Biochemical and Biophysical Research Communications 445 (2014) 151–156

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MiR-361-5p acts as a tumor suppressor in prostate cancer by targeting signal transducer and activator of transcription-6(STAT6)

Dachuang Liu [a,b,c,1], Tao Tao [a,c,1], Bin Xu [a,c,1], Shuqiu Chen [a,c], Chunhui Liu [a,c], Lei Zhang [a], Kai Lu [a], Yeqing Huang [a,c], Liang Jiang [a,c], Xiaowen Zhang [a], Xiaoming Huang [c], Lihua Zhang [d], Conghui Han [b], Ming Chen [a,c,*]

[a] Department of Urology, Zhongda Hospital, Medical School, Southeast University, Nanjing, Jiangsu Province 210009, China
[b] Department of Urology, Xuzhou Central Hospital Affiliated with Southeast University, Xuzhou, Jiangsu Province 221009, China
[c] Surgery Central Laboratory of Southeast University, Nanjing, Jiangsu Province 210009, China
[d] Department of Pathology, Zhongda Hospital Affiliated with Southeast University, Nanjing, Jiangsu Province 210009, China

ARTICLE INFO

Article history:
Received 14 January 2014
Available online 31 January 2014

Keywords:
Castration-resistant prostate cancer (CRPC)
miR-361-5p
STAT6
Bcl-xL

ABSTRACT

Castration-resistant prostate cancer (CRPC), whose pathogenesis is known to be regulated by microRNAs (miRNAs), has a poor prognosis. In our present study, we found that the expression of miR-361-5p in CRPC was lower than in androgen-dependent prostate cancer (ADPC), indicating that miR-361-5p may play an important role in the progression of ADPC to CRPC. The role of miR-361-5p in prostate cancer (PCa) has not been evaluated until date. Our findings suggest that miR-361-5p is a suppressor in CRPC. Signal transducer and activator of transcription-6 (STAT6), a direct target of miR-361-5p, enhances the expression of B-cell lymphoma-extra large (Bcl-xL), while miR-361-5p inhibits its expression through STAT6. Therefore, miR-361-5p has great clinical significance in preventing the malignant progression of PCa.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

According to the figures of the American Cancer Society, the number of new cases of prostate cancer (PCa) and related deaths in the USA for 2013 is estimated at 238,590 and 29,720, respectively. PCa presents the highest morbidity and the second highest mortality among American male cancer patients [1]. In recent years the morbidity and mortality of PCa are in obvious rising in China [2]. Castration-resistant prostate cancer (CRPC) has limited therapeutic options and poor prognosis, and is currently the leading cause of death among male cancer patients [3]. However, the mechanisms of the initiation and progression of PCa remain unclear.

MicroRNAs (miRNAs) are small (approximately 22 nucleotides in length) non-coding single-stranded RNAs that regulate expression of target genes by interacting with and binding to the 3′-untranslated regions (3′-UTR) of their target mRNAs [4]. Altered miRNA regulation is involved in PCa pathogenesis via the modulation of oncogenes and tumor suppressors that subsequently affect the downstream signaling pathways [5–7]. For instance, hsa-let-7c, hsa-miR-21 and hsa-miR-375 were found to be significantly up-regulated in PCa, whereas hsa-miR-143 and hsa-miR-145 were determined to be significantly down-regulated [8]. In a previous study, we revealed that the ubiquitous loss of miR-146a is a critical mechanism for the overexpression of EGFR in CRPC [9].

MiR-361 is encoded on Xq21.2, in an intron between exons 9 and 10 of CHM/choroideremia (Rab escort protein 1), and gives rise to two mature miRNA species, miR-361-3p and the predominant miR-361-5p [10,11]. A previous study revealed that miR-361-5p is down-regulated in a transplantable metastatic versus a non-metastatic PCa xenograft line, both derived from one patient's primary cancer [12]. Another study validated miR-361-5p as being differentially expressed in matched primary and metastatic clear cell renal cell carcinoma pairs, and indicated that miR-361-5p may suppress cell growth and proliferation in tumor development, metastasis and progression [13]. A latest report demonstrates that miR-361 is differentially expressed between both PCa groups [clinical progression free survival (CPFS) vs. clinical failure (CF)] and the non-malignant benign prostate hyperplasia (BPH) samples [14]. Additionally, miRNA-microarray analysis and qRT-PCR demonstrated that miR-361-5p expression in CRPC was lower than in

* Corresponding author at: Department of Urology, Zhongda Hospital, Medical School, Southeast University, 87 Dingjiaqiao Road, Nanjing, Jiangsu Province 210009, China.
E-mail address: mingchenseu@gmail.com (Ming Chen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.01.140
0006-291X/© 2014 Elsevier Inc. All rights reserved.

