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ORIGINAL ARTICLE

miR-205 is frequently downregulated in prostate cancer and acts as a tumor suppressor by inhibiting tumor growth

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The purpose of this study was to elucidate the molecular mechanisms of microRNA-205 (miR-205) as a tumor suppressor in prostate cancer (PCa). In the present study, microRNA microarray analysis suggested that the expression of miR-205 was significantly decreased in advanced PCa compared with early PCa. Real-time PCR analysis also indicated that miR-205 expression was significantly decreased in PCa tissues compared with non-cancerous tissues. Moreover, the expression of miR-205 has been demonstrated to be associated with the clinicopathological stage and total/free prostate-specific antigen (PSA) level of PCa. Functional analyses showed that both the overexpression of miR-205 and the knockdown of *c-SRC* in PCa cell lines could inhibit cell growth, colony formation, migration, invasion and the cell cycle as well as induce cell apoptosis *in vitro*. Furthermore, over-expressing miR-205 reduced tumorigenicity *in vivo*. Through a luciferase activity assay and Western blotting, *c-SRC* was identified as a target of miR-205 in cells. The overexpression of miR-205 suppressed c-SRC and its downstream signaling molecules, including FAK, p-FAK, ERK1/2 and p-ERK1/2, and attenuated cell proliferation, invasion and tumor growth.
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Keywords: *c-SRC*; miR-205; prostate cancer (PCa); tumor growth

INTRODUCTION

Prostate cancer (PCa), which largely affects males, is the second leading cause of cancer-related deaths in the United States, with an estimated 240 000 new cases and 33 000 deaths in 2011, mainly due to its high potential for tumor progression.[1] In China, the current PCa incidence rate, although still not very high, has increased dramatically over the past two decades. Moreover, most new cases also suffer symptomatic and metastatic disease because prostate-specific antigen (PSA) and digital rectal examinations are not routinely used to screen PCa in China.[2] Localized PCa patients have a survival rate of more than 5 years,[3,4] but this survival rate drops rapidly when tumor progression is detected.[5,6] Therefore, many attempts have been made to understand the molecular mechanisms of the development and progression of advanced PCa in China so that novel therapeutic strategies may be devised. In pursuit of this objective, novel molecules, one of which is microRNA (miRNA), are being detected and investigated.

miRNAs are small, non-coding RNAs that regulate gene expression by blocking gene translation or decreasing the stability of mRNAs.[7,8] As a new class of regulatory molecule, miRNAs play critical roles in a variety of biological processes, including cell proliferation, metastasis, development, differentiation, apoptosis, metabolism and immunity.[9,10] microRNA205 (miR-205) has previously been cited as targeting *HER*3, *SHIP*2, *VEGF-A*, *MED*1, *SIP*1 and *ZEB*1 for repression.[11–15] In addition, miR-205 has been shown to activate *IL*24 and *IL*32 by targeting sites on their promoters.[16] In PCa, miR-205 exerts a tumor-suppressive effect by counteracting the epithelial-to-mesenchymal transition and reducing cell migration/invasion, in part through the down-regulation of protein kinase C epsilon.[17]

In this study, we analysed miR-205 expression in advanced and early PCa tissues among Chinese patients using a quantitative real-time PCR assay. By investigating the role of miR-205 in proliferation, cell-cycle progression, apoptosis, migration, invasion and colony formation in DU145 and PC3 cells, we found that forced expression of miR-205 in DU145 cells could inhibit tumor growth in nude mice. Subsequent experiments confirmed that *c-SRC* was a target gene of miR-205 and was down-regulated by miR-205. Furthermore, that the siRNA-mediated knockdown of *c-SRC* partially phenocopied miR-205 restoration suggests that the tumor suppressive role of miR-205 might be mediated through *c-SRC* regulation. Understanding the functional role of miR-205 could help us to identify new targets and biomarkers that can positively impact patients with PCa.

MATERIALS AND METHODS

Cell culture and transfection

The human PCa cell lines DU145 and PC3 were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences, and maintained in DMEM supplemented with 10% foetal bovine serum, 100 units per ml penicillin, and 100 μg ml^{-1} streptomycin in an atmosphere of 5% CO_2 at 37 °C. Has-miRNA-205 mimics, negative controls and siRNAs were chemically designed and synthesized by GenePharma Company (Shanghai, China). Has-miRNA-205 mimics were sense: 5′-UCCUUCAUUCCACCGGAGUCUG-3′ and

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anti-sense: 5′-GACUCCGGUGGAAUGAAGAAUU-3′. Negative controls were sense: 5′-UUCUCCGAACGUGUCACGUTT-3′ and anti-sense: 5′-ACGUGACACGUUCGGAGAATT-3′. siRNA-*SRC* (S1) were sense: 5′-CUCGGCUCAUUGAAGACAATT-3′ and anti-sense: 5′-UUGUCUUCAAUGAGCCGAGTT-3′. Cells of 50%–60% confluence were transfected with oligonucleotides using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

miRNA microarray

Fluorescence targets were taken from 2.5 μg total RNA samples using the miRNA ULS Labeling Kit (Kreatech Diagnostics, Amsterdam, The Netherlands). The miRNA microarray analysis was carried out by a commercial company (Phalanx Biotech, Hsinchu, Taiwan, China) using human miRNA OneArray v3. The Cy5 fluorescent intensities of each spot were analysed by GenePix 4.1 software (Molecular Devices, Silicon Valley, CA, USA).

