Int J Clin Exp Pathol 2020;13(3):587-596
www.ijcep.com /ISSN:1936-2625/IJCEP0104609

Original Article

MiR-22 suppresses the growth and metastasis of bladder cancer cells by targeting E2F3

Junsheng Guo, Jian Zhang, Tianxiao Yang, Wei Zhang, Mingyang Liu

Department of Urology, Affiliated Hospital of Chifeng University, Chifeng, Inner Mongolia, China

Received August 2, 2019; Accepted January 23, 2020; Epub March 1, 2020; Published March 15, 2020

Abstract: Bladder cancer is a common, serious disease worldwide. MicroRNAs (miRNAs) have been reported to participate in the development and progression in many cancers, including bladder cancer. However, the exact roles of miR-22 in bladder cancer process and its underlying mechanism remain largely unknown. The expression levels of miR-22 and E2F3 were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). Western blot was used to detect the protein levels of E2F3, E-cadherin, N-cadherin, and Vimentin in bladder cancer cells. Cell viability, proliferation, migration, and invasion were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay, colony formation assay, and transwell assay, respectively. The potential binding sites between miR-22 and E2F3 were predicted by TargetScan and verified by luciferase report assay. The expression of miR-22 was downregulated and E2F3 expression was upregulated in bladder cancer tissues and cells. Overexpression of miR-22 or E2F3 knockdown inhibited cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) in bladder cancer cells. In addition, E2F3 was a direct target of miR-22 and its knockdown attenuated the promotion of cell proliferation, migration, invasion, and EMT induced by miR-22 inhibitor in bladder cancer cells. In conclusion, miR-22 suppressed cell proliferation, migration, invasion, and EMT in bladder cancer cells by regulating E2F3 expression, providing a novel avenue for treatment of bladder cancer.

Keywords: Bladder cancer, miR-22, E2F3, metastasis, proliferation

Introduction

Bladder cancer, one of the urinary malignancies, is the second most frequent cause of death in patients with genitourinary tract malignancies around the word [12, 24]. Although obvious progress in accurate and effective diagnosis as well as treatment. However, bladder cancer continues to be a frequent cancer with high mortality; the 5-year survival rate remains only 50%-60% [4, 20, 25, 26]. Hence, it is imperative to better understand the molecular basis of pathogenesis and develop effective strategies for treatment of bladder cancer.

MicroRNAs (miRNAs), a class of small (~22 nucleotides) non-coding RNAs, modulate gene expression through binding to the complementary sequences in 3'untranslated regions (UTR) of the target genes [1]. Previous documents have shown that a number of miRNAs are abnormally expressed in bladder cancer and involved in its development and progression, indicating that miRNAs may play crucial roles in its diagnosis and therapy. For example, Bo *et al.* revealed that miR-203 inhibited bladder cancer development via suppressing bcl-w expression [2]. Majid *et al.* proved that miR-23b has been indicated as a tumor suppressor through regulation of Zeb1 in bladder cancer [19]. As for miR-22-3p (miR-22), originally cloned from HeLa cells, is located at chromosome 17p13 [13]. MiR-22 has been confirmed to act as a tumor suppressor or promoter in different cancers [3, 29]. Besides, Xu *et al.* pointed out that miR-22 could limit epithelial-mesenchymal transition (EMT) though repressing Snail and MAPK1/Slug/vimentin feedback loop in bladder cancer [29]. In spite of these findings, the biological function of miR-22 in the development and progression of bladder cancer remains largely unknown.

The miRNAs have been reported to execute their biological function by regulating their downstream target genes [21]. E2F3, located

at the 6p22 bladder amplicon, is a crucial regulator of cell cycle regulation and cell proliferation [22]. E2F3 has been confirmed to be an oncogene and play critical roles in bladder tumorigenesis [10]. However, whether E2F3 is a potential target and the exact functions of E2F3 in progression of bladder cancer are need to be fully investigated.

In this study, the abundances of miR-22 and E2F3 were explored in bladder cancer tissues and cells. Moreover, the biological functions of miR-22 and E2F3 in cell proliferation and metastasis were also investigated. Besides, interaction between miR-22 and E2F3 was first explored in bladder cancer, which might provide new strategies for development of useful therapies against bladder cancer.

Materials and methods

Clinical specimens

In this study, a total of 32 patients with bladder cancer without chemotherapy, radiotherapy, or other therapy were enrolled from Affiliated Hospital of Chifeng University. The collected bladder cancer tissues and adjacent normal tissues should be immediately frozen in liquid nitrogen and then stored at -80°C until RNA or protein extraction. All subjects were informed and signed informed consent and this study protocol was approved by the Research Ethics Committee of Affiliated Hospital of Chifeng University.

Cell culture and transfection

Human bladder cancer cell lines (5637 and T24) were obtained from American Tissue Culture Collection (ATCC, Manassas, VA, USA) and human normal uroepithelial cell line (SVHUC-1) was obtained from the Chinese Academy of Sciences (Shanghai, China). These cells were maintained in RPMI-1640 (Hyclone, Logan, Utah, USA) with 10% FBS (Gibco, Carlsbad, CA, USA) at 37°C with 5% CO_2 in a moist atmosphere.

MiR-22 mimics (miR-22), miR-control, small interfering RNA (siRNA) targeting E2F3 (si-E2F3), si-control miR-22 inhibitor (anti-miR-22), and anti-control were bought from GenePharma (Jiangsu, China). 5637 and T24 cells were transfected with them using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After transfection for 48 h, 5637 and T24 cells were collected for further tests.