androgen-dependent prostate cancer (ADPC) in our study. Our findings indicated that miR-361-5p might play an important role in the progression of ADPC to CRPC. However, miR-361-5p has not been described as an oncomiR until now, and its relevance in the development and progression of PCa remains undetermined. Therefore, we selected miR-361-5p as a subject of our future studies. The inhibitory effects of miR-361-5p on DU145 were revealed by the CCK-8, colony formation, flow cytometry and tumor formation assay in our study. Down-regulation of signal transducer and activator of transcription-6 (STAT6) by utilizing siRNA influenced cell viability and induced apoptosis in DU145, as evaluated by the CCK-8, colony formation and flow cytometry assay. We indicated that miR-361-5p could repress the expression of STAT6 by binding to its 3′-UTR. Further, the down-regulation of STAT6 inhibited B-cell lymphoma-extra large (Bcl-xL) expression at the transcriptional level. Thus, our results suggested that miR-361-5p inhibited PCa and can thus be exploited as a new therapeutic target in CRPC.

2. Materials and methods

2.1. Tissue samples

PCa tissues obtained from 19 early-stage patients that underwent radical prostatectomy and had never received any previous treatment, were considered as having ADPC. All patients were in T_2, including six patients with a Gleason score of <7, eight patients with a Gleason score of 7 and five patients with a Gleason score of >7. Nine patients were diagnosed with CRPC, since their serum prostate-specific antigen (PSA) levels continued to increase during maximum androgen deprivation therapy. All patients with CRPC were in T_4 and had a Gleason score of >7. They underwent transurethral prostatic resection (TURP) because of urinary retention. For each specimen, surgical pathologists examined the clinicopathological features of freshly frozen sections after haematoxylin and eosin (HE) staining. Only those samples with >60% tumor content were included in this study. The samples were snap frozen in liquid nitrogen for microarray analysis (three ADPCs and three CRPCs) and qRT-PCR (sixteen ADPCs and six CRPCs) of miRNAs. These specimens (March, 2012–April, 2013) were used according to institutional guidelines and approved protocols. The ethics committees at Zhongda Hospital Affiliated with Southeast University approved this protocol, and all samples were collected following a written informed consent from the patients.

2.2. miRNA-microarray analysis

To profile differentially expressed miRNA in ADPC and CRPC tissues, we performed miRNA-microarray analysis. In brief, total RNA was first isolated from three ADPC and three CRPC tissues using a mir-Vana™ miRNA isolation kit (Applied Biosystem, Austin, Texas, USA) and then reverse transcribed into cDNA probes using a miRNA complete labeling and Hyb kit (Agilent, Santa Clara, California, USA). After that, the labeled probes were hybridized onto three miRNA microarray (Agilent, Santa Clara, California, USA) according to the manufacturer's instructions. The arrays were scanned and the data were extracted and analyzed using Agilent Feature Extraction (v10.7) software and the Agilent GeneSpring software for data were normalized.

2.3. Cell culture

The CRPC cell line DU145 was obtained from Shanghai Cell Bank, Chinese Academy of Sciences and cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone, Beijing, China) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco, Gaithersburg, Maryland, USA) and 10% foetal bovine serum (FBS, HyClone, Beijing, China), at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4. Oligonucleotides and cell transfection

All miRNA mimics or siRNA oligonucleotide duplexes were designed and chemically synthesised by GenePharma (Shanghai, China) based on the following sequences: hsa-miR-361-5p mimics: 5′-ACCCCUGGAGAUUCUGAUAAUU-3′, negative control (NC): 5′-UUCUCCGAACGUGUCACGUTT-3′; STAT6-specific siRNA: 5′-AGACCUGUCCAUUCGCUCATT-3′ (sense), 5′-UGAGCGAAUGGACAGGUCUTT-3′ (anti-sense), NC: 5′-UUCUCCGAACGUGUCACGUTT-3′ (sense), 5′-ACGUGACACGUUCGGAGAATT-3′ (anti-sense). For transfection, oligonucleotides formed transfection complexes with Lipofectamine 2000 (Invitrogen, Carlsbad, New Mexico, USA). Transfection complexes were added to DU145 cells and incubated for 6–8 h before changing the medium.