Quantitative real-time PCR

Total RNA was extracted and assayed for mature miRNAs. TaqMan MicroRNA Assays (Applied Biosystems, Carlsbad, CA, USA) were introduced in accordance with the manufacturer's instructions. All real time reactions were run in a StepOnePlus Real Time PCR System (Applied Biosystems). Relative expressions were calculated by the $2^{-\Delta C_t}$ method.

Cell proliferation assay

DU145 and PC3 cells with miR-205 mimics, *c-SRC*-siRNA or negative control (NC) that had been transfected for 48 h were seeded into 96-well plates (2000 cells per well) and cultured for 24, 48, 72 and 96 h. Each well received 20 μl of CCK-8 solution in addition to the 200 μl of culture media, and then the plate was incubated for 1.5 h at 37 °C. Absorbance at 450 nm was measured by a V_{max} microplate spectrophotometer (Molecular Devices).

Colony formation assay

Regarding the colony formation assay, cells were seeded into six-well plates at a low density (200 cells per plate) and cultured for 14 days. Then, the cells were fixed with 95% methanol and pigmented with 0.1% crystal violet. The number of colonies was counted.

Migration and invasion assays

Cell migration and invasion were assayed in 24-well plates using a chamber with a 6.5-mm diameter and an 8-μm pore size (BD Biosciences, Silicon Valley, CA, USA). Forty-eight hours post-transfection, DU145 and PC3 cells were added to the upper chamber, which were coated with 1 mg ml^{-1} matrigel for the invasion assays, and 0.7 ml of 20% FBS-DMEM was added to the lower chamber. After 48 h of incubation, the cells in the upper chamber were removed, while the migrated or invaded cells were fixed with 95% methanol, stained with 0.1% crystal violet, and photographed in five independent fields for each well.

Flow cytometry analysis of cell cycle and apoptosis

For the cell cycle analysis, 48 h after transfection, DU145 and PC3 cells were fixed with 75% cold ethanol at −20 °C for 24 h. Propidium iodide and RNase A (Sigma, San Francisco, CA, USA) were added to the cells. The samples were analysed 30 min after staining using the flow cytometry-BD FACSCalibur (BD Biosciences) and CellQuest software. For the apoptosis assays, the apoptosis rates were determined by Annexin V-FITC and PI (KeyGen, Nanjing, China) staining flow cytometry.

Luciferase reporter assay

Human *c-SRC* 3′-UTR was amplified and cloned into the XbaI site of the pGL3-control vector (Invitrogen), downstream of the luciferase gene, to generate the plasmids pGL3-WT-*c-SRC*-3′-UTR. pGL3-Mut-*c-SRC*-3′-UTR was generated from pGL3-WT-*c-SRC*-3′-UTR by changing two base pairs of the miR-205 seed-sequence. For the luciferase reporter assays, the cells were cotransfected with the wild-type or mutant reporter plasmid and the miR-205 or negative control. Luciferase activities were analysed by dual luciferase assays (Promega, Madison, WI, USA) 48 h after cotransfection and normalized against the activity of the Renilla luciferase gene.

Tumorigenicity assays in nude mice

BALB/C nu/nu male mice (4–6 weeks) were purchased from Shanghai SLAC Laboratory Animals. All experimental procedures involving animals followed the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the ethical committee of the First Affiliated Hospital of Nanjing Medical University. Aliquots of DU145 cells (1×10^6 cells in 50 μl) transfected with miR-205 or NC were mixed with 50 μl Matrigel (BD Biosciences) and injected subcutaneously into each side of the flanks of five male nude mice. These mice were then treated with 200 pmol miR-205 or NC mimics in 10 μl Lipofectamine 2000 through a local injection of the xenograft tumor every seven days. The tumor size was measured every 4 days with callipers. Twenty-eight days later, the tumours were removed, stained with antibody against Ki-67 (Abcam, Cambridge, UK), and microscopically visualized.

