Am J Cancer Res 2017;7(12):2545-2553
www.ajcr.us /ISSN:2156-6976/ajcr0067580

Original Article

MiR-491-5p negatively regulates cell proliferation and motility by targeting PDGFRA in prostate cancer

Yanjun Xu[1,2*], Rui Hou[1,2*], Qijie Lu[1,2*], Yang Zhang[1,2], Lei Chen[1,2], Yuanyi Zheng[1,2], Bing Hu[1,2]

[1]Department of Ultrasound in Medicine, [2]Shanghai Institute of Ultrasound in Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China. [*]Equal contributors.

Received October 19, 2017; Accepted November 1, 2017; Epub December 1, 2017; Published December 15, 2017

Abstract: MicroRNA-491-5p (miR-491-5p) has been implicated in several cancers; however, its role in human prostate cancer (PCa) remains unknown. In this study, we observed downregulation of miR-491-5p expression in PCa tissues and cell lines. CCK-8 and EdU assays showed that forced expression of miR-491-5p suppressed PCa cell proliferation, which was further confirmed in a cell cycle assay. Overexpression of miR-491-5p also reduced PCa cell migration and invasion abilities as indicated by Transwell assays. Additionally, miR-491-5p overexpression significantly inhibited PCa growth in a mouse xenograft model. Mechanistically, platelet-derived growth factor receptor α (PDGFRA) was found to be a novel target of miR-491-5p. Re-introduction of PDGFRA antagonized the inhibitory effects of miR-491-5p on the proliferation and motility abilities of PCa cells. In clinical samples of PCa, miR-491-5p was negatively correlated with PDGFRA expression, which was upregulated in PCa. Collectively, these results demonstrate that miR-491-5p acts as a tumor suppressor in PCa by directly targeting PDGFRA and may serve as a therapeutic biomarker for patients with PCa.

Keywords: miR-491-5p, prostate cancer, platelet-derived growth factor receptor α, proliferation, motility

Introduction

Prostate cancer (PCa) is one of the most frequent and aggressive cancers and is the third-leading cause of cancer-related deaths in people in developed countries [1, 2]. The morbidity and mortality rates of PCa are increasing steadily worldwide. In the US, approximately 220,000 PCa cases were diagnosed and 27,540 deaths from PCa occurred in 2015 [3]. Although diagnostic methods for detecting early stage PCa have improved, the long-term survival rates of PCa patients remain poor (~25%) because of its high resistance to chemical treatment [4, 5]. Metastases often occur with no prior symptoms of tumor invasiveness [6]. Hence, there is an urgent need to identify the molecular mechanisms underlying the tendency of PCa to metastasize to other organs, ascertain novel biomarkers for differentiating indolent diseases from aggressive diseases, and provide potential therapeutic targets for PCa.

MicroRNAs (miRNAs) are groups of evolutionally conserved, short-length (20-25 nucleotides (nt) long), noncoding RNAs that play important roles in post-transcriptional regulation [7]. miRNAs can bind to the 3'-untranslated region (3'-UTR) of the corresponding target messenger RNAs (mRNA) to inhibit protein translation or stimulate mRNA degradation [8-10]. Accumulating evidence has shown that miRNAs play critical roles in many cellular functions such as proliferation, death, apoptosis, epithelial mesenchymal transition, and differentiation [11-14]. Various miRNAs are known to function as oncogenes or tumor suppressors in PCa and may act as diagnostic or prognostic biomarkers [12]. miR-491-5p, which is located in the fourth intron of focadhesin, was found to be deregulated in several human cancers [15-17]. However, the role of miR-491-5p in the progression of PCa and its underlying mechanism remain poorly understood.

In the present study, we investigated the potential involvement of miR-491-5p in PCa. We measured the expression of miR-491-5 in human PCa tissues and cells and determined its effects on cell viability, cell-cycle distribution, migration, and invasion. Furthermore, we

explored the underlying mechanism of miR-491-5p roles in PCa. This study improves the understanding of PCa pathogenesis.

Materials and methods

Human tissue specimens and cell culture

Eighteen pairs of clinical samples of PCa tissues and their adjacent normal prostate epithelial tissues were collected from Shanghai Jiao Tong University Affiliated Sixth People's Hospital. The collection and use of patient samples was reviewed and approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital, and written informed consent was obtained from each patient. Immediately upon retrieval, the samples were frozen in liquid nitrogen and stored at -80°C until use. The normal human prostate epithelial cell line RWPE-1 and three human PCa cell lines, PC-3, DU145, and LNCaP, were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultivated in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 medium (Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in a humidified chamber with 5% CO_2.

miRNA mimics, agomir, plasmid, and transfection

miR-491-5p mimics, agomir, and negative controls were purchased from RiboBio (RiboBio Co., Ltd., Guangzhou, China). To force the expression of PDGFRA in PCa cells, a PDGFRA plasmid (pcDNA3.1-PDGFRA) from Hanbio (Shanghai, China) was used. Transfection was conducted with Lipofectamine 2000 reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The effects of miR-491-5p mimics on recipient cells were analyzed 48 h after transfection.