2.5. RNA isolation and quantitative RT-PCR

Total RNA was extracted from tissues or cells using Trizol (Invitrogen, Carlsbad, New Mexico, USA) according to the manufacturer's instructions. RNA concentration was measured using NanoDrop2000c (Thermo Scientific, Wilmington, Delaware, USA). For miR-361-5p, total RNA (10 ng) was transcribed into cDNAs using MMLV reverse transcriptase, and qRT-PCR reactions were performed using the SYBR Green PCR Master Mix of Hairpin-it™ miRNAs RT-PCR Quantitation Kit (GenePharma, Shanghai, China) according to the manufacturer's protocols. The following PCR conditions were used for detecting miRNAs: 95 °C for 3 min, 40 cycles at 95 °C for 12 s and 62 °C for 40 s. The expression of U6 was used as a control. All reactions were performed in triplicate, and negative control reactions that lacked cDNA were included. Relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

2.6. Cell proliferation assay

DU145 cells were seeded in a 6-well plate and cultured overnight. The cells were transfected with oligonucleotides and cultured overnight. Subsequently, the cells were trypsinised and seeded at 2000 cells/well (200 μl/well) in a 96-well plate. The proliferation of the cells was measured using the CCK-8 assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions, every 24 h after transfection. Absorbance was detected at a wavelength of 450 nm. Five wells were measured for cell viability in each treatment group, and all independent treatments were performed in triplicate.

2.7. Colony formation assay

In brief, 24 h after miR-361-5p or NC transfection, 200 cells were plated into each well of 6-well plates and incubated for 2 weeks at 37 °C in the presence of 5% CO_2. Next, cells were washed twice with phosphate buffer solution, fixed with methanol for 15 min, and stained with 0.2% crystal violet for 20 min at room temperature. Colonies containing more than 50 cells were counted. Results were expressed as mean values from three independent experiments.

2.8. Evaluation of apoptosis by flow cytometry

Apoptosis was analyzed by the annexin V-FITC plus propidium iodide (PI) staining method and flow cytometry measurements according to manufacturer's instructions (Ubio Biological

Technology, Ji'nan, China). The treated cells were analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, New Jersey, USA). At least 20,000 cells were acquired for each sample. The experiments were performed in triplicate.

2.9. Western blotting

Cells were lysed using the radioimmunoprecipitation assay buffer (Keygentec, Nanjing, China) for total proteins. The protein concentration was determined by the BCA method (Keygentec, Nanjing, China). Protein samples (30 μg) were electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, Massachusetts, USA). Membranes were blocked with 5% skim milk at room temperature for 1 h and incubated overnight at 4 °C with diluted antibodies specific for either STAT6 (1:1000; Cell Signaling Technology, Danvers, Massachusetts, USA), Bcl-xL or GAPDH (1:1000; Santa-Cruz Biotechnology, Heidelberg, Germany). Membranes were washed 3 times with TBST buffer (pH 7.6, 20 mM Tris–HCl, 137 mM NaCl, 0.01% Tween-20) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody at 25 °C for 2 h. Following washes with TBST buffer, membranes were visualized using enhanced chemiluminescence (ECL, Millipore, Billerica, Massachusetts, USA). Protein levels were determined by normalizing to GAPDH.

2.10. Plasmid construction and luciferase reporter assay

The 3′-UTR segment of STAT6 mRNA with miR-361-5p binding site was amplified by PCR using human DNA. The PCR products were cloned into the XhoI and NotI restriction sites, downstream of the open reading frame of luciferase in the psiCHECK-2™ Vector (Promega, Madison, Wisconsin, USA) to generate the STAT6 3′-UTR reporter. Deleting the binding site for miR-361-5p generated the mutant reporter. For reporter assays, cells were transfected with STAT6 3′-UTR or mutant reporter plasmid and miR-361-5p mimics or NC. We constructed a fragment of the Bcl-xL promoter region containing a STAT6-binding sequence and inserted the fragment into the luciferase reporter plasmid pGL3-basic (Promega, Madison, Wisconsin, USA) as a Bcl-xL pGL3-basic reporter. For reporter assays, cells were transfected with Bcl-xL pGL3-basic plasmid and STAT6 siRNA or NC. Luciferase activity was measured by dual-luciferase assays (Promega, Madison, Wisconsin, USA), 48 h after co-transfection.

2.11. Tumor formation assays in a nude mouse model

Twelve 5-week-old immunodeficient BALB/C nu/nu male mice (SLAC ANIMAL, Shanghai, China) were bred in aseptic conditions and maintained under constant humidity and temperature. Animal experiments were undertaken in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the ethics committee of Zhongda Hospital Affiliated with Southeast University. All mice were subcutaneously injected with DU145 cells in the dorsal scapula region. The tumor volume was measured with a caliper every 2 days, using the formula: volume = (length × width^2)/2. When the tumor volume reached at least 50 mm^3, the mice were randomly divided into two groups (six mice per group). These mice were then treated with 200 pmol NC or hsa-miR-361–5p mimics in 10 μl Lipofectamine 2000 (Invitrogen, Carlsbad, New Mexico, USA), through a local injection of the xenograft tumor at multiple sites. The treatment was performed once every 3 days for 15 days, and tumors were harvested 1 week later. Tumors were formalin fixed immediately following excision and paraffin embedded.