Western blot analysis

Proteins were separated by 10% SDS polyacrylamide gels and electrophoretically transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked overnight with 5% non-fat dried milk and incubated for 2 h with antibodies to c-SRC, ERK1/2, p-ERK1/2 (Cell Signaling Technology, Beverly, MA, USA), p-c-SRC, FAK, p-FAK, c-MYC, CYCLIND1 (Bioworld, Nanjing, China) and GAPDH (Bioworld). The signals were detected by enhanced chemiluminescence (Millipore) after incubation with goat anti-rabbit secondary antibody (Bioworld).

Statistical analysis

All values in the study were reported as the means±s.d. All of the experiments above were repeated three times. The relationship between down-regulated miR-205 expression and total PSA or free PSA in PCa was analysed using the Spearman rank correlation test. All other data were compared by two-sided *t*- tests. Statistical analysis was performed by SPSS v. 17.0 computer software. $P<0.05$ were considered statistically significant.

RESULTS

MiR-205 is significantly downregulated in PCa tissues

To confirm the expression of miRNAs in the PCa tissue samples, we performed microarray experiments using a mixed RNA extract from three advanced PCa tissues (clinical stage ⩾T3) and another mixed RNA extract from three early PCa tissues (clinical stage ⩽T2). We found that miR-205 was more significantly down-regulated in advanced PCa (**Figure 1a**). In addition, to validate the results of the microarrays, we examined the expression of miR-205 in 18 non-cancerous tissues, 15 T2 stage PCa tissues and 13 T3–T4 stage tissues by qRT-PCR. As expected, the results showed that miR-205 expression was significantly weakened in PCa tissues compared with non-cancerous

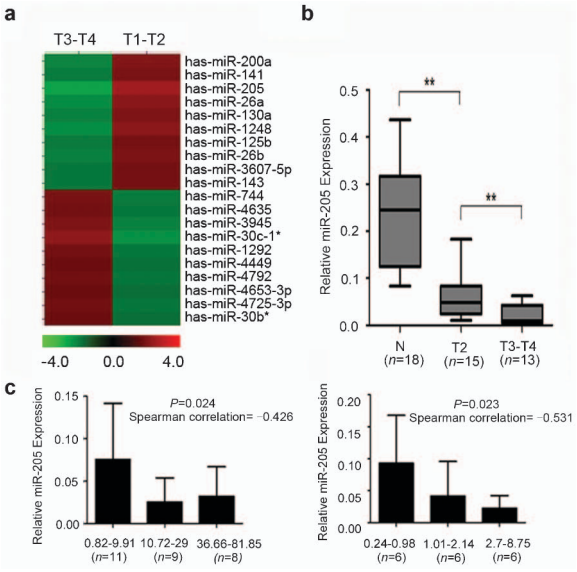

Figure 1 Validation of the selected miR-205 that was predicted to be down-regulated in advanced prostate cancer. (**a**) A heat map comparing the average fold-changes in microRNAs with markedly lower (green) or higher (red) expression in advanced PCa (clinical stage T3–T4) in comparison with early PCa (clinical stage T1–T2), as determined by microarray analysis. (**b**) The expression levels of miR-205 were analysed in 13 advanced prostate cancer tissues, 15 early prostate cancer tissues and 18 non-cancerous tissues by qRT-PCR. $^{**}P<0.01$. (**c**) Tumor samples were divided into three groups with approximately equal sample sizes based on the levels of total or free PSA. The level of total or free PSA in patients with primary tumours is presented on the *x* axis. The *y* axis indicates the total expression of miR-205. There was a statistically significant Spearman correlation that characterized an inverse relationship between miR-205 expression and total or free PSA (Spearman correlation=−0.426, $P=0.024$; Spearman correlation=−0.531, $P=0.023$). miR-205, microRNA-205; PCa, prostate cancer; PSA, prostate-specific antigen.

tissues ($P<0.01$, *t*-test; **Figure 1b**). The results also indicated that miR-205 expression possibly correlated with the clinicopathological stage of PCa (**Figure 1b**). Moreover, the results showed significant inverse correlations between the expression of miR-205 and total PSA levels (Spearman correlation=−0.426, $P=0.024$; **Figure 1c**), as well as a significant inverse correlation between the expression of miR-205 and free PSA levels (Spearman correlation=−0.531, $P=0.023$; **Figure 1c**). The clinical characteristics of the study's PCa population are listed in **Table 1**.

Table 1 Clinical characteristics of the study's prostate cancer population (n=28)

	Values
Age (year)	
Mean (range)	70 (56–78)
Clinical stage	
T2	15
T3	10
T4	3
Total PSA at diagnosis	
Median (range)	24.91 (0.82–81.85)
Free PSA at diagnosis	
Median (range), $n=18$	2.16 (0.24–8.75)

Abbreviations: PCa, prostate cancer; PSA, prostate-specific antigen.