RNA extraction and quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Life Technologies). cDNA synthesis was carried out with reverse transcriptase M-MLV (Takara, Shiga, Japan). Quantitative reverse transcription (qRT)-PCR experiments were conducted using SYBR Premix ExTaq (Takara) on an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The mRNA expression values of PDGFRA and miR-491-5p were normalized to the internal controls β-actin and U6, respectively. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

CCK-8 assay

A CCK-8 assay was performed to measure PCa cell viability. The cells were planted in 96-well plates (2000 cells per well) and incubated for 24, 48, and 72 h. After incubation, the cells were treated using a CCK8 kit (Boster, Wuhan, China). The optical density (OD) was measured at a wavelength of 450 nm.

EdU assay

Cell proliferation was detected using EdU kits (RiboBio, Guangzhou, China), according to the manufacturer's instructions [18]. A fluorescence microscope (Olympus, Tokyo, Japan) was used to acquire images. Nuclei double-labeled with EdU and Hoechst 33342 were considered as positive cells [19].

Cell cycle analysis

Cells were trypsinized, washed twice with cold phosphate-buffered saline (PBS), and fixed with pre-cooling 70% ethanol at -4°C for 12 h. Fixed cells were resuspended in PBS containing 10 mg/mL RNase A, 25 mg/mL propidium iodide (Sigma-Aldrich, St. Louis, MO, USA), and 0.1% Triton-X at 37°C for 30 min. Cell cycle progression was analyzed by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA).

Transwell migration and invasion assays

Cells (5×10^4) were plated in serum-free medium in the upper chamber of a Transwell (Corning, Inc., Corning, NY, USA). The lower chamber was filled with 20% fetal bovine serum as a chemoattractant. After 24 h of incubation, the cells that had migrated to the lower surface were fixed with methanol, stained with 0.1% crystal violet, and photographed. The number of cells was counted under a light microscope in five random fields. The process of Transwell migration assay was similar to that of the Transwell invasion assay except there was no Matrigel (BD Biosciences) in the inner surface of the chamber [20].

Mouse xenograft model

PC-3 cells treated with miR-491-5p agomir or NC agomir (2×10^6 cells in 100 μL PBS) were

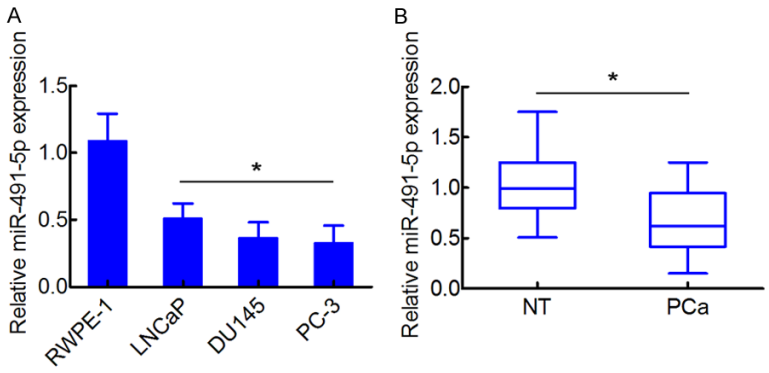

Figure 1. miR-491-5p expression was downregulated in PCa cell lines and tissues. A. Decreased miR-491-5p expression was detected in all three PCa cell lines (LNCaP, DU145, and PC-3) compared to in the normal human prostate epithelial cell line RWPE-1. B. qRT-PCR analysis of miR-491-5p expression in 18 pairs of human PCa tissues and their adjacent normal prostate tissues. The error bars represent the mean ± S.D. of three independent experiments. *$P < 0.05$.

injected subcutaneously into 2 groups of the flanks of BALB/c nude mice (male, 5-6 weeks age). Ten days after tumor cell inoculation, the 2 groups of mice were treated with miR-491-5p agomir or NC agomir by multiple-center intratumor injection separately twice per week for 2 weeks. Tumor volumes (V, mm^3) were estimated every 2 days, $V = 0.5 \times L \times W^2$. Two weeks after treatment, the mice were sacrificed and tumor weights were assessed. The proliferation index was measured with the Ki-67 immunostaining protein, and the ratio of positive cells was calculated from the total number of cells in five randomly selected fields. All animal handling and research protocols were approved by the Animal Care and Use Ethics Committee.

Western blot assay

Cells were harvested, washed with PBS, and lysed in cell lysis buffer containing protease inhibitors. Proteins in the extracts were separated on 10% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat milk and then incubated with primary antibodies against PDGFRA and β-actin (Cell Signaling Technology, Danvers, MA, USA). These membranes were then incubated with secondary antibodies and detected by enhanced chemiluminescence.

Luciferase reporter assay

A luciferase reporter containing the wild-type or mutated type 3'-UTR of PDGFRA was constructed using the pGL3-control vector (Promega, Madison, WI, USA). Cells were seeded in 24-well plates and allowed to settle for 24 h. miR-491-5p mimics and pGL3-PDGFRA-3'-UTR (WT/MUT) were co-transfected into PCa cells using Lipofectamine 2000 according to the manufacturer's instructions. Forty-eight hours after transfection, luciferase activity was determined using a Dual-Luciferase Reporter Assay System.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Measurement data were indicated as the mean ± standard deviation (SD) of three independent experiments. Differences between groups were calculated using 2-tailed Student's *t* test. Pearson's correlation test was employed to evaluate the association between miR-491-5p and PDGFRA mRNA expression. $P < 0.05$ was considered to indicate statistical significance.