2.12. Immunohistochemical staining (IHC)

The paraffin embedded xenograft tumors for IHC were processed according to the manufacturer's instructions [Primary antibody: STAT6 (1:250; Cell Signaling Technology, Danvers, Massachusetts, USA) and Bcl-xL (1:300; Santa-Cruz Biotechnology, Heidelberg, Germany)].

2.13. Statistical analysis

Each value in this study was obtained from at least three independent experiments and presented as mean ± SD. The significance of differences was calculated using *t*-tests for two-group comparisons, using the SPSS 16.0 software package. A probability of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Expression of miR-361-5p in CRPC tissues is lower than in ADPC tissues

In our study, evaluating three ADPC and three CRPC miRNA chips, the expression of miR-361-5p in the CRPC group was lower than in that in the ADPC group (Fig. 1A). To verify our microarray results, we detected the expression of miR-361-5p in 16 ADPC tissues and 6 CRPC tissues by qRT-PCR. A similar trend of regulated miR-361-5p expression was observed (Fig. 1B). Therefore, we concluded that altered expression of miR-361-5p might be associated with the progression of PCa.

3.2. Effects of miR-361-5p in DU145 in vitro

We examined the expression of miR-361-5p in cells 48 h following transfection with miR-361-5p mimics or NC into DU145 by qRT-PCR. Higher expression was observed in the miR-361-5p mimics group than in NC group (Fig. 2A). Overexpression of miR-361-5p significantly inhibited DU145 proliferation in the CCK-8 and colony formation assays (Fig. 2B–D). Flow cytometry assay revealed that cells transfected with miR-361-5p showed a higher rate of apoptosis than NC (Fig. 2E and F).

3.3. STAT6 is a direct target of miR-361-5p and promotes Bcl-xL

We performed bioinformatics analysis to identify potential targets of miR-361-5p in PCa using the website microRNA.org, and found that miR-361-5p could bind to target sequences located in the 3′-UTR of STAT6 mRNA (Fig. 3A). STAT6 protein was inhibited by treatment with miR-361-5p mimics in Western blotting (Fig. 3B). To ascertain the direct miRNA-target interaction, STAT6 3′-UTR was cloned into a dual-luciferase reporter cloning site in the psiCHECK-2 dual-luciferase vector. We observed a dramatic decrease in the luciferase activities with increasing miR-361-5p levels (Fig. 3C). Further, transfection of STAT6 siRNA resulted in a concomitant down-regulation of Bcl-xL, which was shown by Western blotting (Fig. 3D). Based on a previous report [15] that had identified STAT6 binding motifs in the Bcl-xL promoter sequence (Fig. 3E), the Bcl-xL promoter region containing a STAT6-binding sequence was constructed into pGL3-basic plasmids. Consequently, STAT6 siRNA dramatically decreased luciferase activities after co-transfection (Fig. 3F).

3.4. MiR-361-5p suppresses tumor growth in vivo

As expected, miR-361-5p inhibited the tumor volume of DU145 cells (Fig. 4A). MiR-361-5p reduced STAT6 and Bcl-xL staining in

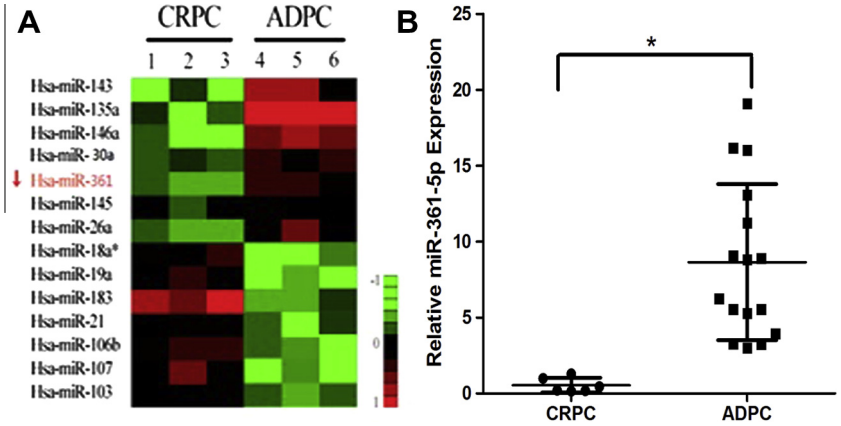

Fig. 1. Expression of MiR-361-5p in prostate cancer tissues. (A) A heat map of the expression profiles of microRNAs in three androgen-dependent prostate cancer (ADPC) tissues and three castration-resistant prostate cancer (CRPC) tissues were analyzed by Agilent microarrays (ADPC: three patients, all in T_2, with one patient with a Gleason score of <7, one patient with a Gleason score of 7, and one patient with a Gleason score of >7; CRPC: three patients all in T_4 and with a Gleason score of >7). (B) qRT-PCR showed decreased miR-361-5p levels in CRPC tissues compared to APDC tissues. $^{*}P < 0.05$.