MiR-205 suppresses tumorigenicity *in vitro*

The significant reduction of miR-205 expression in PCa tissues prompted us to explore the possible biological function of miR-205 in tumorigenesis. The results demonstrated that the proliferative capacity of PC3 and DU145 cells, when transfected with miR-205 mimics, significantly declined in comparison with cells transfected with NC ($P<0.05$, *t*-test; **Figure 2a**). As shown in **figure 2b**, compared with NC transfectants, miR-205-restored PC3 and DU145 cells also had lower colony formation abilities ($P<0.01$, *t*-test) . To further examine whether the decrease in the proliferation of PC3 and DU145 cells reflected cell-cycle arrest and the induction of apoptosis, cell cycle and apoptosis progression were analyzed by flow cytometry. The results revealed that miR-205-restored PC3 and DU145 cells had significant increases in the G_0/G_1 phase of their cell cycles compared with NC transfectants ($P<0.01$, *t*-test, **Figure 2c**). On the other hand, the percentage of total apoptotic cells significantly increased in response to miR-205 transfection, compared with NC, with a corresponding decrease close to 10% in the viable cell population ($P<0.01$, *t*-test; **Figure 2d**).

MiR-205 inhibited PCa cell migration and invasion

Transwell migration and invasion assays were used to investigate whether miR-205-restored PC3 and DU145 cells correlated with tumor metastasis. As shown in **figure 2e**, when the expression of miR-205 was upregulated by mimics-miR-205 in the PC3 and DU145 cell lines, the cells demonstrated low-metastasis potentiality compared with cells treated with NC ($P<0.05$, *t*-test). All of these results suggested a potential tumor suppressor role for miR-205 in PCa *in vitro*.

Knockdown of *c-SRC* mimicked miR-205 inhibition

To determine whether the downregulation of *c-SRC* is involved in miR-205-induced functional assays, we analysed the knockdown effect of *c-SRC* in PCa cells. We initially tested three siRNAs to knockdown the *c-SRC* gene and then confirmed the results at varying protein levels (**Figure 3a**). Then, we chose one siRNA (S-1) for additional experiments. As expected, compared with NC, *c-SRC*-knockdown DU145 and PC3 cells showed decreases in their proliferation, migration and invasion and increases in their G1-phase population and cell apoptosis (**Figure 3b–3e**), similar to the phenotype observed upon miR-205 restoration.

c-SRC was targeted by miR-205

To determine whether *c-SRC* was a bona fide target of miR-205, the human *c-SRC* 3′-UTR fragment containing wild-type or mutant miR-205-binding sequences was subcloned to the downstream Renilla luciferase reporter gene. The binding site was conserved in the region complementary to the seed sequence (**Figure 3a**). When miR-205 mimics were cotransfected with the reporter plasmids, the relative luciferase activity of the reporter containing wild-type *c-SRC* 3′-UTR was obviously suppressed, while the luciferase activity of the reporter containing mutant *c-SRC* 3′-UTR was unaltered (**Figure 4a**). These results indicated that *c-SRC* was a direct target of miR-205, with specific binding sites at the seed sequences. To further ascertain the relationship between miR-205 and c-SRC expression, Western blots were performed to examine the effect of the overexpression of miR-205 on the c-SRC protein levels in PCa cells. As expected, the overexpression of miR-205 suppressed c-SRC and p-c-SRC expression at the protein level in DU145 and PC3 cells (**Figure 4b**).

