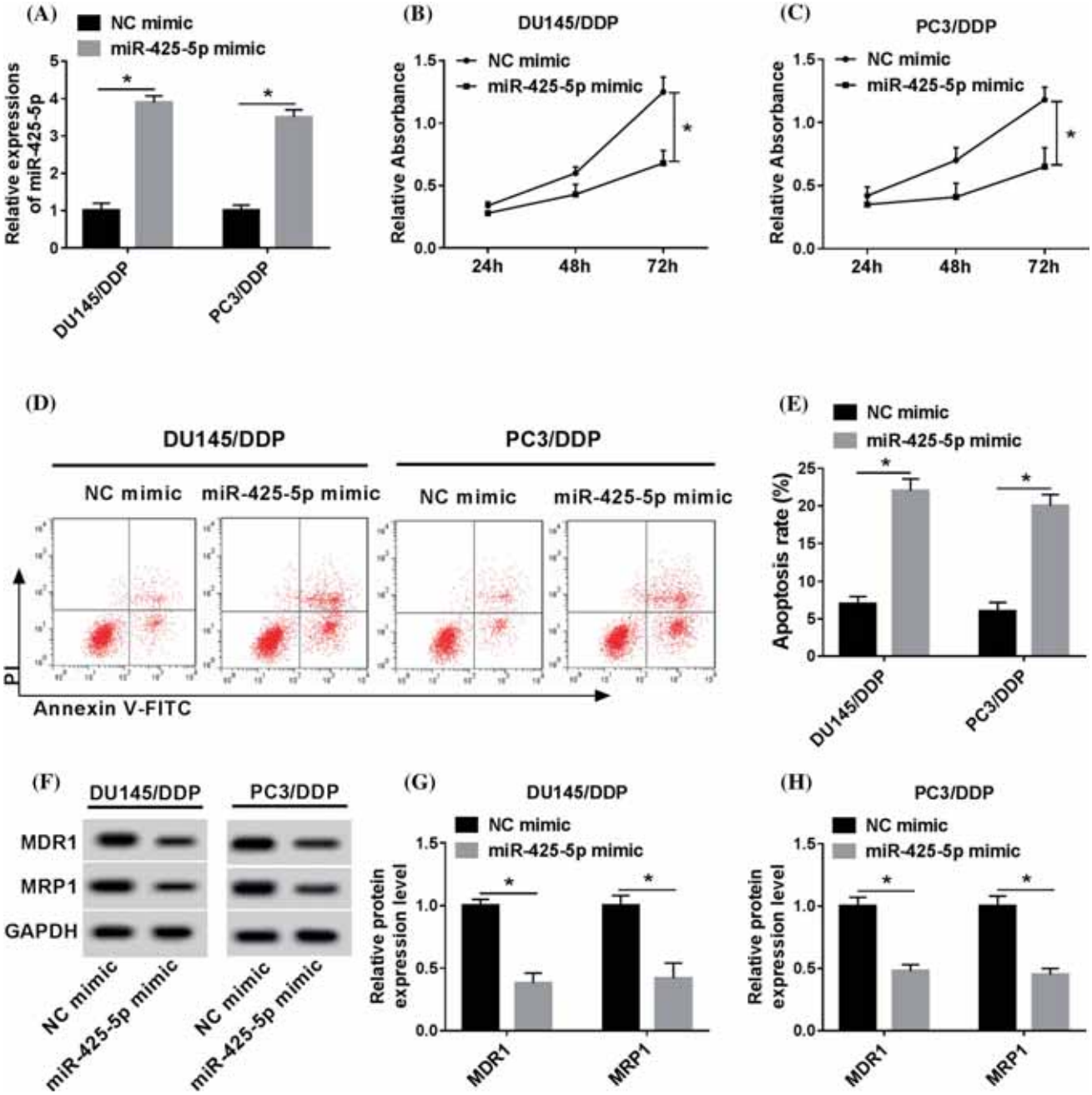

Figure 2. Effects of miR-425-5p overexpression on DDP resistance. Transient transfection of miR-425-5p/NC mimic into DU145/DDP and PC3/DDP cells for further study. (**A**) Levels of miR-425-5p were detected using the qPCR. (**B** and **C**) Cell viability was measured by MTT assay. (**D**) Cell apoptosis assay was conducted on flow cytometry. (**E**) Apoptosis rate was calculated for DU145/DDP and PC3/DDP cells. (**F**) Expressions of drug-resistant genes. Levels of MDR1 and MRP1 were shown depending on western blotting. (**G** and **H**) Gray density was analyzed in ImageJ. GAPDH was the loading control. Data were from three independent experiments and $*P<0.05$ compared with the miR-NC mimic group (NC mimic).

observed to be inhibited after transfection of miR-425-5p mimic and promoted after transfection of miR-425-5p inhibitor (figure 3E and F). These results indicated that miR-425-5p negatively regulated the GSK3β expression by target binding in DDP-resistant PCa cells.

3.4 *miR-425-5p regulated DDP resistance via modulating GSK3β in PCa cells*

To find out that whether miR-425-5p plays biological functions in DDP resistance through GSK3β in PCa,