Results

MiR-491-5p was remarkably downregulated in PCa cell lines and tissues

First, we assessed the expression of miR-491-5p in three PCa cell lines (LNCaP, DU145, and PC-3) by qRT-PCR. The results revealed that expression of miR-491-5p was decreased in all tested PCa cell lines compared to in the RWPE-1 cell line (**Figure 1A**). As DU145 and PC-3 cells showed lower expression of miR-491-5p, they were used in subsequent experiments. Additionally, conspicuously downregulated miR-491-5p expression was observed in PCa tissues compared to in corresponding adjacent normal tissues (n = 18) (**Figure 1B**). These results showed that miR-491-5p may be involved in human PCa.

Forced expression of miR-491-5p suppresses cell proliferation in vitro

To assess the function of miR-491-5p in PCa, the miR-491-5p mimics or its negative control miR-NC were transfected into PCa cells and the

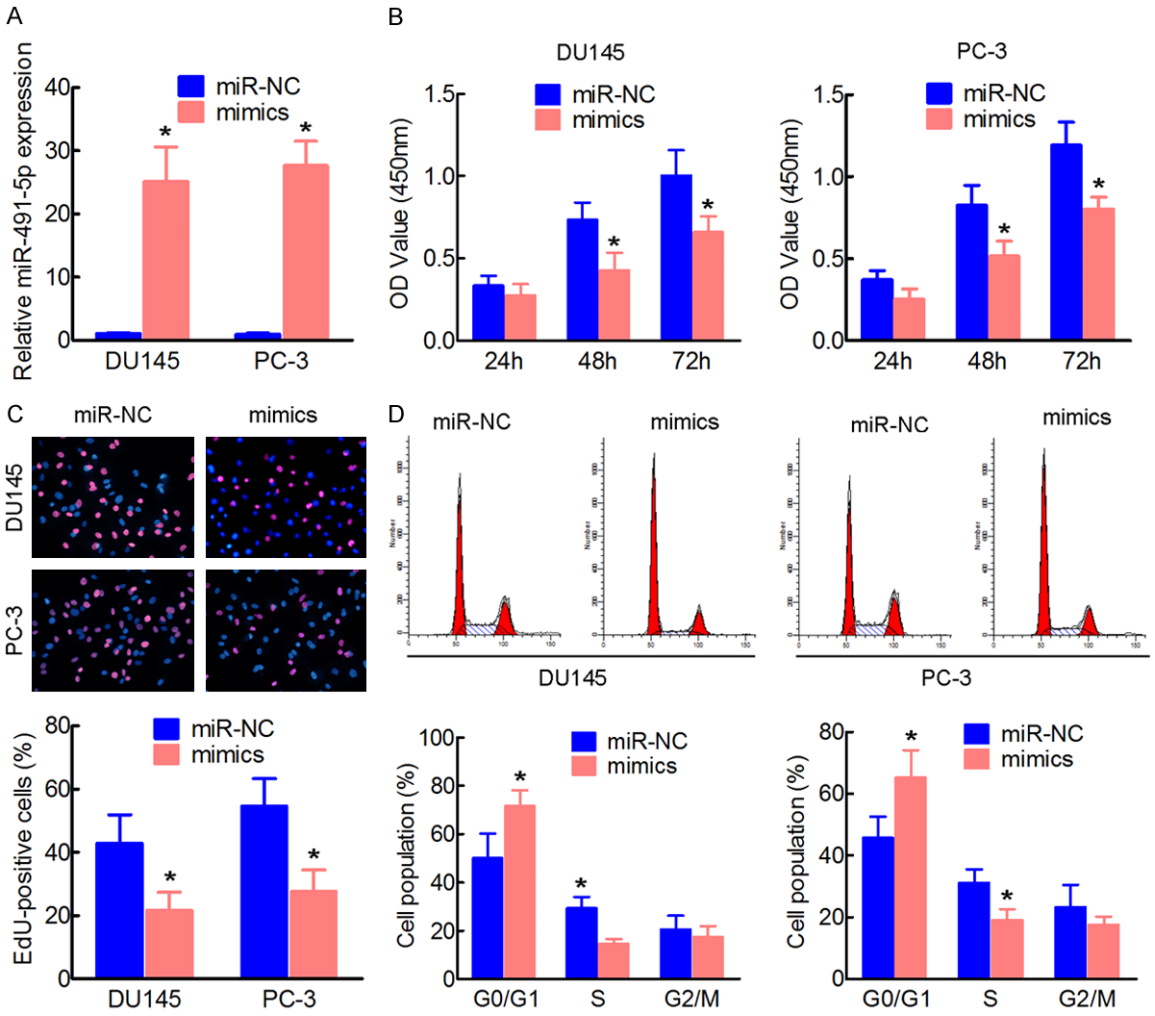

Figure 2. miR-491-5p inhibited PCa progression *in vitro*. A. Cells were transfected with miR-491-5p mimics (mimics) and negative control mimics (miR-NC); 48 h later, the expression of miR-491-5p was determined by qRT-PCR. B. Cell proliferation was evaluated by the CCK-8 assay at 24, 48, and 72 h in the indicated cells. C. DNA synthesis in PCa cells was measured by the EdU assay 48 h after the indicated transfection. D. The cell cycle distribution of DU145 and PC-3 cells transfected with mimics or miR-NC was determined by flow cytometry analysis. The error bars represent the mean ± S.D. of three independent experiments. *$P < 0.05$.