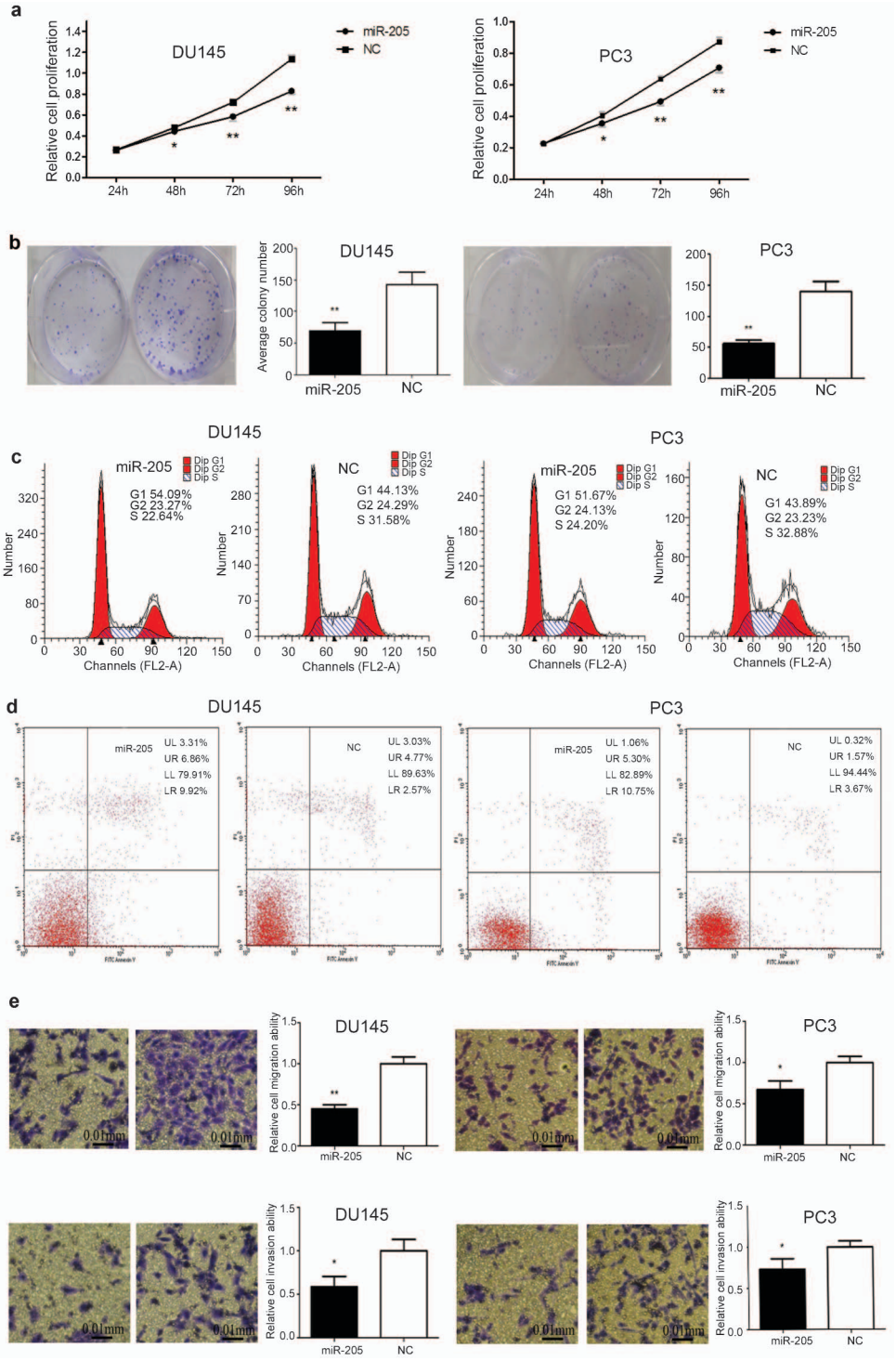

Figure 2 Effect of miR-205 on cell proliferation, colony formation, cell cycle, apoptosis, migration and invasion of DU145 and PC3 cells *in vitro*. (**a**) miR-205 overexpression significantly inhibited the proliferation of DU145 and PC3 cells. (**b**) Colony formation of DU145 and PC3 cells after miR-205 transfection was significantly reduced compared with NC. (**c**) The forced expression of miR-205 led to a significant increase in the G_0/G_1 phase of the cell cycle compared with NC. (**d**) The forced expression of miR-205 increased the sensitivity of DU145 and PC3 cells to apoptosis. (**e**) The forced expression of miR-205 suppressed the migratory capacity and invasive capacity of DU145 and PC3 cells. **P*<0.05, ***P*<0.01. Scale bar=0.01 mm. LL, normal cells; LR, late apoptotic cells; miR-205, microrna-205; NC, negative control; UL, dead cells; UR, early apoptotic cells.

miR-205 inhibits the FAK/ERK1/2 pathway

To investigate the role of miR-205 in the regulation of cellular signaling, we performed Western blotting for the downstream genes of miR-205 in the DU145 and PC3 cells that were either miR-205- or NC-transfected. We found that transient transfection with miR-205 down-regulated FAK, p-FAK, ERK1/2 and p-ERK1/2 in the form of proteins (**Figure 4b**). These data indicated that miR-205 targets *c-SRC*, resulting in suppression of the

