

MicroRNA-124 regulates TGF- α -induced epithelial-mesenchymal transition in human prostate cancer cells

WEI QIN^{1*}, YUJIA PAN^{1*}, XIULAN ZHENG², DONG LI¹, JINGYI BU¹, CHUNYAN XU¹,
JIEBING TANG³, RONGJUN CUI⁴, PING LIN¹ and XIAOGUANG YU¹

¹Department of Biochemistry and Molecular Biology, Harbin Medical University; Departments of ²Ultrasonography and ³Clinical Oncology, Harbin Medical University Cancer Hospital, Harbin, Heilongjiang 150081; ⁴Department of Biochemistry and Molecular Biology, Mudanjiang Medical University, Mudanjiang, Heilongjiang 157011, P.R. China

Received March 27, 2014; Accepted May 23, 2014

DOI: 10.3892/ijo.2014.2506

Abstract. Transforming growth factor- α (TGF- α) is upregulated in advanced stages of prostate cancer and strongly correlated with metastasis. However, the effect of TGF- α on epithelial-mesenchymal transition (EMT) in prostate cancer and the underlying mechanisms remain unclear. Recently, microRNAs have emerged as new regulators of EMT. This study found that treatment of DU145 cells with TGF- α suppressed the expression of epithelial marker E-cadherin and increased the expression of mesenchymal marker Vimentin as well as changed the cell morphology from cobblestone shape to spindle shape. The level of miR-124 was downregulated by TGF- α in several different cancer cell lines. Enforced expression of miR-124 abolished TGF- α -induced EMT. Slug was proven to be a target of miR-124 and mediated the inhibitory effect of miR-124 on TGF- α -induced EMT. Furthermore, overexpression of miR-124 reduced the migratory and invasive capacity of TGF- α -treated DU145 cells. In conclusion, our findings suggest that miR-124 inhibits TGF- α -induced EMT in DU145 cells by targeting Slug. Thus, miR-124 may be a potential target for prostate cancer therapeutic intervention.

Introduction

Prostate cancer is the sixth leading cause of cancer-related death in men in the world (1) and the second in the USA (2). The number of men diagnosed with prostate cancer is increasing every year, especially in the developing country. Although most of the patients survive the initial stage of prostate cancer

with androgen ablation therapy, with tumor progression, it may gradually metastasize to other organs, which lead to poor prognosis and quick death.

Epithelial-mesenchymal transition (EMT) is now viewed as a crucial process during tumor progression and metastasis (3). EMT is characterized by remarkable cell morphology change from epithelial round phenotype to mesenchymal spindle-shaped phenotype. The process involves downregulation of cell adhesion/junction proteins, such as E-cadherin and Zonula occludens (4,5), as well as upregulation of mesenchymal and ECM degradative proteins such as Vimentin, N-cadherin and MMPs (6,7), which results in loss of cell-cell adhesion and increased migratory capacity that contribute to cancer metastasis (8). Many factors promote cancer cell invasion and metastasis by inducing EMT, such as transforming growth factor (TGF) β , platelet derived growth factor (PDGF) D and fibroblast growth factor (FGF) (4,9,10). However, the role of TGF- α in the process of EMT and the mechanism remain to be elucidated.

TGF- α and its receptor epidermal growth factor receptor (EGFR) are crucial oncogenes in the development of different cancers (11,12), including prostate cancer (13). Compared with benign prostate epithelium, TGF- α mRNA and protein levels are increased in carcinoma cells (13,14). Interestingly, studies have shown that compared with the early-stage localized tumors, TGF- α expression is more abundant when prostate cancer metastasizes (13,15-17). However, a reason for the strong correlation between TGF- α expression and high-invasive capacity has not been elucidated. Here we report TGF- α was able to induce EMT in prostate cancer.