efficacy of transfection was confirmed by qRT-PCR (**Figure 2A**). The CCK-8 assay showed that miR-491-5p significantly decreased cell viability in DU145 and PC-3 cells (**Figure 2B**). Additionally, the EdU assay revealed that the proliferation rate of PCa cells transfected with miR-491-5p mimics was significantly decreased compared to that of cells transfected with miR-NC (**Figure 2C**). Furthermore, the effects of miR-491-5p on cell cycle progression of PCa cells were detected by flow cytometric analysis. **Figure 2D** showed that the proportion of cells in miR-491-5p mimics group at S phase were lower than that in the miR-NC group, and the forced expression of miR-491-5p clearly suppressed the G1-S phase transition of these cells. Collectively, these results demonstrate that enhanced expression of miR-491-5p inhibits cell proliferation in PCa cells.

miR-491-5p suppresses PCa cell migration and invasion

To assess whether miR-491-5p is involved in regulating tumor cell migration and invasion in PCa, Transwell assays with and without Matrigel were performed. As shown in **Figure 3A**, overexpression of miR-491-5p remarkably inhibited the migration ability of both DU145 and PC-3 cell lines compared to that of the miR-NC cells.

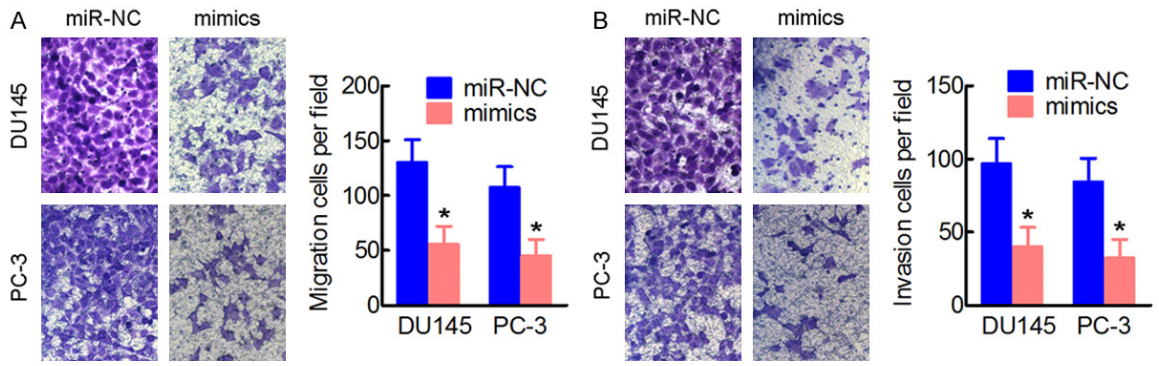

Figure 3. miR-491-5p suppressed the migration and invasion of PCa cells. (A) Transwell migration and (B) invasion assays for DU145 and PC-3 cells were determined after transfection with miR-491-5p mimics or miR-NC. Magnification × 200. The error bars represent the mean ± S.D. of three independent experiments. *$P < 0.05$.