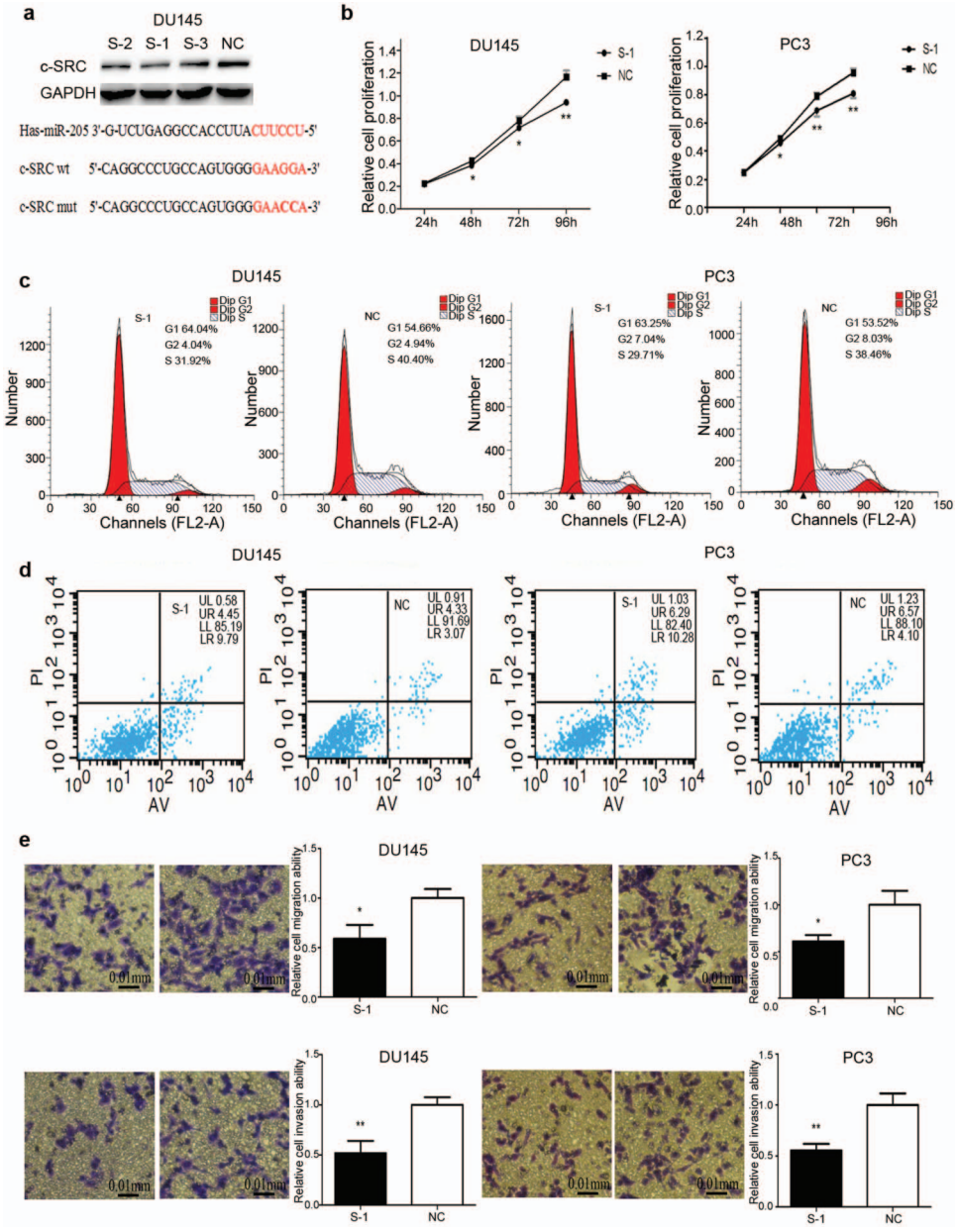

Figure 3 Knockdown of *c-SRC* mimicked miR-205 inhibition in functional tests. (**a**) c-SRC protein levels were assessed by Western blot in DU145 cells transfected with *c-SRC*-siRNA (S-1, S-2 and S-3) and NC. The *c-SRC* 3′-UTR was cloned into the pGL3-control vector (WT), and the mutant construct was obtained by site-directed mutagenesis, which changed two base pairs of the miR-205 seed-sequence (Mut) and inserted them into the vector. (**b**) *c-SRC*-knockdown DU145 and PC3 cells showed decreased proliferation compared with NC. (**c**) Knockdown of *c-SRC* led to a significant increase in the G_0/G_1 phase of the cell cycle compared with NC. (**d**) Knockdown of *c-SRC* increased the sensitivity of DU145 and PC3 cells to apoptosis. (**e**) Knockdown of *c-SRC* suppressed the migratory and invasive capacities of DU145 and PC3 cells. **P*<0.05, ***P*<0.01. Scale bar=0.01 mm. miR-205, microrna-205; NC, negative control.

FAK/ERK1/2 pathway and the genes involved in proliferation. We also found that transient transfection with miR-205 decreased the expression of the cycle-related proteins, c-MYC and CYCLIND1 (**Figure 4b**).

MiR-205 suppresses tumor growth *in vivo*

As expected, the miR-205-restored DU145 cells exhibited a delay in palpable tumor onset and a marked degeneration in tumor growth when compared with NC ($P<0.05$, *t*-test; **Figure 4c**). In addition, miR-205 reduced Ki-67 staining in tumor xenografts ($P<0.01$, *t*-test; **Figure 4d**), suggesting that miR-205 reduced tumorigenicity in a nude mouse model.