MicroRNAs (miRNAs) are small non-coding RNAs that can sequence-specifically bind to the 3'-untranslated regions of target mRNAs resulting in mRNA destruction or translational inhibition (18). Growing evidence suggests that miRNAs are important regulators of EMT. Upregulation of miR-9 provokes the development of EMT (19). Downregulation of miR-200 contributes to PDGF-D induced EMT. However, the involvement of miRNAs in TGF- α -induced EMT has not been elucidated.

In this study, we showed that TGF- α , frequently upregulated in advanced prostate cancer, is able to induce EMT in DU145 cells. Downregulation of miR-124 is ubiquitous in

Correspondence to: Professor Xiaoguang Yu, Department of Biochemistry and Molecular Biology, Harbin Medical University, Baojian Road 157, Harbin, Heilongjiang 150081, P.R. China
E-mail: xiaoguang_yu@hotmail.com

*Contributed equally

Key words: miR-124, TGF- α , epithelial-mesenchymal transition, prostate cancer

TGF- α treated cancer cells and contributes to TGF- α -induced EMT by targeting Slug. This study indicates that miR-124 may be a promising therapeutic candidate for prostate cancer metastasis.

Materials and methods

Cell culture. DU145, HepG2 and Hu7 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). MKN-45, MDA-MB-231, HCT15 and PA1 cell lines were purchased from Cell Resource Center, IBMS, CAMS/PUMC (China). Cells were cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin/streptomycin, and maintained under the conditions of 37°C with 5% CO₂ and 95% air.

Transfection of miRNAs. DU145 cells were transiently transfected with miR-124 mimics or miR-124 inhibitors or negative control (RiboBio Co., Ltd., Guangzhou, China), using Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer's instructions. Briefly, cells were trypsinized and seeded in 60-mm dishes 24 h before transfection. The Opti-MEM serum-free media was used to dissolve the transfection mixture. After 24 h of transfection, the media were removed and RPMI media containing 10% FBS and 1% penicillin/streptomycin was added to the cells.

Western blot analysis. The procedure of western blot analysis was described previously (20). In brief, cells were washed by PBS several times and harvested using RIPA lysis buffer with protease inhibitor. Protein concentration was determined using BCA reagent (Beyotime Institute of Biotechnology, Nanjing, China) according to the manufacturer's protocols. Equal amounts of proteins (60 μ g) were electrophoresed in 12% SDS-PAGE gels and transferred to the nitrocellulose membranes. After being blocked with 5% milk for 2 h at room temperature, the membranes were incubated with primary antibodies for E-cadherin, Vimentin, Slug and Tubulin (Cell Signaling Technology, USA) overnight at 4°C. Tubulin was used as an internal control. The bands were detected with ECL reagents and analysed by Quantity One software.

RNA extraction and real-time RT-PCR. Total RNA was extracted using TRIzol reagent (Gibco, Invitrogen, Carlsbad, CA, USA). Then 1 μ g of extracted RNA was used for reverse transcription and the obtained cDNA was used in real-time PCR. The specific RT-PCR primers for miR-124 and U6 were purchased from Ribo (RiboBio Co., Ltd.).

Immunofluorescence staining. We performed immunofluorescence staining on DU145 cells to determine Vimentin expression. Firstly, cells were fixed with 4% paraformaldehyde for 15 min followed by permeabilized with 0.1% Triton X-100 for 10 min. Then cells were blocked with 10% goat serum (in PBS) blocking solution for 1 h followed by incubation with Vimentin antibodies at a dilution of 1:100 at 4°C overnight. At the end of the incubation, cells were washed and incubated with FITC-conjugated secondary antibody for 1 h. PI was added to the cells for another 10 min. Cells were observed and images were captured by fluorescence microscopy.

Luciferase reporter assay. Luciferase reporter assay was done as previously described (21). Briefly, cells were seeded in a 96-well plate and transfected with plasmids. Twenty-four hours after transfection, cells were harvested and Firefly and Renilla luciferase activities were measured using Luciferase Reporter Gene assay kit of Beyotime (China). Renilla luciferase was used as an internal control and the RLU (relative light unit) ratio of Firefly luciferase relative to Renilla luciferase was calculated.