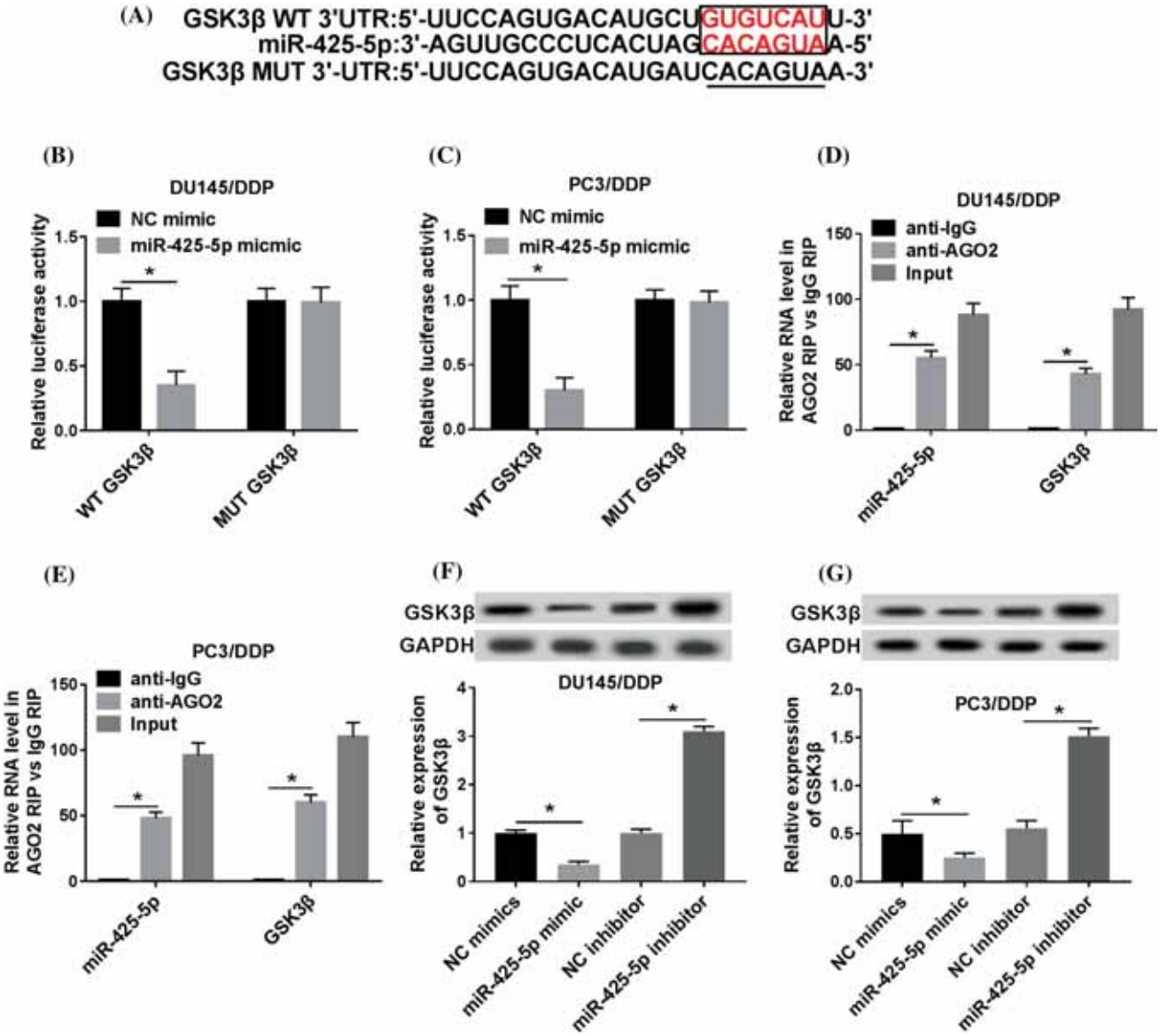

Figure 3. miR-425-5p regulated GSK3β by target binding. The relationship between miR-425-5p and GSK3β expression was uncovered in DU145/DDP and PC3/DDP cells. (**A**) Potential binding sites (red) were predicted using miRcode. (**B** and **C**) Luciferase reporter assay was carried out to verify the luciferase activity after co-transfected with miR-425-5p/NC mimic and GSK3β 3′-UTR WT/mutant (GSK3β WT/MUT). (**D** and **E**) Levels of miR-425-5p and GSK-3β were respectively measured in RIP with AGO2 and IgG. (**F** and **G**) Levels of GSK3β were examined by western blotting after cells were transfected with miR-425-5p mimic or miR-425-5p inhibitor. GAPDH was the loading control in western blotting. Expression of GSK3β protein was quantitated on an image analyzer. *P<0.05. Data were from three independent experiments and *P<0.05 compared with the NC mimic or miR-NC inhibitor group (NC inhibitor).

functional-recovery experiments were performed. As shown in figure 4A and B, miR-425-5p overexpression decreased the expression of GSK3β, whereas ectopic expression of both miR-425-5p and GSK3β resulted in a significant increase of the GSK3β level in DU145/DDP and PC3/DDP cells. Cell viability was dramatically inhibited by miR-425-5p upregulation, which was abolished by GSK3β overexpression (figure 4C and D). In contrast, the apoptosis rate of DU145/DDP and PC3/DDP cells was greatly elevated in the miR-425-5p group, and then was attenuated by GSK3β (figure 4E). We also noted that the suppressive effect of miR-425-5p on expression of MDR1 and MRP1 was blocked by its target gene GSK3β upregulation (figure 4F–H). Above data suggested that the biological regulation role of miR-425-5p on DDP resistances was mediated by GSK3β in PCa cells.