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Cells and tissues were lysed using Trizol (Invitrogen) to obtain total RNA. Complementary DNA (cDNA) was synthesized from total RNA with a PrimeScript RT Reagent Kit (TaKaRa, Osaka, Japan). MiR-22 was converted to cDNA with a TaqMan microRNA reverse transcription kit (Applied Biosystems, Foster City, CA). Subsequently, the cDNA was used for qRT-PCR and the experiment was carried out using SYBR green detection kit (Toyobo, Tokyo, Japan) and a 7900HT Fast Real-Time PCR System (Applied Biosystems). The primers were listed as follows: miR-22 forward, 5'-GGGGGATCCCTGGGGCAGGACCCT-3' and reverse, 5'-GGGGAATTCAACGTATCATCCACCC-3'; E2F3 forward, 5'-CACTTCCACCACCTCCTGTT-3' and reverse, 5'-TGACCGCTTTCTCCTAGCTC-3'; U6 forward, 5'-AGAGAAGATTAGCATGGCCCCTG-3' and reverse, 5'-ATCCAGTGCAGGGTCCGAGG-3'; GAPDH forward, 5'-CTGGGCTACACTGAGCACC-3' and reverse, 5'-AGTGGTCGTTGAGGGCAATG-3'. U6 snRNA and GAPDH were used as normalizing controls for miR-22 and E2F3 quantification, respectively. The $2^{-\Delta\Delta Ct}$ method was employed to evaluate the expression levels.

Western blot assay

Transfected cells were lysed using RIPA lysis buffer (Thermo Fisher, Wilmington, DE, USA) with protease inhibitors (Beyotime, Shanghai, China) to extract the total protein. After quantification by using bicinchoninic acid (BCA) protein assay kit (Sigma-Aldrich, St. Louis, MO, USA), protein samples (about 30 μg) were separated by SDS-PAGE and transferred onto the polyvinylidene fluoride (PVDF; Millipore, Billerica, MA, USA) membranes. Then, 5% nonfat milk (Sangon Biotech, Shanghai, China) was used to block blots. These blots were immunoblotted for 12 h at 4°C by primary antibodies against E2F3 (1:1000, ab50917, Abcam, Cambridge, UK), E-cadherin (1:500, ab15148, Abcam), N-cadherin (1:500, ab18203, Abcam), Vimentin (1:1000, ab1373218, Abcam), and β-actin (1:2000, ab8227, Abcam). Following

washing with TBST, blots were incubated by secondary antibodies (Sangon Biotech). Immunoreactive bands were visualized with the enhanced chemiluminescence (ECL) system (Tanon, Shanghai, China). Protein expression levels were evaluated using Image J software and normalized to the level of β-actin.

Cell viability assay

5637 and T24 cells were placed in 96-well plates and transfected with miR-control, miR-22, si-control, si-E2F3, anti-control, anti-miR-22, anti-miR-22+si-control or anti-miR-22+si-E2F3. After transfection for 48 h, 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) reagent (5 mg/mL, Sigma-Aldrich) was put in the test wells and further incubated for 4 h. Next, the culture medium was gently removed and then dimethyl sulfoxide (DMSO; 150 μL, Sigma-Aldrich) was added to all wells. The absorbance of each well was read at a wavelength of 490 nm with a microplate reader (Bio-TeK Instrument, Winooski, VT, USA).

Colony formation assay

At 48 h post transfection, 5637 and T24 cells were placed in a six-well plates and the medium was updated every 3 d. 2 weeks later, 5637 and T24 cells were carefully washed with cold PBS and fixed with paraformaldehyde (4%, 1 mL) for 30 min at 4°C. Subsequently, 5637 and T24 cells were washed 3 times with PBS, stained with 0.1% crystal violet for 1 h (Sigma-Aldrich), again washed with PBS until per well was clean. If a colony exceeded 50 cells, the colony was counted.

Transwell assay

The migration and invasion capabilities of 5637 and T24 cells were examined by transwell chambers (8 μm, Millipore, Boston, MA, USA). After transfection, suspension of 5637 and T24 cells was placed in the upper chamber with uncoated membrane (migration assay) or the membrane pre-coated with Matrigel (invasion assay) (BD Bioscience, Franklin Lakes, NJ, USA) and cultured in RPMI-1640 medium. In the lower chamber, RPMI-1640 containing 10% FBS would be added as a chemical attractant. The cotton swab was used to clean the nonfiltered cells on the upper chamber, whereas 4% paraformaldehyde was used to fix the cells on the lower surface, followed by staining with 0.1% crystal violet (Sigma-Aldrich) after incubation for 24 h. Each treatment group would be randomly selected for five visual fields and counted by a microscope (Olympus Corp., Tokyo, Japan).

Dual-luciferase reporter assay

The human 3'UTR of E2F3 containing the wild-type or mutant binding sites of miR-22 were synthesized and inserted into pGL3-Basic vector (Promega, Madison, WI, USA) to generate wild-type vectors (WT-E2F3) or mutant-type vectors (MUT-E2F3). 5637 and T24 cells were co-transfected WT-E2F3 or MUT-E2F3 vectors and miR-22 or miR-control for 48 h using Lipofectamine 3000. Dual-Luciferase Reporter Assay System (Promega) was applied to evaluate luciferase activity.

Statistical analysis

All data in this research were expressed in terms of mean ± standard deviation (SD) from at least three independent experiments. Student's *t*-test was chosen for calculation of differences between two groups. GraphPad Prism software version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) was chosen for data analysis. Spearman's correlation tests were performed to analyze the association between miR-22 and E2F3. Statistical significance was accepted at $P<0.05$.