Wound healing assay. DU145 cells were seeded in 6-well plates to achieve 90% confluence. Twenty-four hours after transfection, a vertical wound was created using a 200- μ l pipette tip. Then the cells were washed with PBS for three times and medium without serum was added into the wells. After 24-h incubation, the wound was observed and random fields in each well were selected for imaging. The images were analysed by ImageJ and the distance of wound closure was used to estimate the migration ability.

Migration and invasion assay. Cell migration and invasive ability was evaluated using 24-well transwell plates. For migration assay, 24 h after transfection, 5 \times 10⁴ cells per well were seeded into the top chamber and maintained in serum-free medium. Medium (600 μ l) containing 10% FBS was added into the bottom chamber. After incubated for 24 h at 37°C, cells that migrated through the pore polycarbonate membrane were fixed with methanol and stained with Giemsa. Then the migrated cells were observed and images were captured using microscopy. For invasion assay, before cell seeding, the Matrigel were diluted in serum-free medium at the concentration of 1 mg/ml. Diluted Matrigel (100 μ l) per well were added into the top chamber and incubated for 4 h at 37°C. The following procedures were the same as in the migration assay.

Statistical analysis. The experiments in this study were repeated at least three times and values were expressed as mean \pm SEM. The results were analyzed by One-way ANOVA accompanied with Turkey multiple-comparisons test (GraphPad Prism version 5.0). Student's t-test was used for the comparison between two groups. P<0.05 was considered as statistically significant.

Results

TGF- α induces changes in morphology and EMT related markers of DU145 cells. To identify the possible effect of TGF- α on the progression of prostate cancer, we treated DU145 cells with 50 ng/ml TGF- α . At 0, 12 and 24 h after TGF- α treatment, images of the morphology of cells were taken by microscopy. Interestingly, we found that TGF- α induced a remarkable morphological change from the round and cobblestone form to the long fusiform shape (Fig. 1A), reminiscent of EMT, an important process in cancer development. To further confirm whether TGF- α could induce EMT in DU145 cells, we assessed the expression levels of EMT related markers using western blot analysis. We found that the expression of E-cadherin, an epithelial marker, was significantly decreased after TGF- α treatment, whereas Vimentin, a mesenchymal