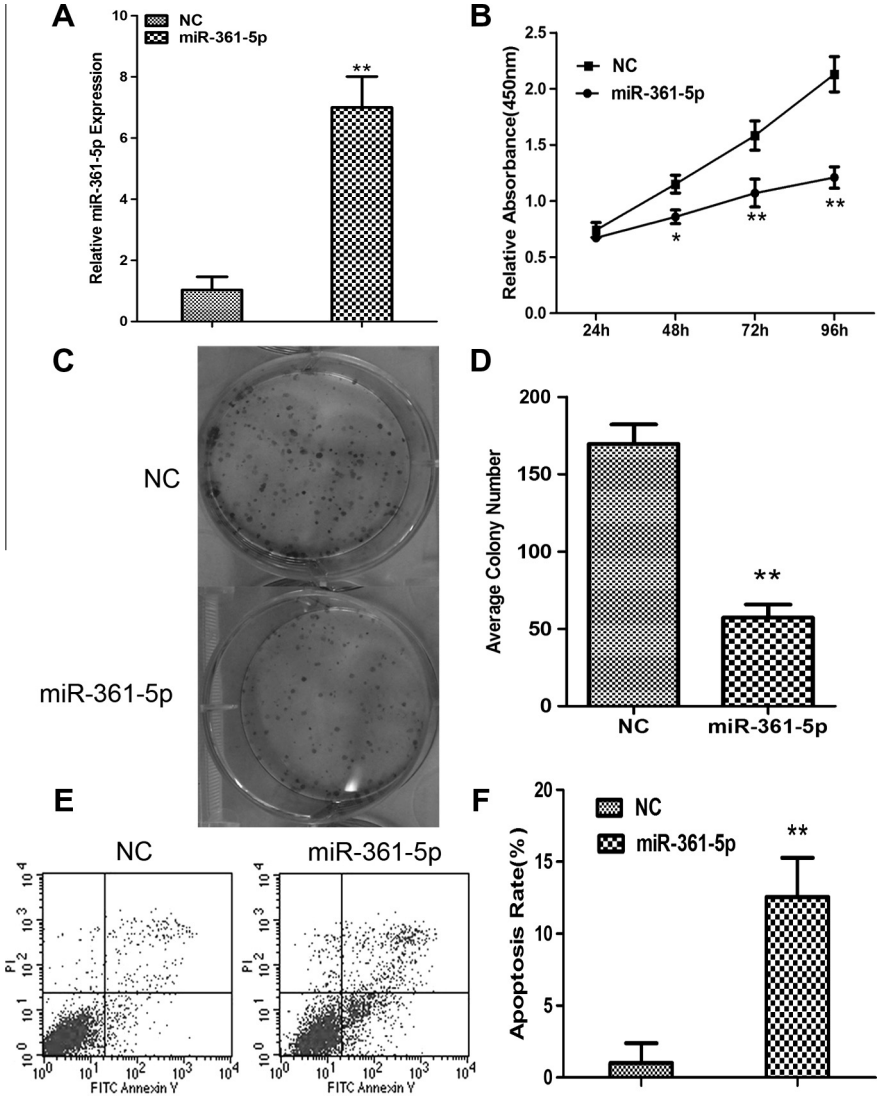

Fig. 2. Effect of miR-361-5p on DU145 *in vitro*. (A) MiR-361-5p mimics increased miR-361-5p expression compared to NC, as evaluated by qRT-PCR. (B) Cell proliferation, determined by CCK-8 assay, revealed that overexpression of miR-361-5p significantly inhibited DU145 proliferation. (C and D) The results showed that forced expression of miR-361-5p in DU145 decreased the colony-forming efficiency of the cells. (E and F) Annexin V-FITC/PI-stained cells transfected with miR-361-5p showed a higher rate of apoptosis, as evaluated by flow cytometry. $^{*}P < 0.05$, $^{**}P < 0.01$.