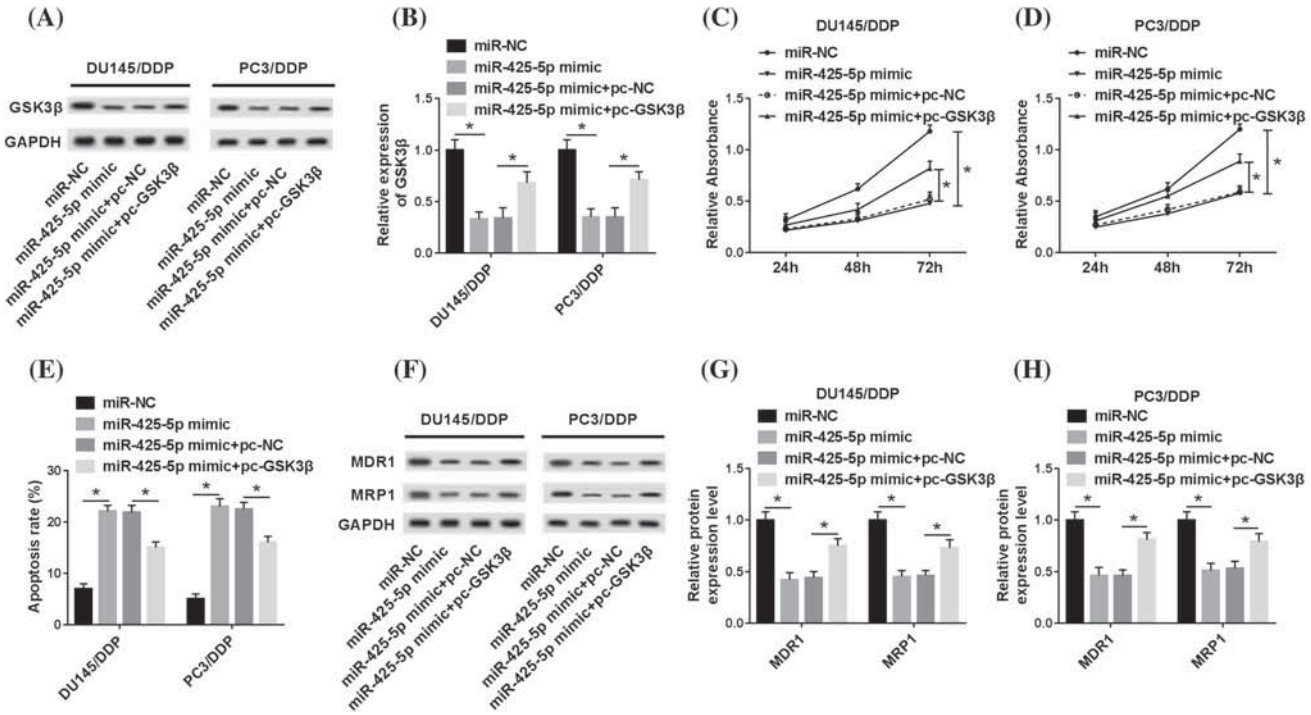

Figure 4. miR-425-5p regulated DDP resistance *via* GSK3β in PCa cells. Overexpressed pc-GSK3β cells were cultured with miR-425-5p mimic or not and overexpressed si-GSK3β cells were incubated with the miR-425-5p inhibitor or not in both DU145/DDP and PC3/DDP cells. (**A** and **B**) Cell viability assay was performed. (**C** and **D**) MTT assay was used to evaluate the cell viability. (**E**) Apoptosis assay was used to evaluate the rate of apoptosis. (**F**–**H**) Expressions of MDR1 and MRP1 were measured, and the gray density was analyzed in ImageJ. GAPDH was the loading control. Data were from three independent experiments and *$P<0.05$ compared with NC mimic or miR-425-5p mimic + pcDNA-NC (pc-NC).

3.5 *miR-425-5p regulated the Wnt/β-catenin signaling pathway via down-regulating GSK3β in DDP-resistant PCa cells*

To further investigate the regulation mechanism of miR-425-5p, we studied the canonical Wnt/β-catenin signaling pathway. As for the miR-425-5p mimic, it served as a blocker of expression of β-catenin, cyclin D1 and C-myc, and their relative levels were restored due to ectopic expression of GSK3β (figure 5A–C) in DU145/DDP and PC3/DDP cells. This indicated that Wnt/β-catenin signaling was involved in miR-425 functions in DDP resistance to PCa and GSK3β mediated the effect of miR-425-5p.