Results

The expression of miR-22 was decreased while E2F3 expression was increased in bladder cancer tissues and cells

To explore the functions of miR-22 and E2F3 in bladder cancer progression, 32 pairs of bladder cancer tissues and adjacent non-tumor tissues were measured by qRT-PCR. Our results indicated that the miR-22 level was greatly downregulated and mRNA expression of E2F3 was upregulated in bladder cancer tissues compared to non-tumor tissues (**Figure 1A** and **1B**). Besides, the relationship between miR-22 and E2F3 was analyzed in bladder cancer tissues. We found that miR-22 abundance was negatively associated with E2F3 mRNA level in bladder cancer tissues (**Figure 1C**). We further

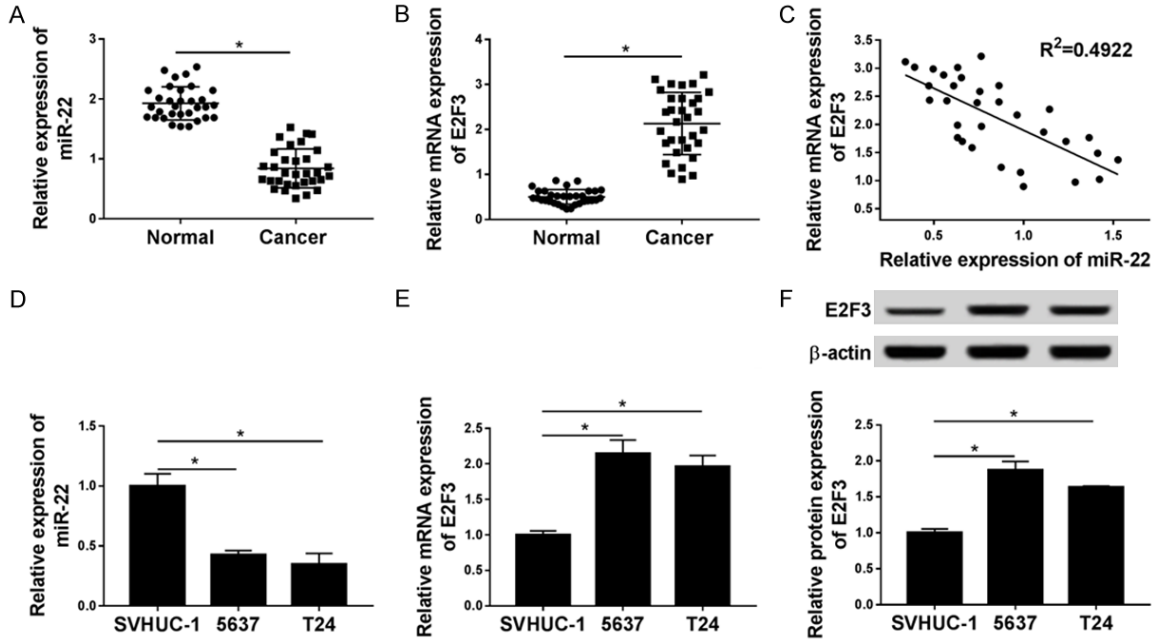

Figure 1. Relative expression levels of miR-22 and E2F3 in bladder cancer tissues and cells. A and B. The levels of miR-22 and E2F3 were measured in bladder cancer tissues and adjacent normal samples by qRT-PCR. C. The correlation between miR-22 level and E2F3 mRNA expression was analyzed in bladder cancer tissues. D and E. The expression levels of miR-22 and E2F3 were measured by qRT-PCR. F. Western blot was used to determine the protein level of E2F3. *P<0.05.

Table 1. Relationship between miR-22 expression and clinicopathological features of bladder cancer patients

Clinicopathological features	n	miR-22		P
		High	Low	
Gender				
Female	10	5	5	0.811
Male	22	12	10	
Age (years)				
≥45	19	11	8	0.417
<45	15	6	7	
Tumor size (cm)				
≥3	14	8	6	0.688
<3	18	9	9	
Distant metastasis				
Yes	19	7	12	*0.026**
No	13	10	3	

*p<0.05.

proved that the expression level of miR-22 was obviously increased and E2F3 mRNA level as well as protein expression were decreased in 5637 and T24 cells compared with SVHUC-1 cells (**Figure 1D-F**). These data suggested that miR-22 was negatively correlated with E2F3 in bladder cancer.

The association between miR-22 expression and the clinicopathological features of patients with bladder cancer

The relationship between the relative expression of miR-22 and the clinicopathological features of the bladder cancer patients was analyzed. As shown in **Table 1**, low expression level of miR-134 was significantly associated with distant metastasis, but not with gender, age, or tumor size.

Overexpression of miR-22 suppressed progression of bladder cancer cells

To study the role of miR-22 in bladder cancer progression, 5637 and T24 cells were transfected with miR-22 or miR-control. QRT-PCR was performed to confirm the transfection efficiency. As shown in **Figure 2A**, transfection of miR-22 mimics notably increased the expression of miR-22. MTT assay revealed that the cell viability was decreased in 5637 and T24 cells transfected with miR-22 compared to those cells transfected with miR-control (**Figure 2B**). Colony formation assay was used to evaluate the role of miR-22 in cell growth. The colony-formation ability of miR-22-transfected

