

MiR-181a promotes epithelial to mesenchymal transition of prostate cancer cells by targeting TGIF2

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Abstract. – OBJECTIVE: Prostate cancer is the most commonly diagnosed cancer, and metastatic prostate cancer often leads to poor outcomes for patients. During the metastasis processes, cancer cells acquire a migratory and invasive phenotype. Epithelial to mesenchymal transition (EMT) has been implicated in multiple processes of prostate cancer development including migration, chemoresistance, and carcinogenesis.

PATIENTS AND METHODS: Expressions of miR-181a in prostate tumor samples and cancer cells were measured by qRT-PCR. Epithelial or mesenchymal markers were detected by Western blot. Nuclear translocation of Smad 2/3 was measured by immunostaining of prostate cancer cells.

RESULTS: In this study, we report an oncogenic role of microRNA-181a in prostate cancer cells and patients. MiR-181a is upregulated in metastatic prostate tumor samples compared with primary prostate tumors. Interestingly, we found that overexpression of miR-181a promotes prostate cancer cell migration and invasion. Moreover, we observed that overexpression of miR-181a contributes to an epithelial to mesenchymal transition phenotype in prostate cancer cells: the epithelial marker, E-cadherin was downregulated, and mesenchymal markers, N-cadherin, vimentin, and snail were upregulated. Consistently, the phosphorylation of Smad 2/3 and the nuclear localization of Smad 2/3 were increased by miR-181a expression. We identified that TGIF2 – a repressor of the Smad pathway – is a direct target of miR-181a in prostate cancer cells. Importantly, restoration of TGIF2 in miR-181a overexpressing prostate cancer cells inhibited the Smad pathway and EMT processes.

CONCLUSIONS: This research identifies a molecular mechanism for microRNA-mediated cancer metastasis and improvement novel therapeutic avenue for metastatic prostate cancer patient treatments.

Key Words:

MiR-181a, TGIF2, Prostate cancer, Epithelial to mesenchymal transition.

Introduction

Prostate cancer is one of the most commonly diagnosed male cancers, and metastatic prostate cancer often leads to poor outcomes for patients^{1,2}. The failures of prostate cancer therapies are chiefly attributable to the metastatic tumors that originate from primary prostate tumors³. At present, the therapeutic approaches for limiting metastatic prostate cancer are still under investigation. The process of epithelial to mesenchymal transition (EMT) – which is an essentially morphological transition process during cancer origination and metastasis – plays a pivotal role in the development of metastatic prostate cancers⁴. Moreover, EMT and its reversal, mesenchymal to epithelial transition (MET), have also been described as important in multiple pathological contexts, such as tissue fibrosis and cancer migration⁵.

The TGF- β induced factor homeobox 2 (TGIF2) protein is a Smad co-repressor, which represses the TGF- β /Smad pathways⁶. Upon TGF- β stimulation, Smad 2 and Smad 3 are phosphorylated directly by the TGF receptor, then translocate to the nucleus where they bind to specific promoters of target genes and activate transcription⁷. Therefore, as a co-repressor, TGIF2 represses Smad-activated transcription⁸.

MicroRNAs (miRNAs) are a family of small, non-coding, single-stranded RNAs that are important regulators of gene expression through direct binding to the 3'-UTR (untranslated re-

gion) of their target mRNAs⁹. Thus, miRNAs result in translational repression and degradation of their target mRNAs¹⁰. It is known that miRNAs modulate a variety of biological processes of prostate cancer, including proliferation, migration, invasion, differentiation, metabolism, chemoresistance and apoptosis¹¹⁻¹³. However, the detailed mechanisms of the oncogenic or tumor suppressive miRNAs in prostate metastasis are still unclear.

Our previous study¹⁴ reported that miR-181 was upregulated in prostate cancer tissues compared with their adjacent normal tissues, which suggested to us an oncogenic role for miR-181a in prostate cancers. Here, we investigate the role of miR-181a in metastatic prostate cancer tumors and cell lines. The direct targets of miR-181a in prostate cancer cells will be identified, and the miR-181a-Smad-EMT pathway will be assessed.

Patients and Methods

Tissue Samples and Cell Culture

A total of 40 prostate cancer samples (20 primary prostate tumors and 20 metastatic prostate tumors) were obtained from patients who underwent surgery at Huashan Hospital Affiliated with Fudan University (Shanghai, China). This investigation was approved by our Hospital Institutional Review Board and written informed consent was obtained from each patient.

PC-3 and DU145 cells were provided by the Institute of Biochemistry and Cell Biology of Chinese Academy of Science (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Life Technologies, Carlsbad, CA, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin sulfate. Cells were incubated at 37°C with 5% CO₂.