3.6 *GSK3β mediated the effect of miR-425-5p on xenograft tumor growth in vivo*

We hypothesized that miR-425-5p-sensitized PCa toward DDP by regulating GSK3β. To identify the anti-tumorigenic role of miR-425-5p in PCa *in vivo*, 5×10^6 DU145/DDP cells overexpressing miR-425-5p/NC mimic and pc-GSK3β/NC or not were subsequently implanted into nude mice. As shown in figure 6A and B, tumors in the miR-425-5p mimic group were grew at the slowest rate and had the smallest size and weight compared with the negative control (NC mimic group); the miR-425-5p mimic + pc-GSK3β group were grew at a higher rate and had larger size and weight compared with the NC (miR-425-5p mimic + pc-NC group). Further, expressions of MDR1 and MRP1 in xenograft tumors were strongly inhibited in the miR-425-5p mimic group, and the down-regulated DR1 and MRP1 levels were improved in the presence of pcDNA-GSK3β (figure 6C). Moreover, levels of GSK3β, β-catenin, cyclin D1 and C-myc were consistent with tumor growth as well as MDR1 and MRP1 expression *in vivo* (figure 6C and D). All the data supported the hypothesis that miR-425-5p retarded PCa tumor growth and suppressed DDP resistance through GSK3β.

4. Discussion

PCa is one of the most aggressive malignant cancers and is the third-leading cause of death from cancer in men (Litwin and Tan 2017). Chemotherapy can only prolong patient survival by a few months in castration-resistant disease due to its insensitivity to conventional chemotherapies, resulting in tumor recurrence (Houede *et al.* 2018). Chemotherapeutic insensitivity remains a big challenge in PCa treatment. Pathak *et al.* (2018) developed a multifunctional polymer-based self-assembled technology to deliver a predefined

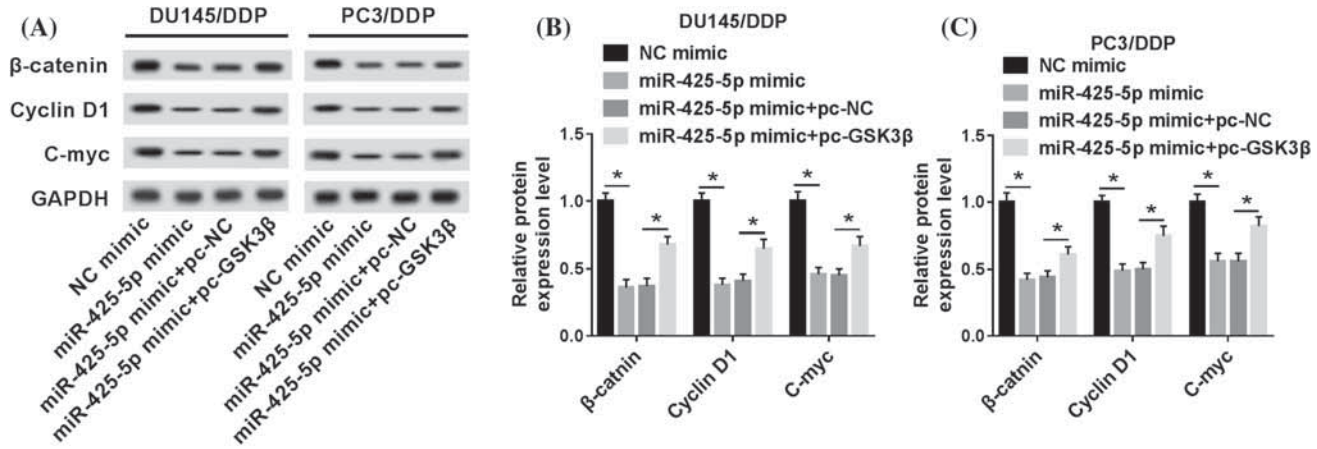

Figure 5. miR-425-5p regulated the Wnt/β-catenin signaling pathway by modulating GSK3β in PCa cells. (**A**) Western blotting detected the expressions of β-catenin, cyclin D1 and C-myc in DU145/DDP and PC3/DDP cells after transfected with miR-425-5p/NC mimic and pc-GSK3β/NC or not. (**B** and **C**) Levels of β-catenin, cyclin D1 and C-myc were quantitated on an image analyzer. Data were from three independent experiments and $*P<0.05$ compared with NC mimic or miR-425-5p mimic + pc-NC.

stoichiometric combination of a chemotherapy and an anti-inflammatory agent in a stimuli responsive manner, to complement and improve the currently established treatment methods of PCa. Su *et al.* (2018) claimed for the first time that adipose stromal cells interaction rendered cancer cells more migratory and resistant to docetaxel, cabazitaxel and cisplatin chemotherapy. Importantly, cisplatin combined with D-CAN, a hunter-killer peptide, was more effective than cisplatin alone in suppressing the growth of mouse PCa allografts and xenografts even in non-obese mice (Su *et al.* 2018).