DISCUSSION

miRNAs are a class of small RNAs that regulate various physiological and pathological mechanisms.[18] Although upregulated in ovarian, bladder and breast carcinomas,[19–21] miR-205 has been reported to be downregulated in oesophageal cancers.[22] Thus, miR-205 might act as a doubled-edged sword by targeting opposite functional genes, as one miRNA can target a dozen mRNAs, impacting many molecules

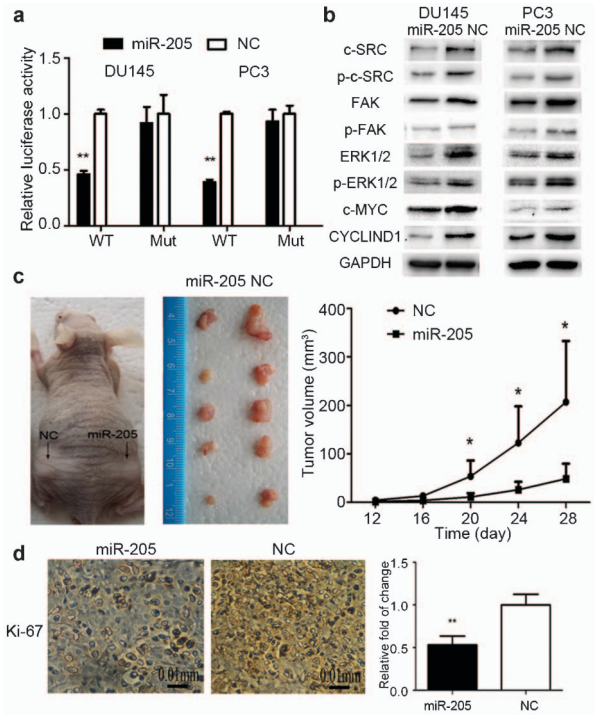

Figure 4 *c-SRC* is a potential target of miR-205. (**a**) The relative luciferase activities of *c-SRC* wild type (WT) and mutant (Mut) 3′-UTR regions were obtained by the cotransfection of miR-205 or NC and the pRL-TK plasmid. The ratio of the firefly/renilla activities was calculated in the DU145 and PC3 cells and normalized to that of the controls. The results were presented as the means±s.d. of each experiment, in triplicate. $^{**}P<0.01$. (**b**) Overexpression of miR-205 suppressed c-SRC and p-c-SRC, as well as the expression of its downstream signaling molecules, FAK, p-FAK, ERK1/2 and p-ERK1/2, at the protein level in DU145 and PC3 cells. Overexpression of miR-205 also suppressed the cycle-related proteins c-MYC and CYCLIND1. (**c**) The tumor volume of the miR-205 group was decreased compared with the NC group. (**d**) miR-205 reduced Ki-67 immunostaining in tumours. $^{*}P<0.05$, $^{**}P<0.01$. Scale bars=0.01 mm. miR-205, microRNA-205; NC, negative control.

in different signal pathways. Here, we used qRT-PCR to profile the expression of miR-205 in PCa and non-cancerous tissues. Consistent with previous studies,[16,17] we demonstrated that the expression of miR-205 in PCa tissues was down-regulated when compared with non-cancerous tissues (**Figure 1b**). Furthermore, miR-205 was shown to be downregulated in advanced-stage PCa in our studies (**Figure 1a and 1b**) and to be down-regulated in metastatic PCa.[23] Chung *et al.*[24] reported that the aberrant expression of miR-205 took place in advanced stages in endometrial cancer. In addition, previous studies have shown that the level of miR-205 was inversely correlated with PSA and tumor stage.[25,26] In our study, the results showed that miR-205 expression was also related to the stage of the disease (**Figure 1b**). We further found that the expression level of miR-205 was inversely correlated with the total/free PSA levels (**Figure 1c**). These results indicated that, as a tumor suppressor gene, miR-205 may be used not only for an early diagnosis of PCa but also as a marker of tumor progression.