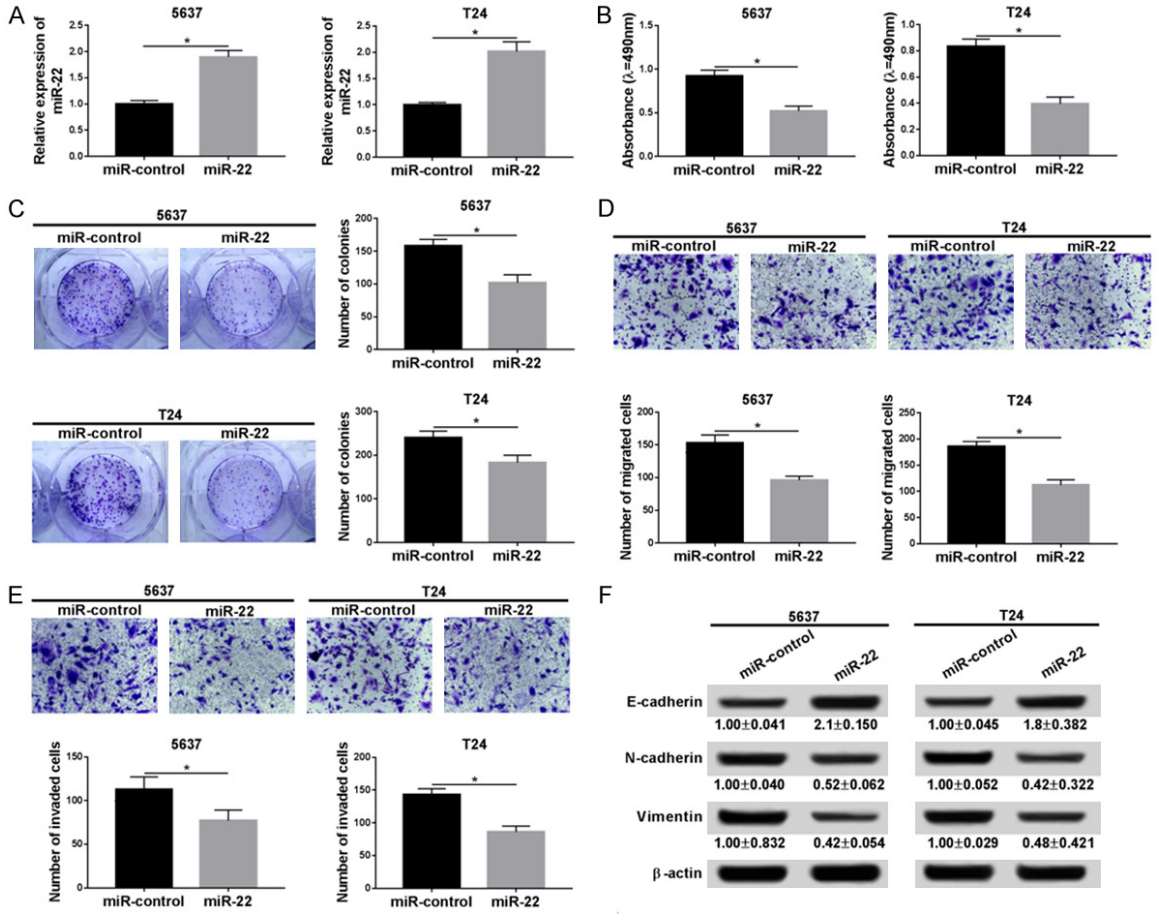

Figure 2. Effects of miR-22 on cell proliferation, apoptosis, migration, invasion, EMT in bladder cancer cells. 5637 and T24 cells were transfected with miR-22 or miR-control. A. QRT-PCR was performed to detect the expression of miR-22. B. Cell viability was analyzed by MTT assay. C. Colony formation assay was used to determine colony-forming ability of 5637 and T24 cells. D and E. The migration and invasion of 5637 and T24 cells were assessed using transwell assay. F. The protein levels of E-cadherin, N-cadherin, and Vimentin were measured by western blot. $^{*}P<0.05$.

5637 and T24 cells was much lower than the cells transfected with miR-control (**Figure 2C**). The transwell assay indicated that overexpression of miR-22 effectively inhibited the migration and invasion capacities of both 5637 and T24 cells (**Figure 2D** and **2E**). To test the effect of miR-22 on EMT progression in bladder cancer cells, the protein levels of EMT markers were determined using western blotting assay. Results showed that addition of miR-22 remarkably elevated the protein level of E-cadherin (an epithelial marker) but reduced the expression of N-cadherin and vimentin (mesenchymal markers) (**Figure 2F**). In conclusion, these results indicated that miR-22 inhibited cell growth, cell motility, and EMT of bladder cancer cells.

Knockdown of E2F3 inhibited progression of bladder cancer cells

In the attempt to understand the biologic function of E2F3 in bladder cancer progression, 5637 and T24 cells were transfected with si-E2F3 or si-control. QRT-PCR and western blot analysis demonstrated that transfection of si-E2F3 resulted in an obvious reduction of E2F3 mRNA and protein expression in 5637 and T24 cells compare with transfection of si-control, suggesting the successful introduction of si-E2F3 into the cells (**Figure 3A** and **3B**). MTT assay proved that inhibition of E2F3 conspicuously limited cell viability of 5637 and T24 cells (**Figure 3C**). The colony-formation experiment disclosed that, compared to the si-control