The multidrug resistance (MDR) phenomenon is the most common justification for refractoriness with increased expression of genes including MDR protein 1 (MDR1) gene and MDR-associated protein 1 (MRP1) (Kimura *et al.* 2007; Sánchez *et al.* 2009). MDR1 encodes the protein P-glycoprotein 1 (P-gp) (also ATP-binding cassette sub-family B member 1, ABCB1), leading to MDR, for example, against taxanes, anthracyclines, Vinca alkaloids and epipodophyllotoxins in a variety of tumors, including various solid tumors and hematological malignancies (Genovese *et al.* 2017). Inhibition of ABC-transporters by low-molecular weight compounds in cancer patients has been extensively investigated in clinical trials, but the results have been disappointing (Lage 2016). In PCa, MDR1 polymorphisms were announced to be related to the risk of Chinese patients with PCa (Shen *et al.* 2013). MDR1 promoter methylation was frequent in prostate carcinoma and inversely correlated with the MDR1 expression level (Henrique *et al.* 2013). Saupe *et al.* (2015) demonstrated that MDR1 was of less importance for drug resistance in PCa cells than in other types of solid cancer. MRP1 is widely expressed in normal tissue and is upregulated in a variety of solid tumors (Li *et al.* 2005). Moreover, the levels of MRP1 tended to increase with disease stage and invasiveness in PCa (Sullivan *et al.* 1998). Functionally, MRP1 transported a wide range of therapeutic agents as well as diverse physiological substrates and conferred chemoresistance in PCa cell lines exposed to the DNA intercalating agent doxorubicin (Munoz *et al.* 2007).

More surprising is the findings that the small-regulatory molecules, miRNAs play a role in the resistance of cancer cells to commonly used anti-cancer drugs (Geretto *et al.* 2017; Razdan *et al.* 2018), such as cisplatin, anthracyclines and taxanes. miR-205 was strongly down-regulated in most archival samples of prostate carcinoma and promoted apoptosis in response to DNA damage by cisplatin and doxorubicin by targeting the anti-apoptotic protein Bcl-2 in the PCa cell lines PC3 and LnCap (Verdoodt *et al.* 2013). Besides, the loss of miR-205 established a permissive autophagic milieu that confers a chemotherapy resistant phenotype to PCa cells by decreasing cisplatin cytotoxicity (Pennati *et al.* 2014). And, miR-29b was reported to enhance cell apoptosis and chemotherapy effects of cisplatin in LNCaP (Yan *et al.* 2015). In this work, we focused on the effect of miR-425-5p on cisplatin resistance, and it was revealed that overexpression of miR-425-5p attenuated cell viability and drug-resistant gene (MDR1 and MRP1) expressions in DDP-resistant PC3 and DU145 cells. About 9 years ago, Folini *et al.* (2010) indicated that the oncogene miR-21 was not differently expressed in prostate carcinomas and matched normal tissues and miR-21 knockdown in PCa cells was not sufficient to affect the proliferative and invasive potential or the chemo- and radio-sensitivity profiles (Yang *et al.* 2015), thus supported the notion that the potential role of a given miRNA as a therapeutic target should be contextualized with respect to the disease.

miR-425-5p has been reported to be implicated in tumorigenesis and exerts a distinct role toward different tumors. On the one hand, there are several evidences that show miR-425-5p serves as an oncogenic gene to promote tumor formation, growth and progression to a higher stage.