Reagents and Antibodies

A-E-cadherin, anti-N-cadherin, anti-vimentin anti-snail rabbit monoclonal antibodies were from the epithelial-mesenchymal transition (EMT) antibody sampler kit #9782 (Cell Signaling, Danvers, MA, USA). Rabbit monoclonal antibody β-actin was purchased from Cell Signaling (#4970, Danvers, MA, USA). The phospho-Smad 2/3 and total Smad 2/3 rabbit monoclonal antibody were purchased from Cell Signaling (#8828,

#8685). Phospho-TGFBR1 rabbit polyclonal antibody was purchased from Invitrogen, #PA5-40298, Carlsbad, CA, USA) total TGFBR1 mouse monoclonal antibody was purchased from Invitrogen, #AHO1552, Carlsbad, CA, USA). Mouse monoclonal anti-TGIF2 antibody was purchased from Santa Cruz Biotechnology, #sc-81989, Santa Cruz, CA, USA). DAPI was purchased from Sigma-Aldrich (Shanghai, China).

MiRNA Mimics and Plasmid DNA Transfection

Human miR-181a mimics and negative controls (NC) were purchased from Qiagen (Shanghai, China). All transfections of PC-3 and DU145 cells were performed using Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions.

Wound Healing Assay

24 h after transfection, PC-3 cells were seeded in 6-well plates and cultured 1-2 days until cells reached 90% confluence. The wound was created using a 200-µl pipette tip. Cells were washed with phosphate buffered saline (PBS) for three times, and regular cell culture medium was added to the wells. After 4 h incubation, the wound was observed, and random fields in each well were selected for imaging. The images were analyzed by ImageJ software, and the distance of wound closure was used to estimate the migration ability.

qRT-PCR

Quantitative Real-time PCR for the detection of miRNAs was performed according to our previous publication¹⁴. Total RNA containing miRNA and mRNA was extracted from tissues or cells using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), in accordance with the manufacturer's instructions. To analyze miR-181a expression, specific stem-loop reverse transcription primers (Invitrogen Life Technologies, Carlsbad, CA, USA) were used. qPCR was performed by TaqMan MicroRNA assay (Qiagen, Shanghai, China) using the Applied Biosystems 7300 system (Applied Biosystems, Foster City, CA, USA). The PCR conditions included an initial holding period at 95°C for 5 min, followed by a two-step PCR program consisting of 95°C for 5 s and 60°C for 30 s for 45 cycles. All samples were normalized against an internal control (U6 small nuclear RNA) and analyzed using the 2^{-ΔΔCt} method.

Luciferase Assay

Luciferase reporter assays were performed as described previously¹⁴. cDNA fragments corresponding to the 3'-untranslated regions of TGIF2 were amplified by RT-PCR from the total RNA extracted from PC-3 cells with KpnI and EcoRI linkers. The PCR products were cloned downstream of the Renilla luciferase open reading frame of the pMir-Report (Qiagen, Hilden, Germany), which contained a constitutively expressed firefly luciferase gene that was used to normalize the transfections. For the luciferase reporter assays, the cells were seeded in 24-well plates and harvested 48 h after transfection. The wild-type and mutant 3'-untranslated region fragments from the human TGIF2 gene were cloned into pMir-Report (Qiagen, Hilden, Germany). Mutations were introduced in potential miR-181a binding sites using a site-directed mutagenesis kit (Qiagen, Hilden, Germany). Luciferase values were determined using the Dual-Luciferase Reporter assay system (Promega Corporation, Madison, WI, USA).

Transwell Invasion Assay

Invasion assay of PC-3 cells was performed with the ThinCert™ Cell Culture Inserts from Greiner Bio-One International as previously described¹⁵. 1×10^4 cells were seeded in serum-free medium in the upper compartment, and DMEM plus 20% fetal bovine serum (FBS) was placed in the lower compartment of the chamber as a chemo-attractant. After 24 h of incubation, the non-invading cells on the upper side of the chamber were removed, and the membranes were fixed with methanol and stained with DAPI solution. Evaluation of invasive capacity was performed by counting the invading cells under a light microscope. All experiments were conducted in triplicate.

Immunofluorescence

Cells with or without miR-181a transfection were fixed by 4% PFA on cover glass for 30 min at 4°C followed by membrane permeabilization with 0.25% triton for 20 mins. Cells were incubated with 5% BSA blocking buffer for one hour then incubated with primary antibody at 4°C overnight. After washing 3 times for 5 min each with PBS, cells were incubated with secondary antibody at room temperature for 1 h. Cover glasses were placed on slides with mounting buffer containing 4',6-diamidino-2-phenylindole (DAPI). The images were acquired at room temperature using a LSM 510 laser scanning con-

focal microscope equipped with 405 nm, 488 nm, 594 nm and 633 nm lasers (Carl Zeiss, Inc., Oberkochen, Germany). Images were analyzed by ImageJ software.

Western Blot

Total cell protein extracts were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% bovine serum albumin (BSA) in phosphate-buffered saline, the membranes were immunoblotted with antibodies as indicated, followed by horseradish peroxidase (HRP)-linked secondary antibodies (Cell Signaling Technology, Inc., Danvers, MA, USA). The signals were detected using a chemiluminescence detection kit (Millipore, Billerica, MA, USA).