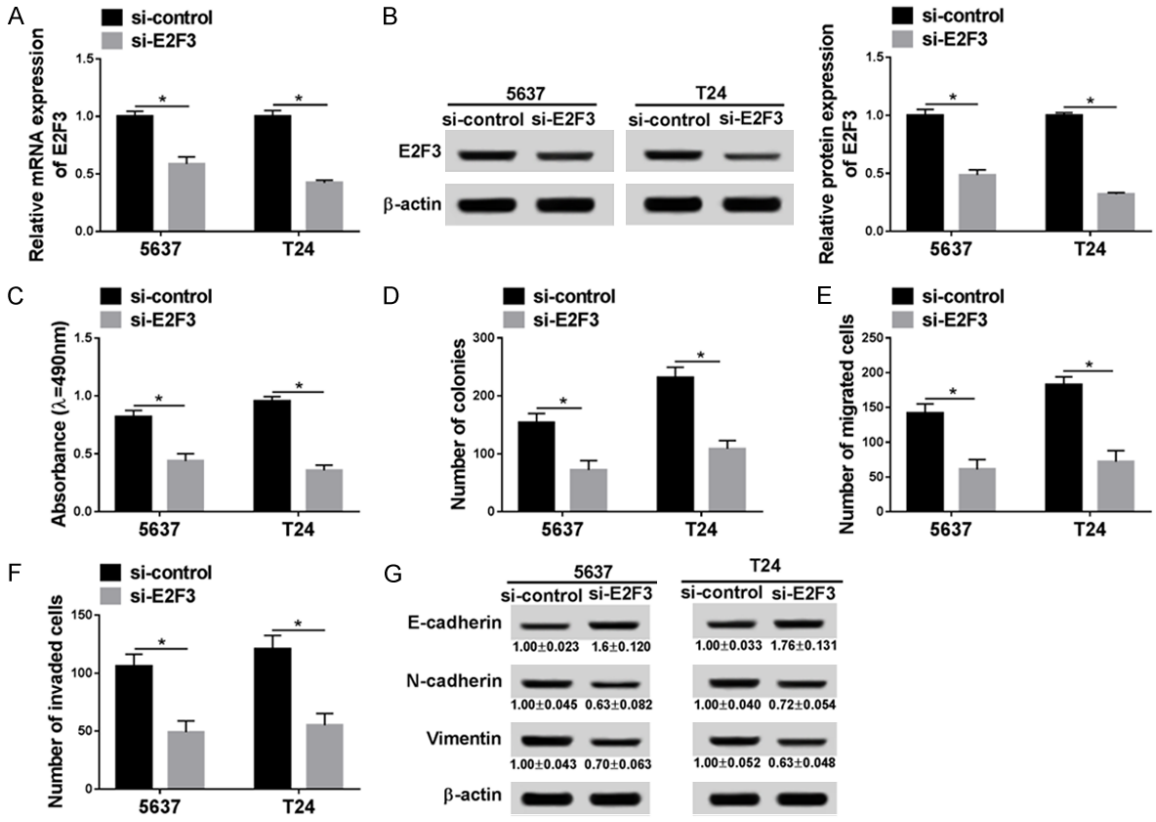

Figure 3. Effects of E2F3 on cell proliferation, apoptosis, migration, invasion, EMT in bladder cancer cells. 5637 and T24 cells were transfected with si-E2F3 or si-control. A and B. The mRNA expression and protein level of E2F3 were detected by qRT-PCR and western blot, respectively. C. Cell viability was examined by MTT assay. D. Cell growth capacity was evaluated using the colony formation assay. E and F. The migration and invasion abilities of 5637 and T24 cells were determined using transwell assay. G. Western blot was conducted to measure the abundances of E-cadherin, N-cadherin, and Vimentin. $^{*}P<0.05$.

group, E2F3 knockdown prominently repressed the colony-forming ability of 5637 and T24 cells (**Figure 3D**). In addition, abrogation of E2F3 greatly decreased the number of migrated and invaded 5637 and T24 cells (**Figure 3E** and **3F**). Moreover, silencing E2F3 increased production of mesenchymal marker N-cadherin and vimentin, but reduced E-cadherin protein level in 5637 and T24 cells (**Figure 3G**). These findings displayed that E2F3 knockdown inhibited the proliferation, migration and invasion, and EMT of bladder cancer cells.

E2F3 was a direct target of miR-22

Since miR-22 was negatively correlated with E2F3 in bladder cancer, we further explore the interaction between miR-22 and E2F3 in bladder cancer cells. TargetScan predicted that the 3'-UTR of E2F3 mRNA contained a complementary site for the seed region of miR-22 (**Figure 4A**). Dual-luciferase reporter assay was then used to examine the interaction between miR-22 and the 3'-UTR of E2F3. Results showed that the relative luciferase activity of the reporter containing wild-type 3'-UTR of E2F3 was strikingly decreased when miR-22 was co-transfected, while the luciferase activity of the reporter containing the mutant miR-22-binding sites was unaffected in 5637 and T24 cells, suggesting that miR-22 might inhibit E2F3 expression by miR-22-binding sequences at the 3'-UTR of E2F3 (**Figure 4B**). Knockdown of miR-22 remarkably downregulated the expression of miR-22 in 5637 and T24 cells (**Figure 4C**). Next, the effect of miR-22 on E2F3 expression was investigated in 5637 and T24 cells. Overexpressed miR-22 drastically reduced mRNA and protein levels of E2F3 and miR-22 knockdown resulted in an opposite effect in 5637 and T24 cells (**Figure 4D** and **4E**). Taken together, our results demonstrated that E2F3 was a downstream target of miR-22.