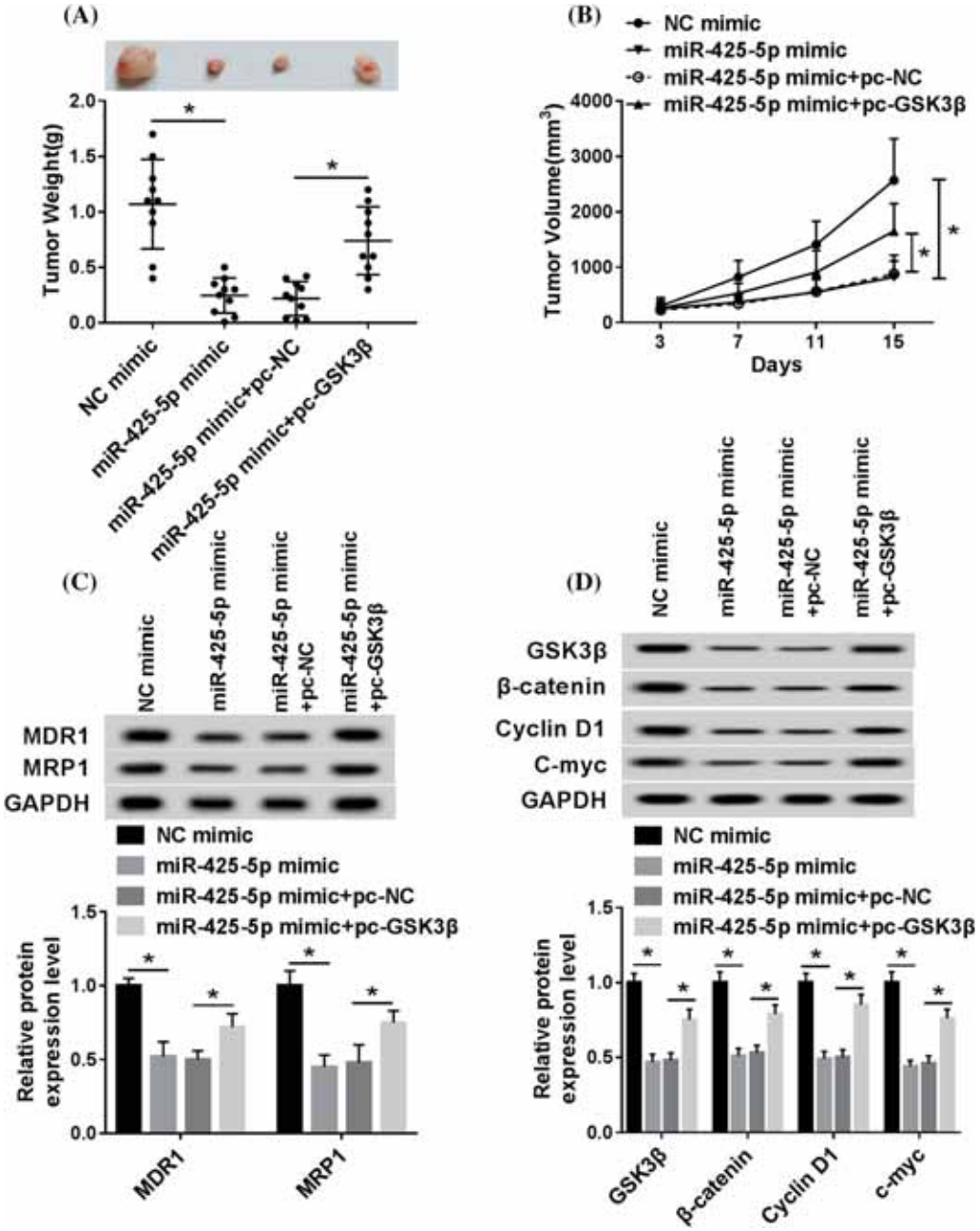

Figure 6. GSK3β mediated the regulatory effects of miR-425-5p on xenograft tumor growth. Female nude mice were subcutaneously injected with 5×10^6 DU145/DDP cells overexpressing miR-425-5p/NC mimic and pc-GSK3β/NC or not. (**A**) Tumor images were taken and tumor weight was recorded after xenograft for 15 days. (**B**) Growth curve of tumor volumes. (**C** and **D**) Expressions of MDR1, MRP1, GSK3β, β-catenin, cyclin D1 and C-myc in xenograft tumors were measured using western blotting. Data were from three independent experiments and $^*P<0.05$ compared with NC mimic or miR-425-5p mimic + pc-NC.