Statistical Analysis

Differences between groups were analyzed using a Student's *t*-test and expressed as the mean $\pm$ standard deviation from three independent experiments. $p < 0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using GraphPad Prism version 5.0 software (GraphPad Software Inc., La Jolla, CA, USA).

Results

miR-181 is Upregulated in Metastatic Prostate Cancer and Promotes Prostate Cancer Cell Invasion and Migration

Our previous work¹⁴ demonstrated that miR-181a is upregulated in prostate cancer cells and human prostate tumor samples. In order to define the potential roles of miR-181a in human metastatic prostate cancer, we compared the expression of miR-181a in human primary and metastatic prostate tumors. As we expected, the miR-181a was significantly upregulated in metastatic prostate tumor samples (Figure 1A), suggesting a positive role of miR-181a in mediating prostate cancer metastasis. To study whether miR-181a promotes prostate cancer cells migration, we overexpressed miR-181a in the PC-3 prostate cancer cell line (Figure 1B). *In vitro* wound healing and transwell assays showed that overexpression of miR-181a enhanced PC-3 cell migration (Figure 1C) and invasion (Figure 1D), which prompted us to explore the mechanisms for the miR-181a-promoted migration and invasion of prostate cancer cells.

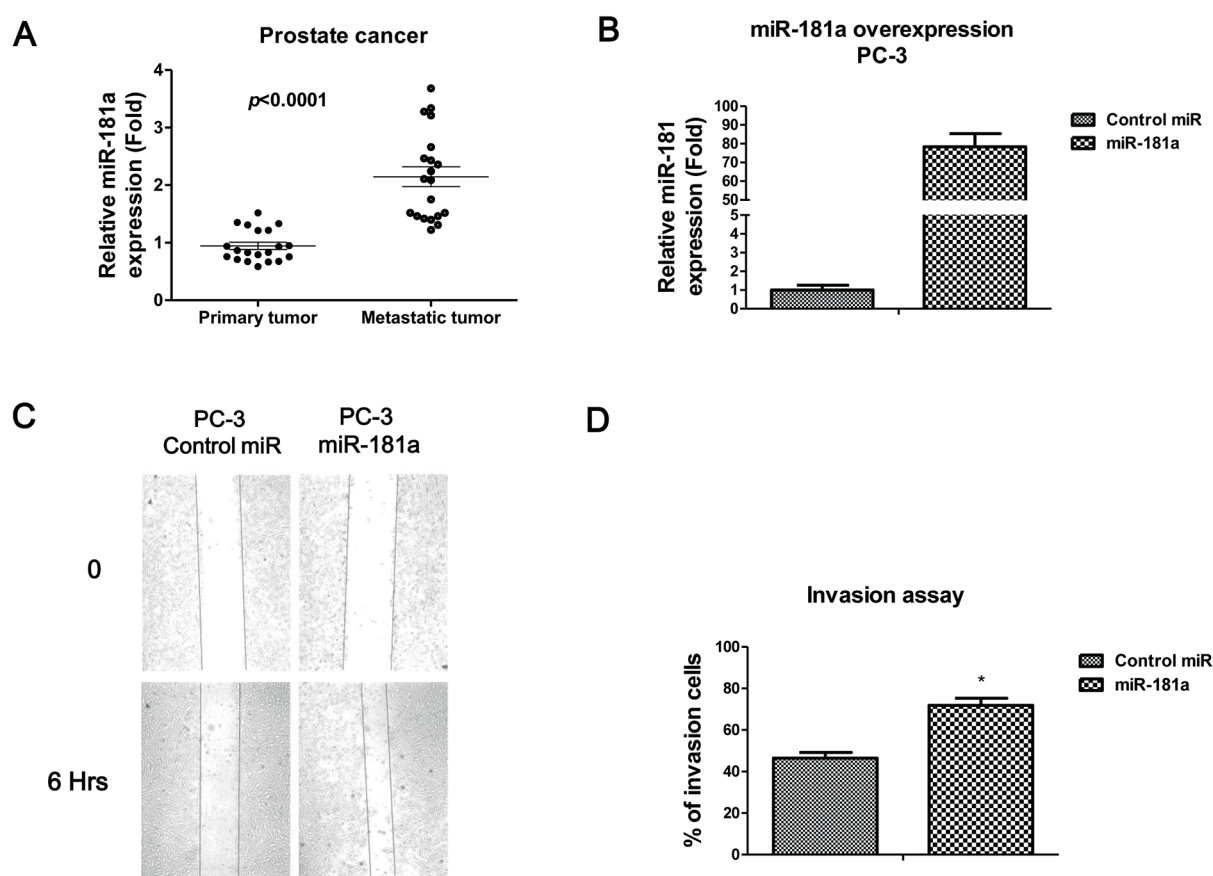


Figure 1. MiR-181a promotes migration and invasion of prostate cancer cells. **(A)** Expression of miR-181a was measured by Real-time PCR (RT-PCR) in human primary and metastatic prostate tumors. Relative levels were normalized to U6. **(B)** Control microRNAs or a miR-181a mimic were transfected into PC-3 prostate cancer cells. Forty-eight hours post-transfection, the expression of miR-181a was measured by RT-PCR. **(C)** PC-3 cells were transfected with control microRNAs or miR-181a mimic for 48 h, followed by wound healing assay for 6 h. **(D)** PC-3 prostate cancer cells were transfected with control microRNAs or miR-181a mimic for 48 h, followed by transwell assay for 16 h. Columns represent the mean of three independent experiments. *Indicates a $p < 0.05$ compared to control conditions.