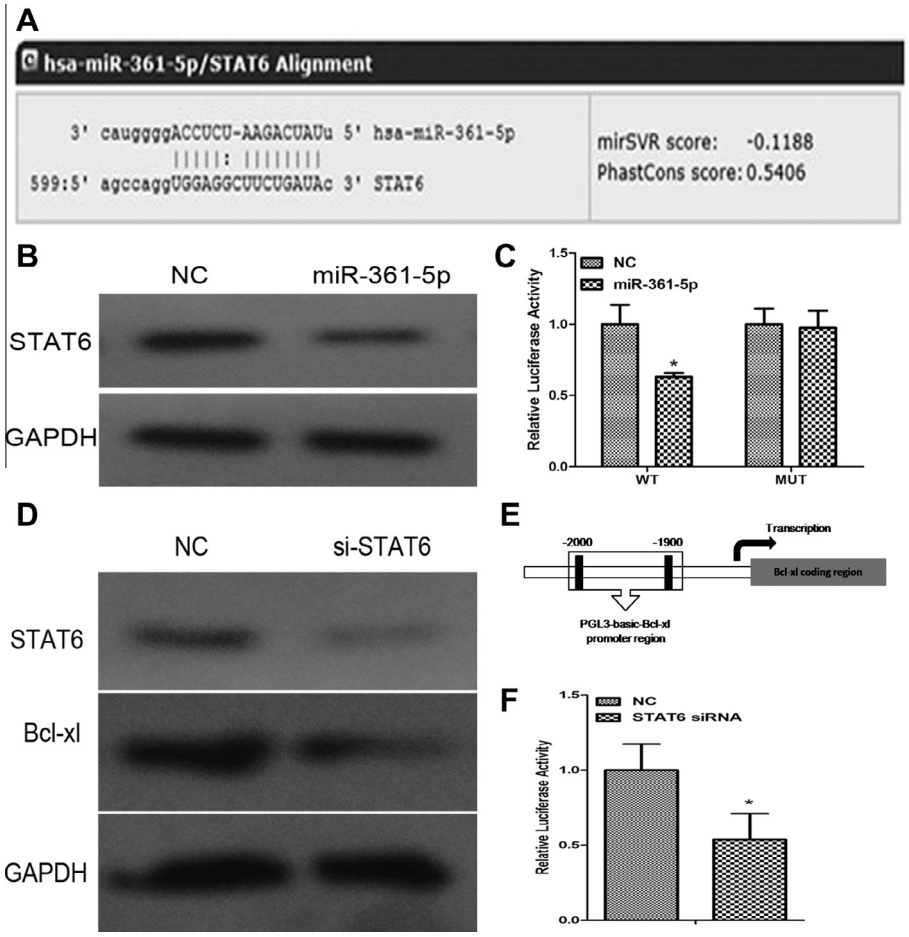

Fig. 3. STAT6 is a direct target of miR-361-5p and promotes Bcl-xL. (A) microRNA.org showed that miR-361-5p could bind to target sequences located in the nucleotides of the 3′-UTR of STAT6 mRNA. (B) STAT6 protein was inhibited by treatment with miR-361-5p mimics, as evaluated by Western blotting. (C) STAT6 3′-UTR was cloned into a luciferase reporter cloning site in the psiCHECK-2 dual luciferase vector. With increasing miR-361-5p level, luciferase activities decreased dramatically. (D) Expression of STAT6 and Bcl-xL was down-regulated using STAT6 siRNA compared with that in the controls in DU145 cells, as evaluated by Western blotting. (E) Schematic representation of putative STAT6 binding motifs in the Bcl-xL promoter sequence. (F) Bcl-xL promoter-containing pGL3-basic plasmid and STAT6 siRNA were co-transfected into cells. Luciferase assays performed after 48 h confirmed the inhibition of Bcl-xL promoter activity by STAT6 siRNA. $^{*}P < 0.05$.

tumor xenografts, as detected by IHC (Fig. 4B), suggesting that miR-361-5p reduced STAT6/Bcl-xL and tumorigenicity in a nude mouse model.

4. Discussion

Androgen ablation therapy for PCa can result in a regression of tumor size and reduce PSA levels. However, most ADPCs inevitably progress to CRPCs [16]. To date, the mechanisms for the progression of PCa remain unclear. Recently, accumulating evidence has associated dysregulated expression patterns of miRNAs to various types of human cancers, including PCa [17]. Previous studies have reported that miRNA play a critical role in tumorigenicity, tumor progression and drug resistance [18]. We observed that the expression of miR-361-5p in CRPC tissues was significantly lower than those in ADPC tissues, which suggests that miR-361-5p could be useful for the preventing the progression of ADPC to CRPC. Functional analyses showed that the forced expression of miR-361-5p suppressed proliferation and triggered apoptosis in DU145 cells and reduced tumorigenicity *in vivo*. However, a contradictory report identified miR-361-5p as an oncogene that enhanced the proliferation of cervical carcinoma cells, promoted cell invasion and was accompanied with epithelial-to-mesenchymal transition, suggesting that miR-361-5p is an important factor in the progression of cervical cancer [19]. These conflicting roles of miR-361-5p in different cancers necessitate the verification of the precise role of this miRNA in various tumors in the future.