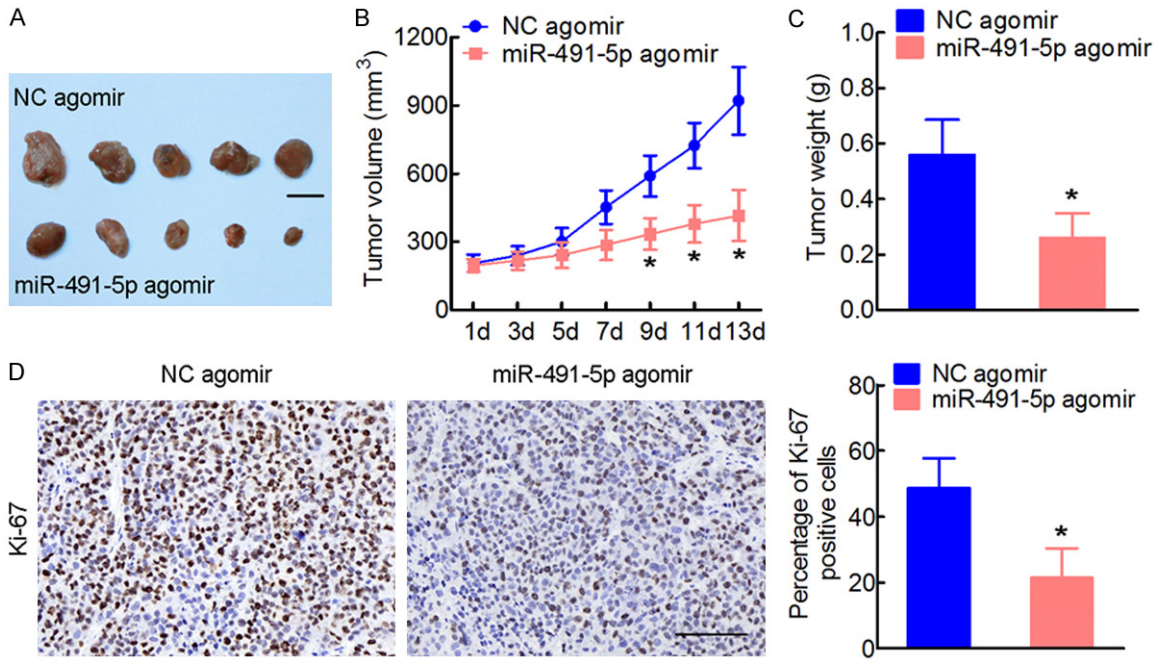

Figure 4. miR-491-5p inhibited PCa cell growth *in vivo*. A. PC-3 cells were injected into mice subcutaneously. After the tumor was established, tumors were directly injected with 4 nmol miR-491-5p agomir or NC agomir followed by monitoring of tumor size for 2 weeks and representative tumor samples were taken. Scale bar, 1 cm. B. Tumor growth of miR-491-5p agomir and NC agomir-treated PC-3 xenografts in nude mice. C. The tumors were weighed after harvest. D. Ki-67 staining of xenograft sections. Scale bar, 100 μm. The error bars represent the mean ± S.D. of three independent experiments. *$P < 0.05$.

Consistent with this result, miR-491-5p overexpression resulted in diminished invasive abilities in both cell lines (**Figure 3B**). These results indicate that miR-491-5p suppresses the migration and invasion of PCa cells.

miR-491-5p inhibits tumor growth in a subcutaneous PCa model

Based on the tumor suppressive roles of miR-491-5p *in vitro*, we further investigated the function of miR-491-5p *in vivo*. As shown in **Figure 4A** and **4B**, tumors treated with miR-491-5p agomir grew much slower than those treated with NC agomir. After 2 weeks, all mice were sacrificed to harvest the xenograft. Treatment with miR-491-5p agomir clearly led to a remarkable decrease in tumor weight compared to in the control group (**Figure 4C**). In addition, immunohistochemical staining showed that there were fewer Ki-67-positive cells in tumors treat-

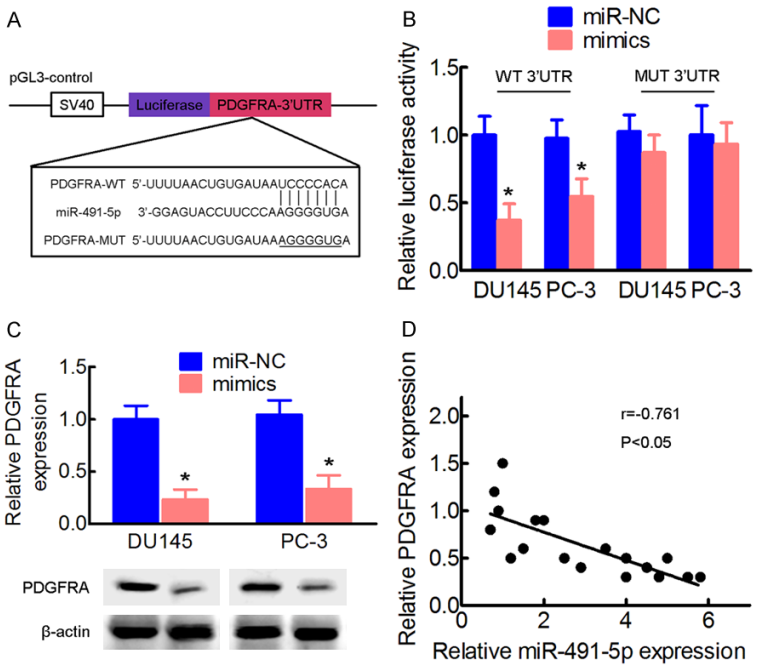

Figure 5. miR-491-5p targeted PDGFRA by binding to its 3'-UTR. A. Predicted miR-491-5p binding sites in the 3'-UTR of PDGFRA (PDGFRA-3'-UTR-WT) and mutant containing 7 altered nucleotides in the 3'-UTR of PDGFRA (PDGFRA-3'-UTR-MUT). B. Luciferase assay of DU145 and PC-3 cells transfected with pGL3-PDGFRA-3'UTR-WT or pGL3-PDGFRA-3'UTR-MUT reporter with miR-491-5p mimics. C. qRT-PCR and western blot analysis of PDGFRA in DU145 and PC-3 cells transfected with miR-491-5p mimics or miR-NC. D. Relationship between relative miR-491-5p and PDGFRA expression. The error bars represent the mean ± S.D. of three independent experiments. *$P < 0.05$.

ed with miR-491-5p agomir than in tumors treated with NC agomir (**Figure 4D**). These data suggest that miR-491-5p suppresses tumor growth *in vivo* and is a potential therapeutic target for treating PCa.

PDGFRA is a direct target of miR-491-5p

To gain an insight into the molecular mechanism by which miR-491-5p regulates the development of PCa, we conducted bioinformatic analysis to explore the potential regulatory targets of miR-491-5p using the miRanda, TargetScan, and PicTar databases, which revealed PDGFRA as a target. There is one predicted binding site between miR-491-5p and PDGFRA the 3'-UTR (**Figure 5A**). We constructed luciferase vectors containing PDGFRA-3'-UTR-WT or PDGFRA-3'-UTR-MUT. The PDGFRA-3'-UTR-WT or PDGFRA-3'-UTR-MUT vector was cotransfected into DU145 and PC-3 cells with miR-491-5p mimics or miR-NC. The results showed that miR-491-5p suppressed luciferase activity in cells transfected with PDGFRA-3'-UTR-WT, but did not affect luciferase activity in cells transfected with PDGFRA-3'-UTR-MUT (**Figure 5B**). Additionally, miR-491-5p modulated the expression of PDGFRA in PCa cells; overexpression of miR-491-5p decreased PDGFRA gene and protein expression (**Figure 5C**). Furthermore, PDGFRA mRNA expression was negatively correlated with miR-491-5p in PCa tissues from patients (**Figure 5D**, n = 18). Collectively, these data demonstrate that PDGFRA is a direct target of miR-491-5p.