MiR-181a Activates EMT in Prostate Cancer Cells

As described above, epithelial to mesenchymal transition is a unique process which plays a critical role in tumor invasion and metastasis^{4,5}. We, therefore, investigated whether miR-181a could regulate the EMT process of prostate cancer cells. DU145 is an epithelial prostate cancer cell line with relative low metastatic capacity. To investigate whether overexpression of miR-181a could induce EMT in DU145 cells, we assessed the expressions of the EMT-related markers by Western blot analysis. We found that the expression of E-cadherin, an epithelial marker, was significantly decreased in miR-181a overexpressing cells compared with control cells (Figure 2A), whereas mesenchymal markers N-cadherin, vimentin, and snail were significantly upregulated (Figure 2A).

To assess mechanisms for the regulation of these EMT markers by miR-181a, we further compared the mRNA levels in miR-181a overexpressing or control DU145 cells. Consistently, our results showed the mRNA expressions of E-cadherin and ZO-1 were downregulated and N-cadherin, vimentin, and snail, were upregulated by miR-181a (Figure 2B). Given the central role of the TGF- β pathway in the induction of cellular EMT process, we evaluated TGF- β receptor activation by measuring the protein expressions of TGFBR1 and TGFBR2 as well as the phosphorylation of Smad 2/3. Western blotting analysis showed significantly increased Smad 2/3 phosphorylation and nuclear translocation of Smad 2/3 proteins in DU145 miR-181a overexpressing cells as compared with control cells (Figure 2C-D), while the expressions of TGF- β receptors did not change

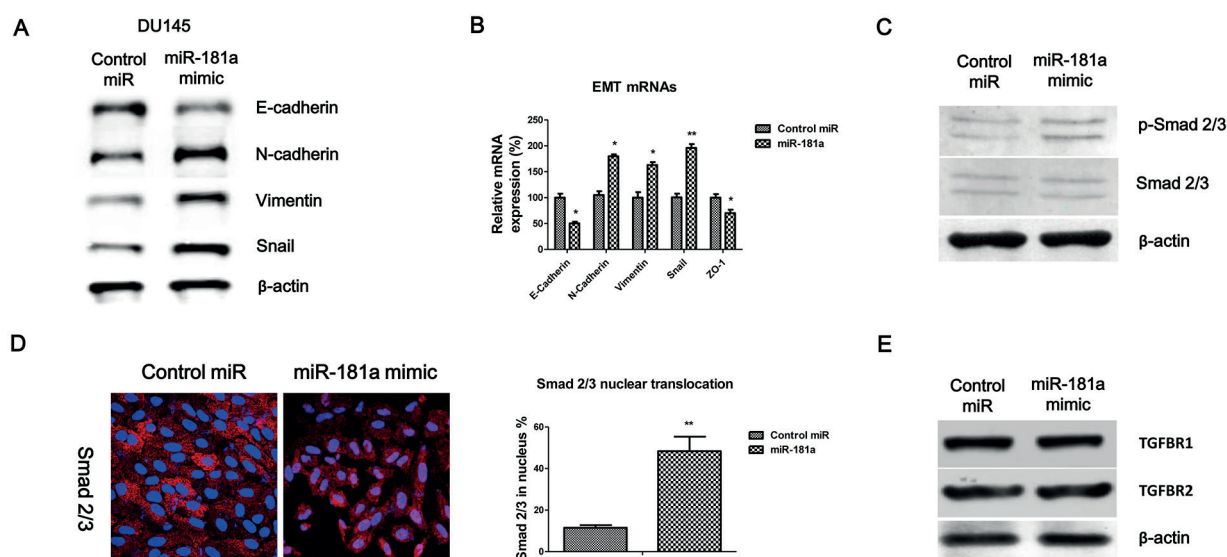


Figure 2. Overexpression of miR-181a inhibits EMT. **(A)** DU145 cells were transfected by control microRNAs or miR-181a mimic for 48 hours, then protein expression of E-cadherin, N-cadherin, Vimentin, and Snail were analyzed by Western blot. β -actin was used as a loading control. **(B)** The mRNA expressions of E-cadherin, ZO-1, N-cadherin, Vimentin, and Snail were detected by Real-time PCR in control microRNAs or miR-181a mimic transfected DU145 cells. **(C)** The phosphorylation of Smad 2/3 was detected by Western blot in control microRNAs or miR-181a mimic transfected DU145 cells. **(D)** DU145 cells were transfected by control microRNAs or miR-181a for 48 hours, cells were fixed and stained with Smad 2/3 antibody (*left*), and the translocation of Smad 2/3 into the nucleus was analyzed by confocal microscopy (*right*). **(E)** The total protein levels of TGFB1 and TGFB2 were measured by Western blot in control microRNA or miR-181a mimic transfected DU145 cells. Columns represent the mean of three independent experiments. *Indicates $p < 0.05$; **Indicates $p < 0.01$ as compared to control conditions.