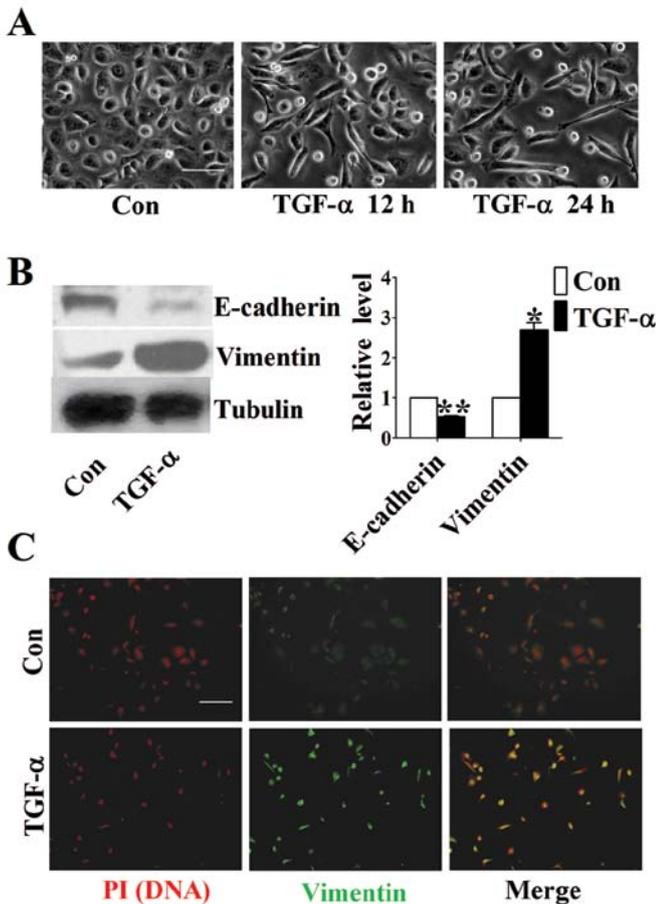


Figure 1. TGF- α treatment induces EMT in DU145 cells. (A) The morphology of DU145 cells. Cells in control group display round and cobblestone-like epithelial cell shape and TGF- α (50 ng/ml) treated cells exhibit spindle-shaped phenotype. Scale bar indicates 200 μ m. (B) Western blot analysis of epithelial marker E-cadherin and mesenchymal marker Vimentin in DU145 cells treated with or without TGF- α (50 ng/ml) for 24 h. Tubulin was used as internal control. n=3. *P<0.05; **P<0.01 vs con. (C) Representative images of DU145 cells stained with antibody to Vimentin (green). Red indicates PI staining. Scale bar, 200 μ m.

marker, was increased concomitantly (Fig. 1B). Similar results were obtained from immunofluorescent staining (Fig. 1C). These data suggest that TGF- α is able to induce EMT in DU145 cells.

Downregulation of miR-124 is ubiquitous in TGF- α treated cancer cells. To test whether miR-124 could contribute to TGF- α -induced EMT, we first detected miR-124 levels using real-time RT-PCR in DU145 cells. After TGF- α treatment for 24 h, the expression of miR-124 was significantly decreased compared with control group (Fig. 2A). To further confirm the effect of TGF- α on miR-124 expression, TGF- α was applied to gastric cancer MKN-45 cells, breast cancer MDA-MB-231 cells, colon cancer HCT15 cells, ovarian teratocarcinoma PA1 cells, liver cancer HepG2 cells and Hu7 cells. As expected, the levels of miR-124 was significantly decreased by TGF- α in all of the six different cancer cell lines (Fig. 2B). These results indicate that miR-124 may function as a key downstream effector of TGF- α in cancer cells.

Overexpression of miR-124 inhibits TGF- α induced-EMT in DU145 cells. The effect of miR-124 on TGF- α -induced EMT was evaluated by western blot analysis. Treatment with 50 ng/ml TGF- α on DU145 cells led to a significant EMT change as indicated by E-cadherin and Vimentin protein levels. Overexpression of miR-124 inhibited TGF- α -induced downregulation of E-cadherin and upregulation of Vimentin, whereas transfection with microRNA negative control had no effect on these proteins (Fig. 3A). In addition, we found that TGF- α treated DU145 cells with miR-124 overexpression displayed round-like morphology, indicating acquisition of mesenchymal-epithelial transition (MET) phenotype (Fig. 3B). The above results indicate that overexpression of miR-124 is able to reverse TGF- α -induced EMT.

Involvement of Slug in miR-124-mediated suppression of TGF- α -induced EMT. Slug was reported to regulate