In gastric cancer, miR-425-5p was highly expressed in tumor tissues than adjacent mucosa, and up-regulation of miR-425-5p implied the depth of invasion, TNM stages and poor outcome (Zhang *et al.* 2015; Zhang *et al.* 2017). Cell viability and cells arrested in the G2/M and S stages were lower; the apoptosis rate and cells arrested in the G0/G1 stage were higher by miR-425-5p. In colorectal cancer, miR-425-5p is of important potential therapeutic values, the inhibitor of miR-425-5p-sensitized HCT116-R (5-FU and oxaliplatin-resistant HCT116 cells) xenografts to chemo drugs *in vivo* (Cristobal *et al.* 2016; Zhang *et al.* 2016). On the other, it has been reported in several studies that miR-425-5p acts as a tumor suppressor and suppresses cell proliferation and migration. Downregulation of miR-425-5p was recorded in nasopharyngeal carcinoma and resumption of miR-425 expression suppressed cell viability and invasion

in SUNE-1 cells by targeting HDGF (Zhu *et al.* 2018). In triple-negative breast cancer tissues, miR-425-5p was down-regulated by miRNA microarray analysis than hormone receptor positive BC and expression of miR-425-5p was higher in MDA-MB-231 and MDA-MB-468 cells compared with MCF-7 cells (Li *et al.* 2017). In consideration of the results in the YM500v3 database, our work demonstrated the downregulation of miR-425-5p and the anti-tumor role in the enhancement of DDP resistance to PCa cells (PC3 and DU145) by promoting apoptosis and suppressing xenograft tumor growth and drug-resistant gene (MDR1 and MRP1) expressions.

The Wnt-signaling pathway has been widely implicated in the acquisition of resistance. The field of WNT-signaling research has progressed both at the level of basic research, in which noncanonical-WNT signaling is now recognized as an important contributor to PCa progression and new regulators of canonical-WNT signaling at the cell membrane have been identified, and at the clinical level, as WNT-signaling inhibitors are now in early stage clinical trials (Murillo-Garzon and Kypta 2017). In the absence of WNT ligands, β-catenin is recruited and degraded by the destruction complex, whose components include axin, GSK3, CK1 and APC. A novel branch of WNT signaling, WNT-dependent stabilization of proteins (WNT-STOP), was described in 2014 and WNT-STOP signals were proposed to be important in cancer initiation and progression (Acebron *et al.* 2014). The genetic and epigenetic changes have been discovered that activate WNT-β-catenin signaling and could, therefore, contribute to PCa progression (Ahmad and Sansom 2018). Studies in mice showed that genetic activation of WNT/β-catenin signaling by a stabilized form of β-catenin (Munoz-Moreno *et al.* 2013) or by APC deletion resulted in high-grade prostate intraepithelial neoplasia (Valkenburg *et al.* 2014). In our investigation, downregulation of GSK3β mediated by miR-425-5p decreased DDP resistance and tumor growth, during which β-catenin was involved to be down-regulated, suggesting miR-425-5p-sensitized DDP-resistant PCa cells to cisplatin by inactivating the WNT/β-catenin signaling pathway.

In conclusion, the essence of this study is that miR-425-5p is one tumor-suppressor, and is down-regulated in PCa tissues and cell lines with acquired resistance to DDP. Overexpression of miR-425-5p contributes to cell apoptosis and inhibits cell viability, tumor growth and DDP resistance *via* targeting GSK3β through inactivating the Wnt/β-catenin signaling pathway. This provides us with a new target for treatment of human PCa, especially DDP-resistant PCa and a concept of deserving of further studies.

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Corresponding editor: Sorab Dalal