(Figure 2E). Taken together, these data demonstrate that miR-181a promotes the EMT process of prostate cancer cells through the downstream signaling of TGF- β receptors.

miR-181a Direct Targets TGIF2

The above results demonstrated an indirect regulation of TGF- β -Smad-EMT pathway. As we discussed, after TGF- β stimulation, Smad proteins enter the nucleus and form transcriptional activation complexes or interact with TGIF- β , which acts as a co-repressor to inhibit downstream signaling⁷. To investigate the mechanisms for the miR-181a-mediated EMT of prostate cancer cells, we searched the potential targets of miR-181a from the public miRNA database TargetScan, which predicted that the 3'-UTR of TGIF2 contains highly conserved binding sites for miR-181 family (Figure 3A). Moreover, the binding sites on 3'UTR of TGIF2 are conserved in multiple species (Figure 3B). To determine whether miR-181a targets TGIF2 in prostate cancer cells, we transiently transfected a miR-181a mimic into PC-3 cells. Overexpression of miR-181a significantly suppressed the

TGIF2 protein expression (Figure 3C). We next sought to verify whether miR-181a could directly bind the 3'-UTR of TGIF2 mRNA. Luciferase reporter assays were performed by co-transfecting a reporter-luciferase vector fused with either the wild-type 3'-UTR sequence of TGIF2 or a sequence with mutations in the predicted binding site of miR-181a, and with either miR-181a mimic or control mimic. The results in Figure 3D illustrated that overexpression of miR-181a significantly decreased the luciferase activity of the reporter containing the wild-type 3'-UTR of TGIF2. However, transfection of miR-181a did not affect the activity of the luciferase reporter fused with the mutated predicted binding site in the 3'-UTR (Figure 3D). Taken together, these results demonstrate that miR-181a directly targets TGIF2 in prostate cancer cells.

TGIF2 Inhibits EMT of Prostate Cancer Cells

We next assessed whether downregulation of TGIF2 by siRNA could inhibit the EMT process in prostate cancer cells. Knockdown of TGIF2 by siRNA upregulated phosphoryla-

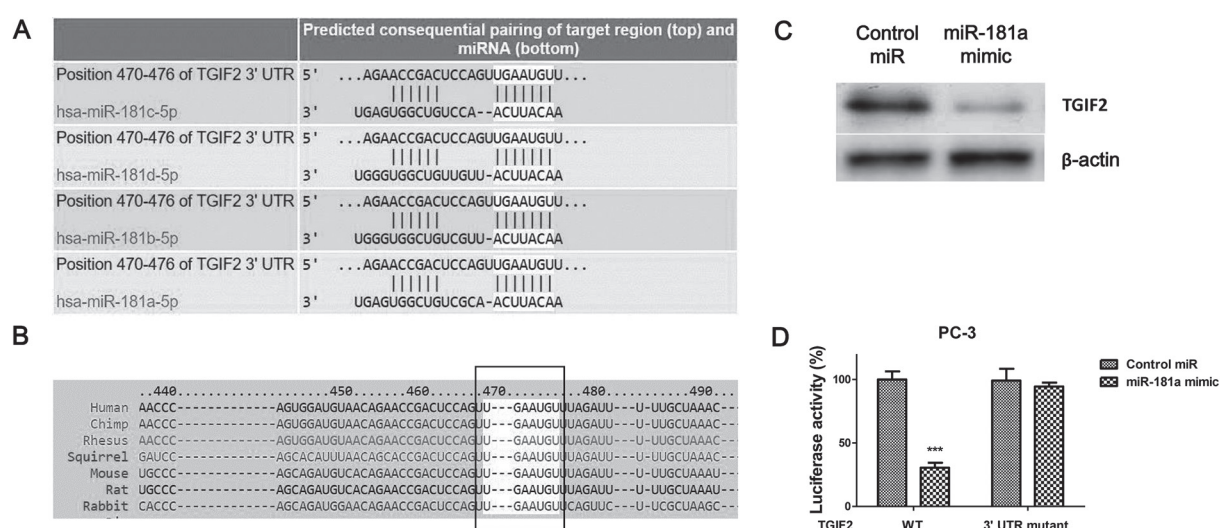


Figure 3. Targeting TGIF2 by miR-181a in prostate cancer cells. **(A)** Target prediction from Targetscan. The position 470-476 of TGIF2 3'-UTR contains conserved binding sites for miR-181a. **(B)** The predicted binding sites are conserved in multiple species. **(C)** PC-3 cells were transfected with 100 nM negative control (control miRNA) or a miR-181a mimic for 48 h. Cell lysates were analyzed by Western blot. β-actin was used as a loading control. **(D)** PC-3 cells were co-transfected with luciferase reporter plasmids containing either wild-type 3'-UTR or mutant 3'-UTR of TGIF2 and miR-181a mimic, or control microRNAs using lipofectamine 2000 reagent. Forty-eight hours post-transfection, cells were harvested and lysed with passive lysis buffer. Luciferase activity was measured by a dual luciferase reporter assay. Columns represent the mean of three independent experiments. ***Indicates $p < 0.001$ as compared to control conditions.