STAT proteins are a family of latent cytoplasmic transcription factors that are activated by cytokines and growth factors. The homogenous or heterogeneous polymerization dimers of activation of transcription activating factor could translocate into the nucleus and target the gene promoter sequences of specific sites after phosphorylation [20]. STAT6 is a member of the STAT family that has been found to be constitutively activated in cancers [21,22]. STAT6 activity in prostate neoplasms was observed to be significantly higher than matched normal tissues adjacent to tumors and in normal prostate from donors, and STAT6 was proved to be a survival factor in CRPC [23–25]. We found that STAT6 directly regulated the expression of Bcl-xL at the transcriptional level in CRPC. Furthermore, our experiments identified STAT6 as a direct target of miR-361-5p. Overexpression of miR-361-5p reduced the malignant progression of PCa and diminished the expression of the STAT6/Bcl-xL signaling pathway *in vitro* and *in vivo*.

In summary, our results clearly showed that miR-361-5p, which suppressed cell proliferation and triggered apoptosis through STAT6/Bcl-xL pathway *in vitro* and *in vivo*, was down-regulated in CRPC. These results suggest that the role of miR-361-5p in preventing the progression of ADPC to CRPC requires further evaluation.

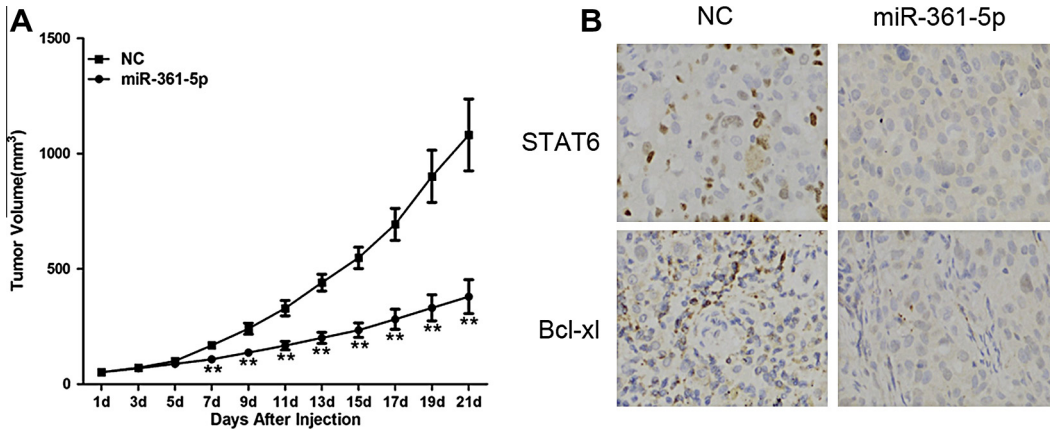

Fig. 4. MiR-361-5p suppresses tumor growth *in vivo*. (A) After establishment of subcutaneous tumors, miR-361-5p was injected as a multi-site injection every 3 days for 15 days. Tumor volumes were measured every 2 days. The tumor volume of the miR-361-5p group was significantly decreased compared to that of the NC group. (B) IHC was performed on xenograft tumors after miR-361-5p treatment. MiR-361-5p reduced STAT6 and Bcl-xL immunostaining in tumors. (Magnification, ×200 bar). $^{**}P < 0.01$.

Acknowledgments

This study was supported by National Natural Science Foundation of China (81370849, 81300472, 81070592 and 81202034), Natural Science Foundation of Jiangsu Province (BL2013032 and BK2012336) and Nanjing City (201201053) and Southeast University (3290002402), Science Foundation of Ministry of Education of China (20120092120071). Fundamental Research Funds for the Central Universities and Graduate Innovative Foundation of Jiangsu Province (CXZZ13_0133).