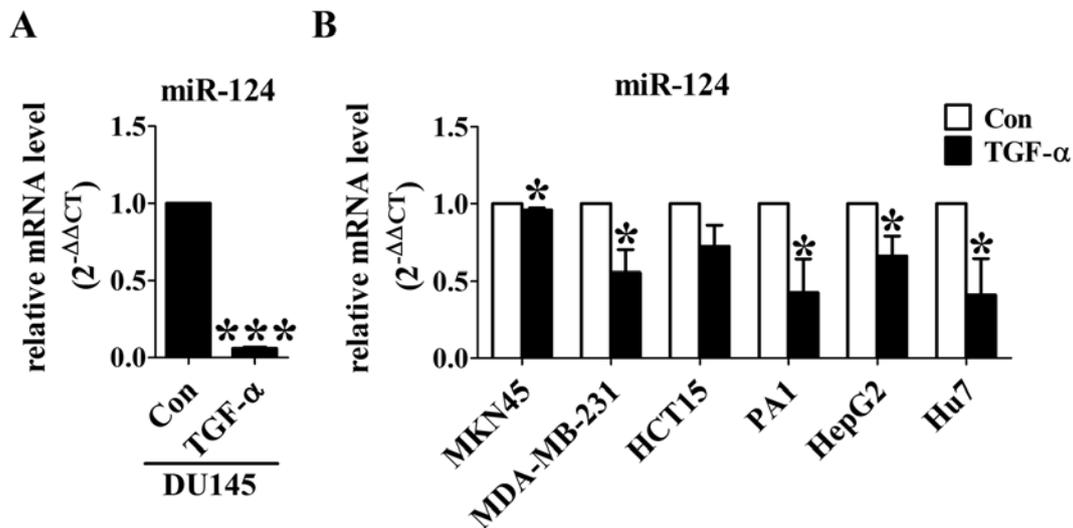


Figure 2. Expression of miR-124 is suppressed by TGF- α in cancer cells. Real-time RT-PCR was performed to determine the expression of miR-124. Relative levels were normalized to U6. TGF- α (50 ng/ml) treatment significantly downregulated miR-124 level in (A) prostate cancer DU145 cells, (B) gastric cancer MKN-45 cells, breast cancer MDA-MB-231 cells, colon cancer HCT15 cells, ovarian teratocarcinoma PA1 cells, liver cancer HepG2 cells and Hu7 cells. n=3. *P<0.05; ***P<0.001 vs con.

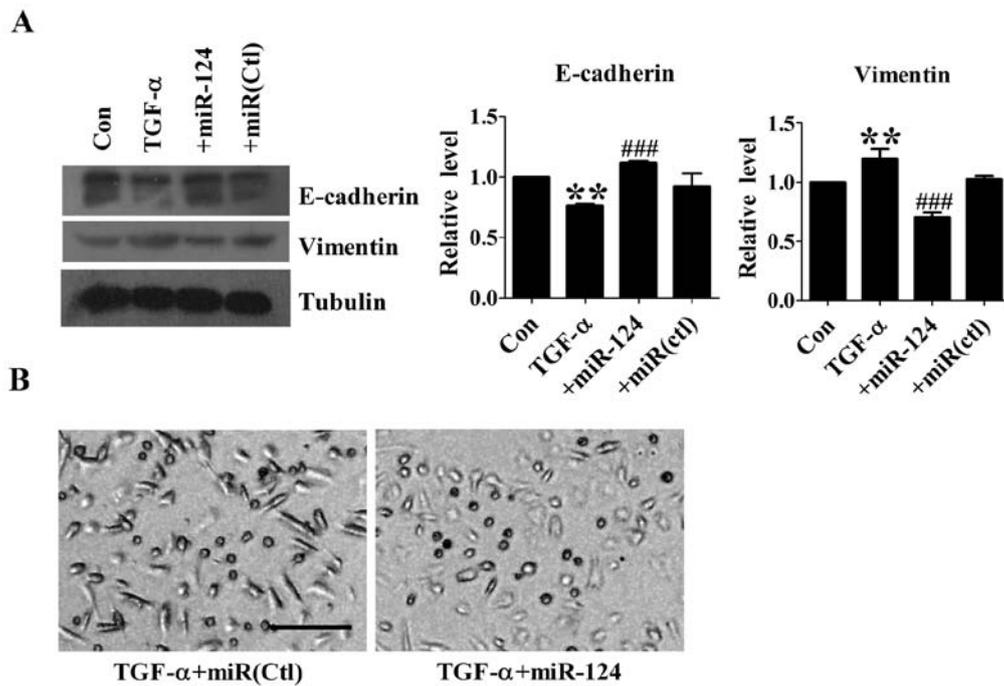


Figure 3. Overexpression of miR-124 inhibits TGF- α -induced EMT. (A) Protein expression and the summarized data of E-cadherin and Vimentin detected by western blot analysis in DU145 cells. +miR-124, co-application of miR-124 mimics and TGF- α (50 ng/ml). +miR(ctl), co-application of miRNA negative control and TGF- α (50 ng/ml). n=3. **P<0.01 vs con; ***P<0.001 vs TGF- α . (B) Representative images of DU145 cells. Scale bar, 200 μ m.