tion of Smad 2/3 (Figure 4A). Consistently, we observed the nuclear translocation of Smad 2/3 was increased by depletion of TGIF2 (Figure 4B). Further, knockdown of TGIF2 by siRNA promoted migration and invasion of PC-3 cells (Figure 4C-D).

miR-181a Activates EMT Through Inhibition of TGIF2

Because our results demonstrated a correlation of the miR-181a-mediated TGIF2 downregulation and the EMT process of prostate cancer cells, we hypothesized that miR-181a activates EMT by directly targeting TGIF2. To verify this miR-181a-TGIF2-Smad 2/3-EMT axis, we rescued TGIF2 expression in PC-3 cells that overexpressed miR-181a (Figure 5A). Subsequently, restoration of TGIF2 inhibited the nuclear translocation of Smad 2/3 (Figure 5B). Additionally, the migration and invasion abilities of TGIF2-recovered cells were significantly decreased compared with miR-181a overexpressing cells (Figure 5C-D). Restoration of TGIF2 suppressed migration and invasion of miR-181a overexpressing cells, clearly demonstrating that miR-181a activates the EMT process in prostate cancer cells through inhibition of TGIF2.

Discussion

Previously, we reported that miR-181a was up-regulated in prostate cancer tissues compared with adjacent normal tissues¹⁴. In this study, we explored the molecular mechanisms causing prostate cancer cells metastasis through upregulation of miR-181a. We demonstrated that upregulation of miR-181a is correlated with prostate cancer cells migration and invasion. Moreover, miR-181 overexpression markedly promoted the proliferation of prostate cancer cells. Other labs have also described miR-181a as playing a role in chemoresistance¹⁶ and alteration of metabolism in prostate cancer cells¹⁷, data which are consistent with our conclusions. In this work, we continue to explore the oncogenic roles of miR-181a and demonstrate that miR-181a is highly correlated with metastatic prostate cancer. We observed that miR-181a promoted the EMT process of cancer cells, resulting from the suppression of the Smad repressor, TGIF2, which suggests that inhibition of miR-181a might contribute to the development of anti-metastatic agents for prostate cancer treatments.

Epithelial-mesenchymal transition (EMT) is a regulatory process in which an epithelial cell undergoes morphological and functional alterations