References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (2013) 11–30.
[2] E.M. Peyromaure, K. Mao, Y. Sun, S. Xia, N. Jiang, S. Zhang, G. Wang, Z. Liu, B. Debré, A comparative study of prostate cancer detection and management in China and in France, Can. J. Urol. 16 (2009) 4472–4477.
[3] D. Shapiro, B. Tareen, Current and emerging treatments in the management of castration-resistant prostate cancer, Expert Rev. Anticancer Ther. 12 (2012) 951–964.
[4] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[5] J. Shen, S.A. Stass, F. Jiang, MicroRNAs as potential biomarkers in human solid tumors, Cancer Lett. 329 (2013) 125–136.
[6] J.J. Yu, S.J. Xia, Novel role of microRNAs in prostate cancer, Chin. Med. J. (Engl.) 126 (2013) 2960–2964.
[7] Y. Pang, C.Y. Young, H. Yuan, MicroRNAs and prostate cancer, Acta Biochim. Biophys. Sin. 42 (2010) 363–369.
[8] J. Szczyrba, E. Löprich, S. Wach, V. Jung, G. Unteregger, S. Barth, R. Grobholz, W. Wieland, R. Stöhr, A. Hartmann, B. Wullich, F. Grässer, The microRNA profile of prostate carcinoma obtained by deep sequencing, Mol. Cancer Res. 8 (2010) 529–538.
[9] B. Xu, N. Wang, X. Wang, N. Tong, N. Shao, J. Tao, P. Li, X. Niu, N. Feng, L. Zhang, L. Hua, Z. Wang, M. Chen, MiR-146a suppresses tumor growth and progression by targeting EGFR pathway and in a p-ERK-dependent manner in castration-resistant prostate cancer, Prostate 72 (2012) 1171–1178.
[10] A. Kanitz, J. Imig, P.J. Dziunycz, A. Primorac, A. Galgano, G.F. Hofbauer, A.P. Gerber, M. Detmar, The expression levels of microRNA-361-5p and its target VEGFA are inversely correlated in human cutaneous squamous cell carcinoma, PLoS One 7 (2012) e49568.
[11] E.A. Afanasyeva, A. Hotz-Wagenblatt, K.H. Glatting, F. Westermann, New miRNAs cloned from neuroblastoma, BMC Genomics 9 (2008) 52.
[12] A. Watahiki, Y. Wang, J. Morris, K. Dennis, H.M. O'Dwyer, M. Gleave, P.W. Gout, Y. Wang, MicroRNAs associated with metastatic prostate cancer, PLoS One 6 (2011) e24950.
[13] H.W. Khella, N.M. White, H. Faragalla, M. Gabril, M. Boazak, D. Dorian, B. Khalil, H. Antonios, T.T. Bao, M.D. Pasic, R.J. Honey, R. Stewart, K.T. Pace, G.A. Bjarnason, M.A. Jewett, G.M. Yousef, Exploring the role of miRNAs in renal cell carcinoma progression and metastasis through bioinformatic and experimental analyses, Tumour Biol. 33 (2012) 131–140.
[14] M. Schubert, M. Spahn, S. Kneitz, C.J. Scholz, S. Joniau, P. Stroebel, H. Riedmiller, B. Kneitz, Distinct microRNA expression profile in prostate cancer patients with early clinical failure and the impact of let-7 as prognostic marker in high-risk prostate cancer, PLoS One 8 (2013) e65064.
[15] A. Natoli, R. Lüpertz, C. Merz, W.W. Müller, R. Köhler, P.H. Krammer, M. Li-Weber, Targeting the IL-4/IL-13 signaling pathway sensitizes Hodgkin lymphoma cells to chemotherapeutic drugs, Int. J. Cancer 133 (2013) 1945–1954.
[16] B.J. Feldman, D. Feldman, The development of androgen independent prostate cancer, Nat. Rev. Cancer 1 (2001) 34–45.
[17] M.D. Jansson, A.H. Lund, MicroRNA and cancer, Mol. Oncol. 6 (2012) 590–610.
[18] T. Tao, Y. Wang, H. Luo, L. Yao, L. Wang, J. Wang, W. Yan, J. Zhang, H. Wang, Y. Shi, Y. Yin, T. Jiang, C. Kang, N. Liu, Y. You, Involvement of FOS-mediated miR-181b/miR-21 signalling in the progression of malignant gliomas, Eur. J. Cancer 49 (2013) 3055–3063.
[19] X. Wu, X. Xi, Q. Yan, Z. Zhang, B. Cai, W. Lu, X. Wan, MicroRNA-361-5p facilitates cervical cancer progression through mediation of epithelial-to-mesenchymal transition, Med. Oncol. 30 (2013) 751.
[20] K. Takeda, S. Akira, STAT family of transcription factors in cytokine-mediated biological responses, Cytokine Growth Factor Rev. 11 (2000) 199–207.
[21] T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, Oncogene 19 (2000) 2474–2488.
[22] O. Ritz, C. Guiter, K. Dorsch, I. Dusanter-Fourt, S. Wegener, H. Jouault, P. Gaulard, F. Castellano, P. Möller, K. Leroy, STAT6 activity is regulated by SOCS-1 and modulates BCL-XL expression in primary mediastinal B-celllymphoma, Leukemia 22 (2008) 2106–2110.
[23] Z. Ni, W. Lou, S.O. Lee, R. Dhir, F. DeMiguel, J.R. Grandis, A.C. Gao, Selective activation of members of the signal transducers and activators of transcription family in prostate carcinoma, J. Urol. 167 (2002) 1859–1862.
[24] S. Das, C.P. Roth, L.M. Wasson, J.K. Vishwanatha, Signal transducer and activator of transcription-6 (STAT6) is a constitutively expressed survival factor in human prostate cancer, Prostate 67 (2007) 1550–1564.
[25] S. Das, P. Shetty, M. Valapala, S. Dasgupta, Z. Gryczynski, J.K. Vishwanatha, Signal transducer and activator of transcription 6 (STAT6) is a novel interactor of annexin A2 in prostate cancer cells, Biochemistry 49 (2010) 2216–2226.