Expression of PDGFRA reverses the inhibited proliferation and invasion function of miR-491-5p

Because we demonstrated that miR-491-5p can target and regulate the expression of PDGFRA, next we investigated the ability of PDGFRA to alter the effect of miR-491-5p on cell proliferation and invasion. First, we observed that PDGFRA expression was remarkably enhanced in PCa tissues compared to in adjacent normal prostate tissues by immunohistochemistry (**Figure 6A**), demonstrating the opposite situation to miR-491-5p. Next, we overexpressed PDGFRA in DU145 and PC-3 cells by pcDNA3.1-PDGFRA transfection. As shown in **Figure 6B**, cotransfection of miR-491-5p mimics and pcDNA3.1-PDGFRA abrogated the effects of miR-491-5p on PDGFRA expression (**Figure 6B**). Moreover, ectopic expression of PDGFRA reversed the miR-491-5p-induced inhibition of cell proliferation and invasion. These results indicate that PDGFRA mediates the miR-491-5p-induced tumor-suppressive function.

Discussion

miRNAs are known to influence the expression of various genes involved in human cancer progression and play a crucial role in human diseases [21]. In this study, we explored the func-

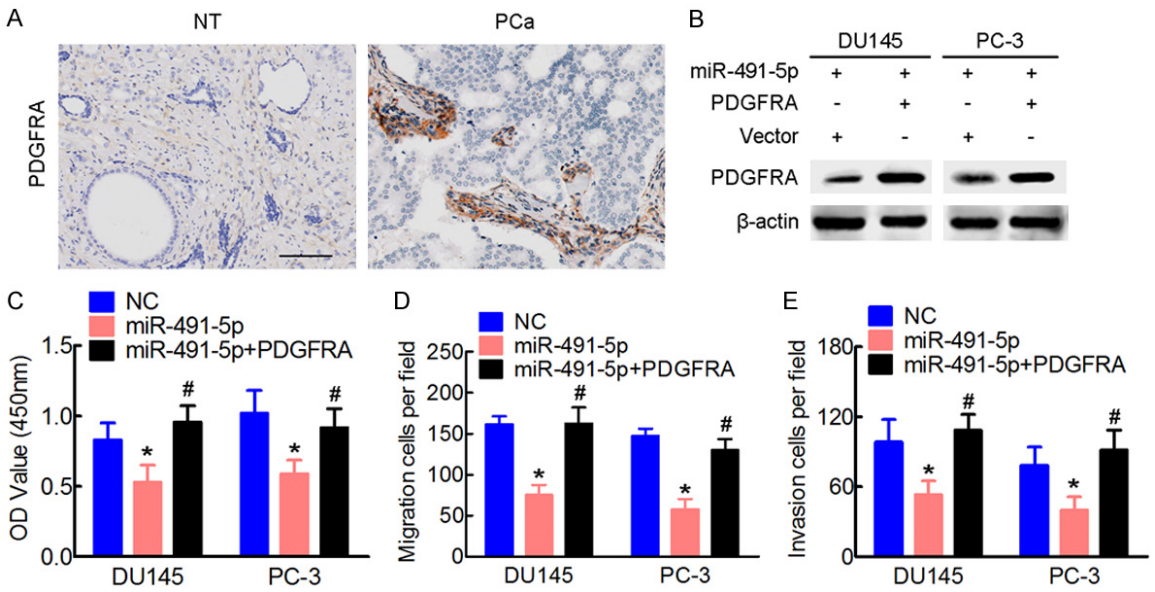

Figure 6. PDGFRA mediated multiple biological actions of miR-491-5p. A. PDGFRA expression was detected in PCa tissues and adjacent normal prostate tissues by immunohistochemistry. Scale bar, 100 μm. B. Cells were cotransfected with miR-491-5p mimics and pcDNA3.1-PDGFRA, or a negative control vector (Vector). After 48 h, PDGFRA protein levels were detected by western blotting. C. Cell proliferation was evaluated by the CCK-8 assay after 48 h of incubation. D. The cell cycle was determined by flow cytometry analysis. E. Cell invasive ability was detected by Transwell assay. The error bars represent the mean ± S.D. of three independent experiments. $^{*}P < 0.05$ vs NC, $^{\#}P < 0.05$ vs miR-491-5p.

tion of miR-491-5p in PCa. We demonstrated that miR-491-5p depresses cell proliferation and invasion *in vitro* and tumor growth *in vivo*. In addition, we found that miR-491-5p may serve as a mediator of these effects by targeting PDGFRA.