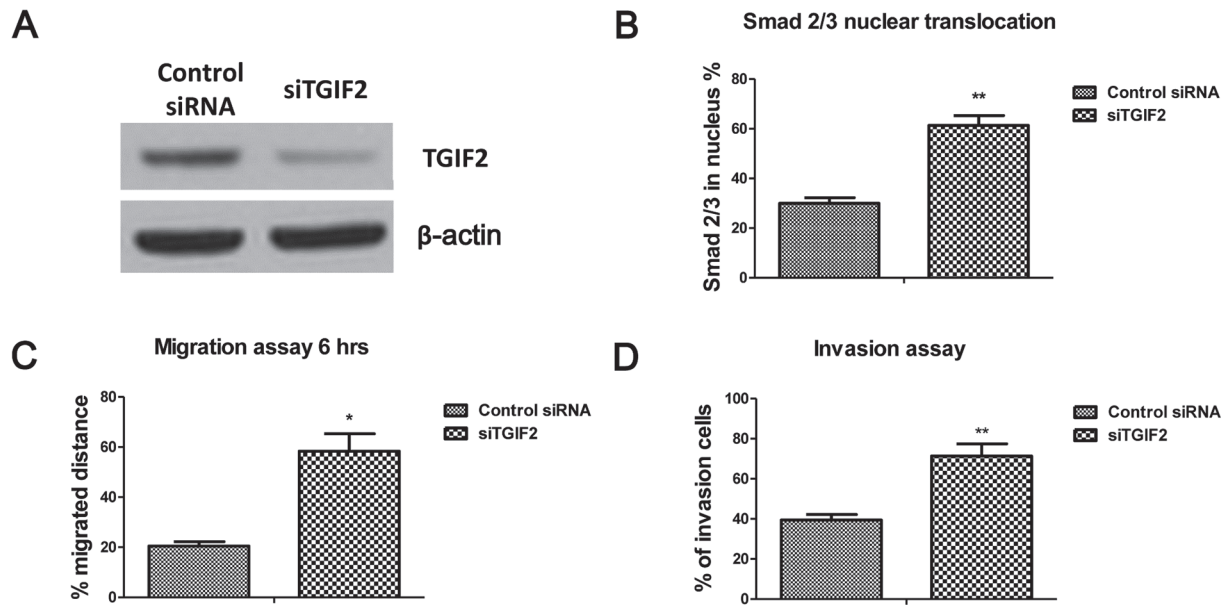


Figure 4. Knockdown of TGIF2 activates Smad and promotes migration and invasion of prostate cancer cells. **(A)** PC-3 cells were transfected with 100 nM control siRNA or siTGIF2 for 48 h. Cell lysates were prepared for Western blotting analysis. β -actin was used as a loading control. **(B)** PC-3 cells were transfected with 100 nM control siRNA or siTGIF2 for 48 h. Cells were fixed then the nuclear TGIF2 was analyzed by confocal and ImageJ software. **(C)** PC-3 cells were transfected with 100 nM control siRNA or siTGIF2 for 48 h. The migration assay and **(D)** invasion assay were performed. Columns represent the mean of three independent experiments. *Indicates $p < 0.05$; **Indicates $p < 0.01$ as compared to control conditions.

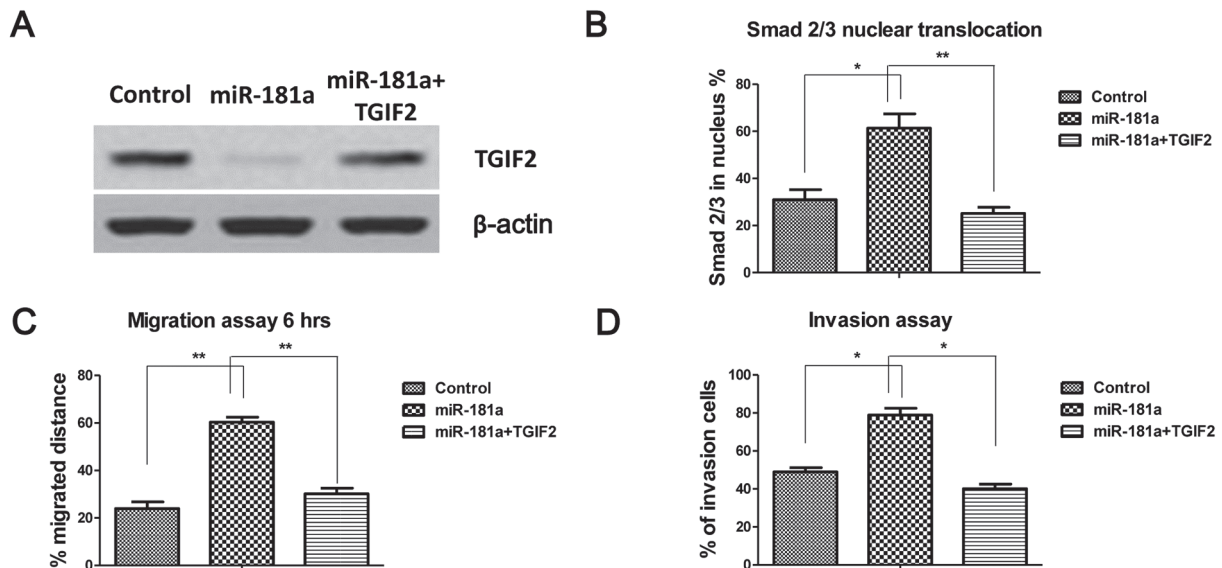


Figure 5. Restoration of TGIF2 inhibits migration and invasion of miR-181a overexpressing DU145 cells. **(A)** PC-3 cells were transfected with 100 nM control vector, miR-181a mimics or miR-181a mimics plus TGIF2 overexpressing vector for 48 h, cells were collected and subjected to Western blot analysis. β -actin is a loading control. **(B)** PC-3 cells were transfected with 100 nM control vector, miR-181a mimics or miR-181a mimics plus TGIF2 overexpressing vector for 48 h, then nuclear translocation of Smad 2/3 was analyzed by confocal and ImageJ software. **(C)** The migration and **(D)** invasion capacities were measured in PC-3 cells transfected with control vector, miR-181a mimics or miR-181a mimics plus a TGIF2 overexpressing vector. Columns represent the mean of three independent experiments. *Indicates $p < 0.05$; **Indicates $p < 0.01$ as compared to control conditions.

to assume a mesenchymal cell phenotype⁵. EMT has been described to contribute to multiple cellular processes including loss of normal polarity and adhesion, enhanced migration and invasion, resistance to apoptosis and alteration of metabolic pathways⁴. Several miRNAs are known as important EMT regulators such as miR-143 and miR-145, which have been identified as potential stimulators of EMT. Both are downregulated in bone metastasis arising from prostate cancer, and transfection of miR-143 and miR-145 into PC3 cells has been shown to lead to an epithelial phenotype, with increased E-cadherin and reduced fibronectin expression¹⁸. Our study reports that miR-181a promotes EMT in prostate cancer cells, revealing an oncogenic function of miR-181a in prostate cancer metastasis.