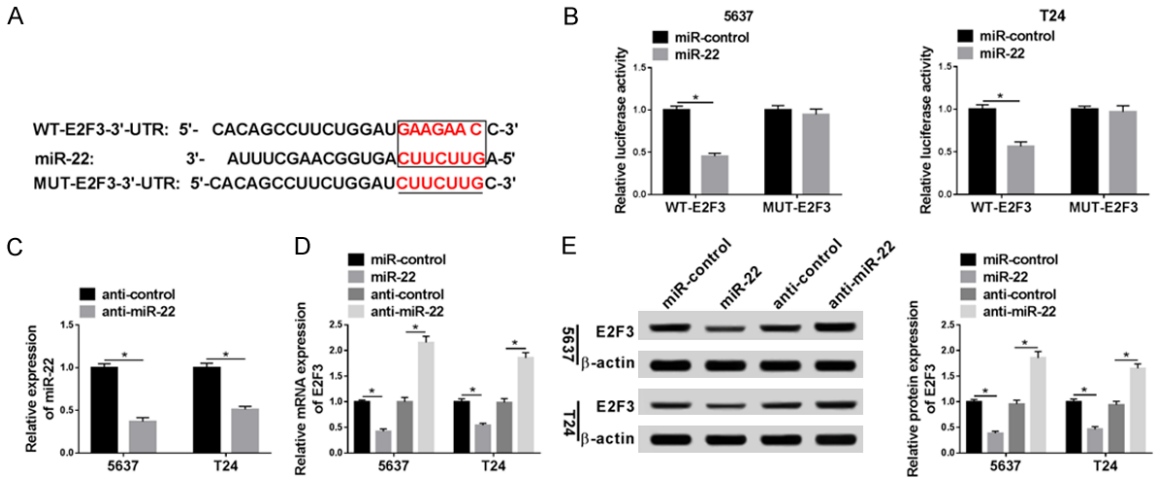

Figure 4. MiR-22 directly targeted E2F3. A. The predicted binding sites of miR-22 in 3'-UTR of E2F3 were predicted by TargetScan. B. Luciferase activity was measured in 5637 and T24 cells co-transfected with E2F3-WT or E2F3-MUT and miR-22 or miR-control. C and D. The expression levels of miR-22 and E2F3 were evaluated by qRT-PCR. E. The protein level of E2F3 was detected and western blot. $^{*}P<0.05$.

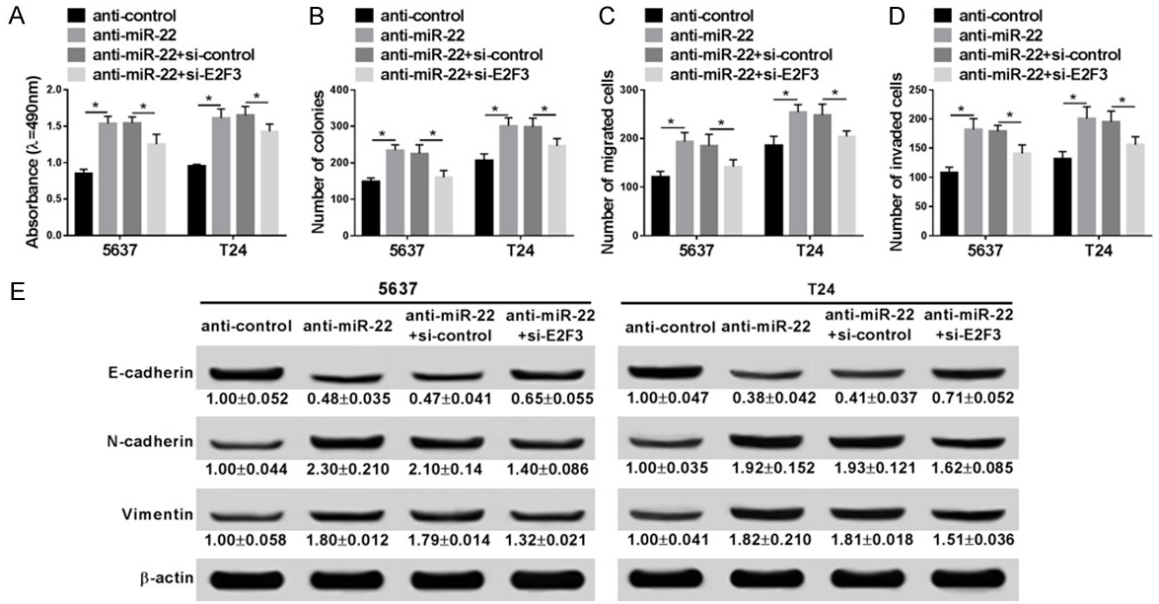

Figure 5. E2F3 knockdown attenuated the anti-miR-22-mediated promotion in bladder cancer progression. 5637 and T24 cells were transfected with anti-control, anti-miR-22, anti-miR-22+si-control, or anti-miR-22+si-E2F3. A. MTT assay was used to examine cell viability. B. Cell growth capacity was evaluated using the colony formation assay. C and D. Transwell assay was performed to determine migration and invasion of 5637 and T24 cells. E. The protein levels of E-cadherin, N-cadherin, and Vimentin were measured by western blot. $^{*}P<0.05$.

Knockdown of E2F3 reversed miR-22 inhibition-mediated progression of bladder cancer

To assess whether E2F3 could regulate the biological function of miR-22 in bladder cancer, 5637 and T24 cells were transfected with anti-control, anti-miR-22, anti-miR-22+si-control or anti-miR-22+si-E2F3. MTT analysis displayed that abrogation of miR-22 apparently increased cell viability and number of colonies, while the effect was abated by inhibition of E2F3 in 5637 and T24 cells (**Figure 5A** and **5B**). Moreover, interference of E2F3 attenuated the promotion of migration and invasion caused by silencing

miR-22 in 5637 and T24 cells (**Figure 5C** and **5D**). Further, absence of miR-miR-22 led to an obvious reduction of E-cadherin protein level but promotion of N-cadherin and vimentin expression, whereas it was abolished by inhibition of E2F3 in 5637 and T24 cells (**Figure 5E**). Collectively, these data indicated that E2F3 was an important mediator of miR-22 in bladder cancer cell progression.

Discussion

Bladder cancer is considered to be a very common and fatal malignancy. Recently, deregulation of miRNAs expression has been confirmed in various cancers [5]. Nevertheless, the molecule mechanisms of miRNAs in bladder tumorigenesis remain relatively unclear, and the specific tumors as well as therapeutic targets for bladder cancer have not yet been identified. Hence, it is of great significance to understand more about the miRNAs involved in this disease.