Previous studies demonstrated that miR-491-5p is downregulated in pancreatic cancer tissues [22], ovarian carcinoma tissues [23], and colorectal adenocarcinoma cells [24]. Ectopic expression of miR-491-5p depresses cell proliferation and colony formation and promotes apoptosis in gastric cancer cells [25]. Other studies showed that miR-491-5p inhibited the invasive capabilities of human oral squamous cancer and breast cancer cells [26]. In addition, miR-491-5p inhibited the transforming growth factor-β/SMAD3/nuclear factor-κB signal pathway by targeting the SMAD3 3'-UTR, thereby, weakening the angiogenic ability of hepatocellular carcinoma cells [27]. However, the function of miR-491-5p in PCa remains unknown. In this study, we conducted expression analysis of miR-491-5p by qRT-PCR in human PCa tissues and cell lines. Lower miR-491-5p expression levels were detected in PCa tissues and cell lines compared to in non-tumor tissues and cell lines. Ectopic expression of miR-491-5p decreased PCa cell proliferation, which was further confirmed by an EdU assay and cell cycle assay. Moreover, overexpression of miR-491-5p notably inhibited the migratory and invasive capacities of PCa cells *in vitro*. Importantly, subcutaneous xenograft tumor growth analysis *in vivo* revealed decreased tumor growth following treatment with the miR-491-5p agomir, demonstrating its therapeutic potential for treating Pca patients. These results confirm the tumor-suppressive role of miR-491-5p in PCa cells and support its potential utility in miRNA-based cancer therapy.

miRNAs can regulate multiple downstream targets [28], as one miRNA may modulate various cells through different mechanisms because of their diverse cell types and microenvironments. It is important to identify the specific miR-491-5p-mediated signaling pathway in PCa cells. A search of the miRanda, TargetScan, and PicTar databases revealed that PDGFRA-encoding mRNA contains a 3'-UTR biding site for miR-491-5p. A previous study demonstrated that PDGFRA is promoted by miR-146a-mediated BRCA1 reduction to strengthen the angiogenic activity of endothelial cells in hepatocellular

carcinoma [14]. Additionally, the inhibitory effects of miR-218-2 with SLIT3 on cell proliferation, invasion, and migration and of thyroid cancer by targeting PDGFRA and PLCG1 were observed [29]. Moreover, another study showed that inhibition of PDGFRA reduced the proliferation of PCa cells [30]. Here, we found that miR-491-5p binds to the 3'-UTR of PDGFRA by conducting luciferase reporter assays. In addition, miR-491-5p decreased PDGFRA mRNA and protein expression levels. Furthermore, we found that PDGFRA expression was negatively correlated with miR-491-5p expression in PCa samples. Ectopic expression of PDGFRA reversed the effect of suppression on cell proliferation and invasion by miR-491-5p. Together, these results indicated that a reduction of miR-491-5p enhances human PCa cell proliferation and invasion through a PDGFRA-mediated signaling pathway.

In conclusion, these *in vitro* and *in vivo* studies demonstrated that upregulation of miR-491-5p inhibited human PCa cell proliferation and invasion by inhibiting PDGFRA. The results of this study provide potential new therapeutic targets for the treatment of PCa.

Acknowledgements

We acknowledge the excellent work of all the research staff in this study. This study was funded by a grant from Shanghai Hospital Development Center (grant number: SHDC2201-5001) and the National Natural Science Foundation of China (grant number: 81401421).

Disclosure of conflict of interest

None.

Address correspondence to: Bing Hu, Department of Ultrasound in Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600th Yishan Road, Shanghai 200233, China. E-mail: binghu_stan@163.com

References

[1] Damber JE and Aus G. Prostate cancer. Lancet 2008; 371: 1710-1721.

[2] Attard G and Antonarakis ES. Prostate cancer: AR aberrations and resistance to abiraterone or enzalutamide. Nat Rev Urol 2016; 13: 697-698.

[3] Siegel RL, Fedewa SA, Miller KD, Goding-Sauer A, Pinheiro PS, Martinez-Tyson D and Jemal A. Cancer statistics for Hispanics/Latinos, 2015. CA Cancer J Clin 2015; 65: 457-480.

[4] Moore TH, King AJ, Evans M, Sharp D, Persad R and Huntley AL. Supportive care for men with prostate cancer: why are the trials not working? A systematic review and recommendations for future trials. Cancer Med 2015; 4: 1240-1251.

[5] Hudson SV, O'Malley DM and Miller SM. Achieving optimal delivery of follow-up care for prostate cancer survivors: improving patient outcomes. Patient Relat Outcome Meas 2015; 6: 75-90.

[6] Hughes C, Murphy A, Martin C, Sheils O and O'Leary J. Molecular pathology of prostate cancer. J Clin Pathol 2005; 58: 673-684.

[7] Doench JG and Sharp PA. Specificity of microRNA target selection in translational repression. Genes Dev 2004; 18: 504-511.

[8] Behm-Ansmant I, Rehwinkel J and Izaurralde E. MicroRNAs silence gene expression by repressing protein expression and/or by promoting mRNA decay. Cold Spring Harb Symp Quant Biol 2006; 71: 523-530.

[9] Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, Dorner S, Bork P, Boutros M and Izaurralde E. Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. Genes Dev 2007; 21: 2558-2570.

[10] Maroney PA, Yang Y, Fisher J and Nilsen TW. Evidence that microRNAs are associated with translating messenger RNAs in human cells. Nat Struct Mol Biol 2006; 13: 1102.

[11] Wang Q, Chen W, Bai L, Chen W, Padilla MT, Lin AS, Shi S, Wang X and Lin Y. Receptor-interacting protein 1 increases chemoresistance by maintaining inhibitor of apoptosis protein levels and reducing reactive oxygen species through a microRNA-146a-mediated catalase pathway. J Biol Chem 2014; 289: 5654-5663.