Conclusions

As a co-repressor of Smad, TGIF2 has been reported to associate with cancer development and metastasis¹⁹. We identified TGIF2 as a direct target of miR-181a in prostate cancer cells, which suggests that miR-181a might be a therapeutic target in metastatic prostate tumors. Moreover, our results showed inhibition of TGIF2 by either overexpression of miR-181a or siRNA could suppress the Smad pathway. We observed that the overexpression of miR-181a significantly inhibited the phosphorylation of Smad 2/3, resulting in decreased nuclear translocation of Smad 2/3. These findings illustrate a miR-181a-TGIF2-Smad-EMT axis in metastatic prostate cancer cells. However, the detailed cellular events, which regulate upstream signaling of miR-181a in these cells, remain unclear. In our next research, we will establish *in vivo* models and continue to explore the oncogenic roles of miR-181a. Our results provide a rationale for miRNA-based prostate tumor metastasis therapies and define a direct target of miR-181a for the development of novel therapies for advanced prostate cancer.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- BIENZ M, SAAD F. Management of bone metastases in prostate cancer: a review. *Curr Opin Support Palliat Care* 2015; 9: 261-267.
- GARTRELL BA, COLEMAN R, EFSTATHIOU E, FIZAZI K, LOGOTHETIS CJ, SMITH MR, SONPAVDE G, SARTOR O, SAAD F. Metastatic prostate cancer and the bone: significance and therapeutic options. *Eur Urol* 2015; 68: 850-858.
- TREWARTHA D, CARTER K. Advances in prostate cancer treatment. *Nat Rev Drug Discov* 2013; 12: 823-824.
- DE CRAENE B, BEXX G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer* 2013; 13: 97-110.
- SETON-ROGERS S. Epithelial-mesenchymal transition: Untangling EMT's functions. *Nat Rev Cancer* 2016; 16: 1.
- HU Y, YU H, SHAW G, RENFREE MB, PASK AJ. Differential roles of TGIF family genes in mammalian reproduction. *BMC Dev Biol* 2011; 11: 58.
- BU JQ, CHEN F. TGF- β 1 promotes cells invasion and migration by inducing epithelial mesenchymal transformation in oral squamous cell carcinoma. *Eur Rev Med Pharmacol Sci* 2017; 21: 2137-2144.
- LO RS, WOTTON D, MASSAGUE J. Epidermal growth factor signaling via Ras controls the Smad transcriptional co-repressor TGIF. *EMBO J* 2001; 20: 128-136.
- HA M, KIM VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014; 15: 509-524.
- JONAS S, IZAURRALDE E. Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet* 2015; 16: 421-433.
- JACKSON BL, GRABOWSKA A, RATAN HL. MicroRNA in prostate cancer: functional importance and potential as circulating biomarkers. *BMC Cancer* 2014; 14: 930.
- ALBERTI C. Prostate cancer cell both microRNA aberrant expression-mediated autophagy and radiation therapy resistance with short cross-reference to epigenetic/genetic mechanism implications. *Eur Rev Med Pharmacol Sci* 2016; 20: 1001-1002.
- TAO ZQ, SHI AM, LI R, WANG YQ, WANG X, ZHAO J. Role of microRNA in prostate cancer stem/progenitor cells regulation. *Eur Rev Med Pharmacol Sci* 2016; 20: 3040-3044.
- TONG SJ, LIU J, WANG X, QU LX. microRNA-181 promotes prostate cancer cell proliferation by regulating DAX-1 expression. *Exp Ther Med* 2014; 8: 1296-1300.
- QIN W, PAN YJ, ZHENG XL, LI D, BU JY, XU CY, TANG JB, CUI RJ, LIN P, YU XG. MicroRNA-124 regulates TGF- α -induced epithelial-mesenchymal transition in human prostate cancer cells. *Int J Oncol* 2014; 45: 1225-1231.

- 16) NIU J, XUE A, CHI Y, XUE J, WANG W, ZHAO Z, FAN M, YANG CH, SHAO ZM, PFEFFER LM, WU J, WU ZH. Induction of miRNA-181a by genotoxic treatments promotes chemotherapeutic resistance and metastasis in breast cancer. *Oncogene* 2016; 35: 1302-1313.
- 17) WEI ZH, CUI L, MEI ZB, LIU M, ZHANG DX. miR-181a mediates metabolic shift in colon cancer cells via the PTEN/AKT pathway. *FEBS Lett* 2014; 588: 1773-1779.
- 18) PENG XS, GUO W, LIU TJ, WANG X, TU XA, XIONG DF, CHEN S, LAI YR, DU H, CHEN GF, LIU GL, TANG YB, HUANG S, ZOU XN. Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. *PLoS One* 2011; 6: e20341.
- 19) HAMABE A, KONNO M, TANUMA N, SHIMA H, TSUNEKUNI K, KAWAMOTO K, NISHIDA N, KOSEKI J, MIMORI K, GO-TOH N, YAMAMOTO H, DOKI Y, MORI M, ISHII H. Role of pyruvate kinase M2 in transcriptional regulation leading to epithelial-mesenchymal transition. *Proc Natl Acad Sci U S A* 2014; 111: 15526-15531.