Deregulated cell proliferation is a key mechanism in neoplastic progression.[27] Our cell proliferation assay showed that miR-205 overexpression in PCa cells markedly attenuated cell proliferation (**Figure 2a**). In the present study, we found that PCa cells transfected with miR-205 mimics had obvious cell cycle arrest at the G_0/G_1 phase, coupled with a decrease in cellular apoptosis, as shown by the flow cytometry analysis (**Figure 2c and 2d**). However, whether miR-205 could affect the proliferation of PCa cells remains controversial. Gandellini *et al.*[17] reported that the aberrant expression of miR-205 did not induce appreciable perturbations of cell proliferation or cell cycle progression. However, a previous report described that miR-205 impaired tumor cell growth by the induction of apoptosis and cell cycle arrest. We used nude mouse xenograft models to confirm that miR-205 could suppress the tumorigenicity of PCa cells. Our results clearly demonstrated that miR-205 overexpression in DU145 cells delayed xenograft tumor growth, highlighting the relevance of miR-205 as a putative therapeutic agent for the treatment of this disease (**Figure 4c**). Furthermore, to confirm the indications that miR-205 may reduce tumor xenograft growth by decreasing proliferation, we evaluated proliferation in tumor xenograft tissue sections by Ki-67 immunohistochemistry. As expected, miR-205 overexpression markedly decreased the expression of Ki-67, which was consistent with the marked reduction in tumor growth (**Figure 4d**). To our knowledge, this is the first demonstration that miR-205 overexpression decreases proliferation in human PCa cells xenografted into mice.

The loss of miR-205 is associated with enhanced metastatic potential in both model tumor systems and in human cancers, particularly of the breast.[13,15] miR-205 may halt PCa progression through the downregulation of protein kinase C epsilon.[17] Regarding the human PCa cell lines DU145 and PC3, which have a high metastatic ability and low expression of miR-205,[17] the upregulation of the expression of miR-205 permitted these PCa cell lines to gain low metastatic potentiality (**Figure 2e**). Further, we concluded that the siRNA knockdown of *c-SRC* inhibited cell proliferation, impairing the cells' invasion and migration abilities and inducing G_0/G_1 arrest and late apoptosis in PCa cell lines (**Figure 3b–3e**). All of these results suggested that miR-205 may function as a tumor suppressor, partially through the down-regulation of c-SRC expression.

The *SRC* family kinases (*SFKs*) are non-receptor tyrosine kinases and prototypical modular signaling proteins.[28–30] The *SFKs* play key roles in cell proliferation, invasion, migration, apoptosis, survival, adhesion, morphology and motility.[29,31,32] Among the *SFKs*, *c-SRC* is arguably the best characterized and most often implicated *SFK* in cancer. Advanced stages of cancer progression have been associated with high *c-SRC* expression.[33] Increased *c-SRC* activity takes place in bladder carcinoma,[34] head and neck,[35] colon[36] and breast[37] cancers. Majid *et al.*[38] reported that the *SRC* family members were direct targets of miR-205 in renal cancer. In PCa, we also confirmed that *c-SRC* was a target of miR-205, with the forced expression of miR-205 suppressing c-SRC and p-c-SRC protein expression (**Figure 4b**). Moreover, the *c-SRC* 3′-UTR reporter experiment showed a marked decline in luciferase activity after miR-205 over-expression (**Figure 4a**).

The remarkable influence of miRNA on the regulation of cell function may depend on the essential target genes involved in different signal pathways. In this model, miR-205 targeted *c-SRC* and its downstream signal pathway, which play a major role in the regulation of tumor cell growth. It has been reported that *c-SRC* is involved in multiple signaling pathways, including *RAS/RAF/ERK*1/2, *PI3K/AKT/HIF*-1α, *STAT*3/*c-MYC/CYCLIND*1, *B-CATENIN/c-MYC/CYCLIND*1, *FAK/p*130*CAS/MMP*9 and *RAC/NADPH*, which induce the growth, survival and migration of various types of cancer cells.[39–41] The ability to target *c-SRC* transcripts signifies that miR-205 might be a potential regulator of the *FAK/ERK*1/2 pathways in PCa cells. Western blot analysis showed that the expression levels of the members of the FAK/ERK1/2 pathway were reduced through targeting with c-SRC after miR-205 overexpression (**Figure 4b**). Moreover, our results showed

that miR-205 can inhibit the constitutive activity of p-FAK, p-ERK1/2, c-MYC and CYCLIND1 in PCa cells with suppressed p-c-SRC expression (**Figure 4b**).

We concluded that there was markedly low expression of miR-205 in PCa tissues compared with non-cancerous tissues. Moreover, the expression of miR-205 was found to be associated with the clinicopathological stage and level of the total PSA/free PSA in PCa patients. Our results clearly confirmed that miR-205 suppressed the proliferation and metastasis of the PCa cells by targeting *c-SRC* through the *FAK/ERK*1/2 pathway. As no effective approaches for curing advanced PCa exist at present, we propose that gene therapy targeting miR-205/*c-SRC* could serve as a potential therapeutic strategy for PCa.

AUTHOR CONTRIBUTIONS

All authors discussed the results and implications and commented on the manuscript at all stages. LXH, CJY and CQ conceived and designed the study. NW, NHF and GC gathered and analyzed the data. NW, QL, ZLG and YW performed the experiment. NW wrote and revised the paper, which was read and approved by all authors.

COMPETING FINANCIAL INTERESTS

All authors declare that there are no competing financial interests.

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