[12] Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F and Negrini M. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004; 101: 2999-3004.

[13] Ory B, Ramsey MR, Wilson C, Vadysirisack DD, Forster N, Rocco JW, Rothenberg SM and Ellisen LW. A microRNA-dependent program controls p53-independent survival and chemosensitivity in human and murine squamous cell carcinoma. J Clin Invest 2011; 121: 809-820.

[14] Zhu K, Pan Q, Zhang X, Kong LQ, Fan J, Dai Z, Wang L, Yang XR, Hu J and Wan JL. MiR-146a enhances angiogenic activity of endothelial cells in hepatocellular carcinoma by promoting

PDGFRA expression. Carcinogenesis 2013; 34: 2071-2079.
[15] Zeng H, Chen Y, You W, Huang XQ, Zhou CY and Li H. miR-491-5p functions as a tumor suppressor by targeting JMJD2B in ERα-positive breast cancer. FEBS Lett 2015; 589: 812.
[16] Zhang Q, Li Q, Xu T, Jiang H and Xu LG. miR-491-5p suppresses cell growth and invasion by targeting Notch3 in nasopharyngeal carcinoma. Oncol Rep 2016; 35: 3541-3547.
[17] Sun R, Liu Z, Tong D, Yang Y, Guo B, Wang X, Zhao L and Huang C. miR-491-5p, mediated by Foxi1, functions as a tumor suppressor by targeting Wnt3a/β-catenin signaling in the development of gastric cancer. Cell Death Dis 2017; 8: e2714.
[18] Zhang T, Hu Y, Ju J, Hou L, Li Z, Xiao D, Li Y, Yao J, Wang C, Zhang Y and Zhang L. Downregulation of miR-522 suppresses proliferation and metastasis of non-small cell lung cancer cells by directly targeting DENN/MADD domain containing 2D. Sci Rep 2016; 6: 19346.
[19] Zhang T, Hu Y, Ju J, Hou L, Li Z, Xiao D, Li Y, Yao J, Wang C and Zhang Y. Downregulation of miR-522 suppresses proliferation and metastasis of non-small cell lung cancer cells by directly targeting DENN/MADD domain containing 2D. Sci Rep 2016; 6: 19346.
[20] Wang J, Tian X, Han R, Zhang X, Wang X, Shen H, Xue L, Liu Y, Yan X and Shen J. Downregulation of miR-486-5p contributes to tumor progression and metastasis by targeting protumorigenic ARHGAP5 in lung cancer. Oncogene 2014; 33: 1181-1189.
[21] Gu Y, Cheng Y, Song Y, Zhang Z, Deng M, Wang C, Zheng G and He Z. MicroRNA-493 suppresses tumor growth, invasion and metastasis of lung cancer by regulating E2F1. PLoS One 2014; 9: e102602.
[22] Guo R, Wang Y, Shi WY, Liu B, Hou SQ and Liu L. MicroRNA miR-491-5p targeting both TP53 and Bcl-XL induces cell apoptosis in SW1990 pancreatic cancer cells through mitochondria mediated pathway. Molecules 2012; 17: 14733-14747.
[23] Denoyelle C, Lambert B, Meryetfiguière M, Vigneron N, Brotin E, Lecerf C, Abeilard E, Giffard F, Louis MH and Gauduchon P. miR-491-5p-induced apoptosis in ovarian carcinoma depends on the direct inhibition of both BCL-XL and EGFR leading to BIM activation. Cell Death Dis 2014; 5: e1445.
[24] Hanisch C, Sharbati J, Kutzlohroff B, Huber O, Einspanier R and Sharbati S. TFF3-dependent resistance of human colorectal adenocarcinoma cells HT-29/B6 to apoptosis is mediated by miR-491-5p regulation of lncRNA PRINS. Cell Death Discov 2017; 3: 16106.
[25] Sun R, Liu Z, Tong D, Yang Y, Guo B, Wang X, Zhao L and Huang C. miR-491-5p, mediated by Foxi1, functions as a tumor suppressor by targeting Wnt3a/beta-catenin signaling in the development of gastric cancer. Cell Death Dis 2017; 8: e2714.
[26] Rutnam ZJ and Yang BB. The non-coding 3' UTR of CD44 induces metastasis by regulating extracellular matrix functions. J Cell Sci 2012; 125: 2075-2085.
[27] Jiang F, Wang X, Liu Q, Shen J, Li Z, Li Y and Zhang J. Inhibition of TGF-β/SMAD3/NF-κB signaling by microRNA-491 is involved in arsenic trioxide-induced anti-angiogenesis in hepatocellular carcinoma cells. Toxicol Lett 2014; 231: 55.
[28] Ventura A and Jacks T. MicroRNAs and cancer: short RNAs go a long way. Cell 2009; 136: 586-591.
[29] Guan H, Wei G, Wu J, Fang D, Liao Z, Xiao H, Li M and Li Y. Down-regulation of miR-218-2 and its host gene SLIT3 cooperate to promote invasion and progression of thyroid cancer. J Clin Endocrinol Metab 2013; 98: 1334-1344.
[30] Mccabe CD, Spyropoulos DD, Martin D and Moreno CS. Genome-wide analysis of the homeobox C6 transcriptional network in prostate cancer. Cancer Res 2009; 69: 709.