MiR-22 has been identified to play crucial roles in multiple cellular process, including cell growth, migration, apoptosis, and autophagy [15, 16]. Increasing evidence has shown that miR-22 was commonly deregulated in a variety of cancers. For example, the miR-22 level was reduced in gastric cancer [9], colon cancer [14], lung cancer [17], and ovarian cancer [27]; while in prostate cancer [3], pancreatic cancer [7], and esophageal squamous cell carcinoma [32], miR-22 expression was commonly enhanced, acting as an oncogene. Here, results indicated that miR-22 abundance was remarkably reduced in bladder cancer tissues and cells, which was in line with a previous study [31]. Previous study indicated that miR-22 dysregulation was closely associated with cancer proliferation and EMT [28]. EMT, one of the major molecular mechanisms, is a process that promotes invasion and metastasis of cancers and transforms cells from epithelial state to mesenchymal state, accompanied by morphological adhesion loss, promoted motility, and decreased levels of epithelial adhesive molecules (such as E-cadherin and increased levels of mesenchymal molecules (such as N-cadherin and vimentin)) [8, 30]. Next, we further probed the biological functions of miR-22 in cell growth, migration, invasion, and EMT. Results suggested that addition of miR-22 obviously limited progression of bladder cancer cells. These results proved that miR-22 might serve as a tumor suppressor and repressed bladder cancer progression.

Recently, increasing evidence demonstrated that miR-22 largely determined the fate of many cancers, namely death or survival, by targeting and inhibiting downstream transcription factors through complex known or unknown mechanisms [28]. To the best of our knowledge, this research first demonstrated that miR-22 execute its biological functions by targeting E2F3. It was proved that E2F3 was tightly linked to the progression of bladder cancer [6]. Hurst *et al.* revealed that silencing E2F3 led to the suppression of bladder cancer cell proliferation [11]. A large number of literatures demonstrated that upregulation of E2F3 accelerated cell growth as well as metastasis in bladder cancer cells, which was in line with an oncogenic effect of E2F3 [18, 22, 23]. In our study, we observed a negative correlation between miR-22 level and E2F3 abundance in bladder cancer tissues. Silencing E2F3 repressed the cell growth, migration, invasion, and EMT in bladder cancer cells, which was similar to the role of miR-22 accumulation in the bladder cancer cells. Besides, downregulation of E2F3 partly reversed the promotion of cell growth and metastasis caused by miR-22 inhibitor in bladder cancer cells. Therefore, our findings suggested that miR-22 exerted its biological function by modulating the target gene E2F3 in bladder cancer cells.

In conclusion, these findings proved the altered expression of miR-22 in bladder cancer tissues and cells and miR-22 expression was negatively correlated with E2F3. Our findings also proved that upregulation of miR-22 repressed the cell growth and metastasis in bladder cancer cells. Knockdown of E2F3 caused similar effects to upregulated miR-22 in the bladder cancer cells. We provided the first evidence that miR-22 directly targeted E2F3. Moreover, E2F3 absence partly reversed the effects of miR-22 knockdown on cell growth and metastasis in bladder cancer cells. Collectively, miR-22 blocked the progression of bladder cancer cells through regulating E2F3, which would provide potential therapeutic strategy of bladder cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Junsheng Guo, Department of Urology, Affiliated Hospital of Chifeng University, No. 42 Wangfu Street, Xincheng District, Chifeng 024005, Inner Mongolia, China. Tel: +86-0476-5973011; E-mail: 19759922@163.com

References

[1] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[2] Bo J, Yang G, Huo K, Jiang H, Zhang L, Liu D and Huang Y. microRNA-203 suppresses bladder cancer development by repressing bcl-w expression. FEBS J 2011; 278: 786-792.

[3] Budd WT, Seashols-Williams SJ, Clark GC, Weaver D, Calvert V, Petricoin E, Dragoescu EA, O'Hanlon K and Zehner ZE. Dual action of miR-125b as a tumor suppressor and oncomiR-22 promotes prostate cancer tumorigenesis. PLoS One 2015; 10: e0142373.

[4] Chen J, Wang L, Tang Y, Gong G, Liu L, Chen M, Chen Z, Cui Y, Li C, Cheng X, Qi L and Zu X. Maspin enhances cisplatin chemosensitivity in bladder cancer T24 and 5637 cells and correlates with prognosis of muscle-invasive bladder cancer patients receiving cisplatin based neoadjuvant chemotherapy. J Exp Clin Cancer Res 2016; 35: 2.

[5] Deng S, Calin GA, Croce CM, Coukos G and Zhang L. Mechanisms of microRNA deregulation in human cancer. Cell Cycle 2008; 7: 2643-6.

[6] Feber A, Clark J, Goodwin G, Dodson AR, Smith PH, Fletcher A, Edwards S, Flohr P, Falconer A, Roe T, Kovacs G, Dennis N, Fisher C, Wooster R, Huddart R, Foster CS and Cooper CS. Amplification and overexpression of E2F3 in human bladder cancer. Oncogene 2004; 23: 1627-30.

[7] Ganepola GA, Rutledge JR, Suman P, Yiengpruksawan A and Chang DH. Novel blood-based microRNA biomarker panel for early diagnosis of pancreatic cancer. World J Gastrointest Oncol 2014; 6: 22-33.

[8] Guarino M, Rubino B and Ballabio G. The role of epithelial-mesenchymal transition in cancer pathology. Pathology 2007; 39: 305-18.

[9] Guo MM, Hu LH, Wang YQ, Chen P, Huang JG, Lu N, He JH and Liao CG. miR-22 is down-regulated in gastric cancer, and its overexpression inhibits cell migration and invasion via targeting transcription factor Sp1. Med Oncol 2013; 30: 542.

[10] Huang L, Luo J, Cai Q, Pan Q, Zeng H, Guo Z, Dong W, Huang J and Lin T. MicroRNA-125b suppresses the development of bladder cancer by targeting E2F3. Int J Cancer 2011; 128: 1758-1769.

[11] Hurst CD, Tomlinson DC, Williams SV, Platt FM and Knowles MA. Inactivation of the Rb pathway and overexpression of both isoforms of E2F3 are obligate events in bladder tumours with 6p22 amplification. Oncogene 2008; 27: 2716-27.

[12] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[13] Lagos-Quintana M, Rauhut R, Lendeckel W and Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001; 294: 853-8.

[14] Li B, Song Y, Liu TJ, Cui YB, Jiang Y, Xie ZS and Xie SL. miRNA-22 suppresses colon cancer cell migration and invasion by inhibiting the expression of T-cell lymphoma invasion and metastasis 1 and matrix metalloproteinases 2 and 9. Oncol Rep 2013; 29: 1932-1938.

[15] Li G, Wang G, Ma L, Guo J, Song J, Ma L and Zhao X. miR-22 regulates starvation-induced autophagy and apoptosis in cardiomyocytes by targeting p38α. Biochem Biophys Res Commun 2016; 478: 1165-1172.

[16] Li J, Liang S, Jin H, Xu C, Ma D and Lu X. Tiam1, negatively regulated by miR-22, miR-183 and miR-31, is involved in migration, invasion and viability of ovarian cancer cells. Oncol Rep 2012; 27: 1835-1842.

[17] Ling B, Wang GX, Long G, Qiu JH and Hu ZL. Tumor suppressor miR-22 suppresses lung cancer cell progression through post-transcriptional regulation of ErbB3. J Cancer Res Clin Oncol 2012; 138: 1355-1361.

[18] Liu L, Qiu M, Tan G, Liang Z, Qin Y, Chen L, Chen H and Liu J. miR-200c Inhibits invasion, migration and proliferation of bladder cancer cells through down-regulation of BMI-1 and E2F3. J Transl Med 2014; 12: 305.

[19] Majid S, Dar AA, Saini S, Deng G, Chang I, Greene K, Tanaka Y, Dahiya R and Yamamura S. MicroRNA-23b functions as a tumor suppressor by regulating Zeb1 in bladder cancer. PLoS One 2013; 8: e67686.

[20] Marta GN, Hanna SA, Gadia R, Correa SF, Silva JL and Carvalho Hde A. The role of radiotherapy in urinary bladder cancer: current status. Int Braz J Urol 2012; 38: 144-53; discussion 153-4.

[21] O'Day E and Lal A. MicroRNAs and their target gene networks in breast cancer. Breast Cancer Res Bcr 2010; 12: 201.

[22] Oeggerli M, Tomovska S, Schraml P, Calvano-Forte D, Schafroth S, Simon R, Gasser T, Mihatsch MJ and Sauter G. E2F3 amplification and overexpression is associated with invasive tumor growth and rapid tumor cell proliferation in urinary bladder cancer. Oncogene 2004; 23: 5616-23.

[23] Olsson AY, Feber A, Edwards S, Te Poele R, Giddings I, Merson S and Cooper CS. Role of E2F3 expression in modulating cellular proliferation rate in human bladder and prostate cancer cells. Oncogene 2007; 26: 1028-37.
[24] Parkin DM, Bray F, Ferlay J and Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.
[25] Racioppi M, D'Agostino D, Totaro A, Pinto F, Sacco E, D'Addessi A, Marangi F, Palermo G and Bassi PF. Value of current chemotherapy and surgery in advanced and metastatic bladder cancer. Urol Int 2012; 88: 249-258.
[26] Schultz PK, Herr HW, Zhang ZF, Bajorin DF, Seidman A, Sarkis A, Fair WR, Scherr D, Bosl GJ and Scher HI. Neoadjuvant chemotherapy for invasive bladder cancer: prognostic factors for survival of patients treated with M-VAC with 5-year follow-up. J Clin Oncol 1994; 12: 1394-1401.
[27] Wan WN, Zhang YQ, Wang XM, Liu YJ, Zhang YX, Que YH, Zhao WJ and Li P. Down-regulated miR-22 as predictive biomarkers for prognosis of epithelial ovarian cancer. Diagn Pathol 2014; 9: 178.
[28] Wang J, Li Y, Ding M, Zhang H, Xu X and Tang J. Molecular mechanisms and clinical applications of miR-22 in regulating malignant progression in human cancer. Int J Oncol 2017; 50: 345-355.
[29] Xu D, Takeshita F, Hino Y, Fukunaga S, Kudo Y, Tamaki A, Matsunaga J, Takahashi RU, Takata T, Shimamoto A, Ochiya T and Tahara H. miR-22 represses cancer progression by inducing cellular senescence. J Cell Biol 2011; 193: 409-424.
[30] Xu J, Lamouille S and Derynck R. TGF-β-induced epithelial to mesenchymal transition. Cell Res 2009; 19: 156-72.
[31] Xu M, Li J, Wang X, Meng S, Shen J, Wang S, Xu X, Xie B, Liu B and Xie L. MiR-22 suppresses epithelial-mesenchymal transition in bladder cancer by inhibiting Snail and MAPK1/Slug/vimentin feedback loop. Cell Death Dis 2018; 9: 209.
[32] Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Chen L, Zhang Y, Li Y, Qiu H, Xing J, Liang Z, Ren B, Yang C, Zen K and Zhang CY. Expression profile of microRNAs in serum: a fingerprint for esophageal squamous cell carcinoma. Clin Chem 2010; 56: 1871-1879.