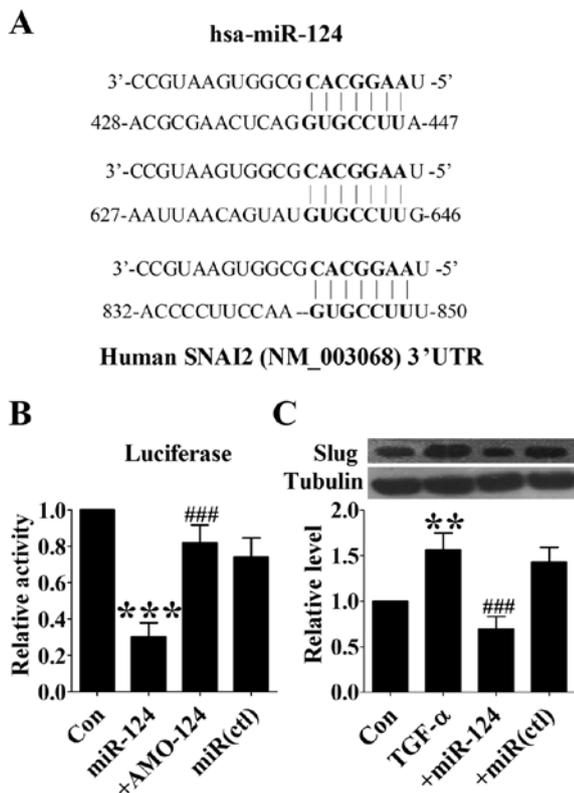


Figure 4. Post-transcriptional repression of Slug by miR-124. (A) Complementarities of sequences between miR-124 and SNAI2 (Slug) 3'-UTR. The text in bold letters indicates the matched bases. (B) Luciferase assay showed miR-124 could target Slug. +AMO-124, co-application of miR-124 and its antisense AMO-124. n=3. ***P<0.001 vs con; ***P<0.01 vs miR-124. (C) Protein expression and the summarized data of Slug detected by western blot analysis in DU145 cells. +miR-124, co-application of miR-124 mimics and TGF- α (50 ng/ml). +miR(ctl), co-application of miRNA negative control and TGF- α (50 ng/ml). n=3. **P<0.01 vs con; ***P<0.001 vs TGF- α .

expression of E-cadherin and the EMT process of cancer cells. Computational analysis using the miRNA TargetScan predicted that Slug might be a target of miR-124. The 3'-UTR of Slug mRNA contains 3 binding sites for miR-124 in humans (Fig. 4A). A previous study has proved that Slug could be regulated by miR-124. In this study, we sought to test whether or not miR-124 could inhibit TGF- α -induced EMT via regulating Slug. First we confirmed the regulation of Slug by miR-124 using luciferase assay. The results showed that miR-124 markedly suppressed the luciferase activity of Slug 3'-UTR luciferase vector (Fig. 4B). In DU145 cells, TGF- α treatment induced significant upregulation of Slug expression as indicated by western blot analysis, whereas overexpression of miR-124 abolished this effect (Fig. 4C). These data suggest that miR-124 may inhibit TGF- α -induced EMT by regulating Slug.

Overexpression of miR-124 inhibites migration and invasion of TGF- α treated DU145 cells. EMT is a major process leading to increased migration and invasive ability in cancer cells. The migration ability of DU145 cells was first assessed by wound healing. The migration rate of TGF- α -treated cells significantly increased compared with control group, while overexpression of miR-124 obviously attenuated cell movement (Fig. 5A). Moreover, migration assay was also used to evaluate the migration ability. We found that transfection of miR-124 markedly reduced the amount of migrated cells induced by TGF- α (Fig. 5B). Additionally, miR-124 overexpression resulted in a significant repression of the invasion ability as evidenced by the invasion assay (Fig. 5B). These results clearly indicate that re-expression of miR-124 suppresses migration and invasion of TGF- α treated DU145 cells.

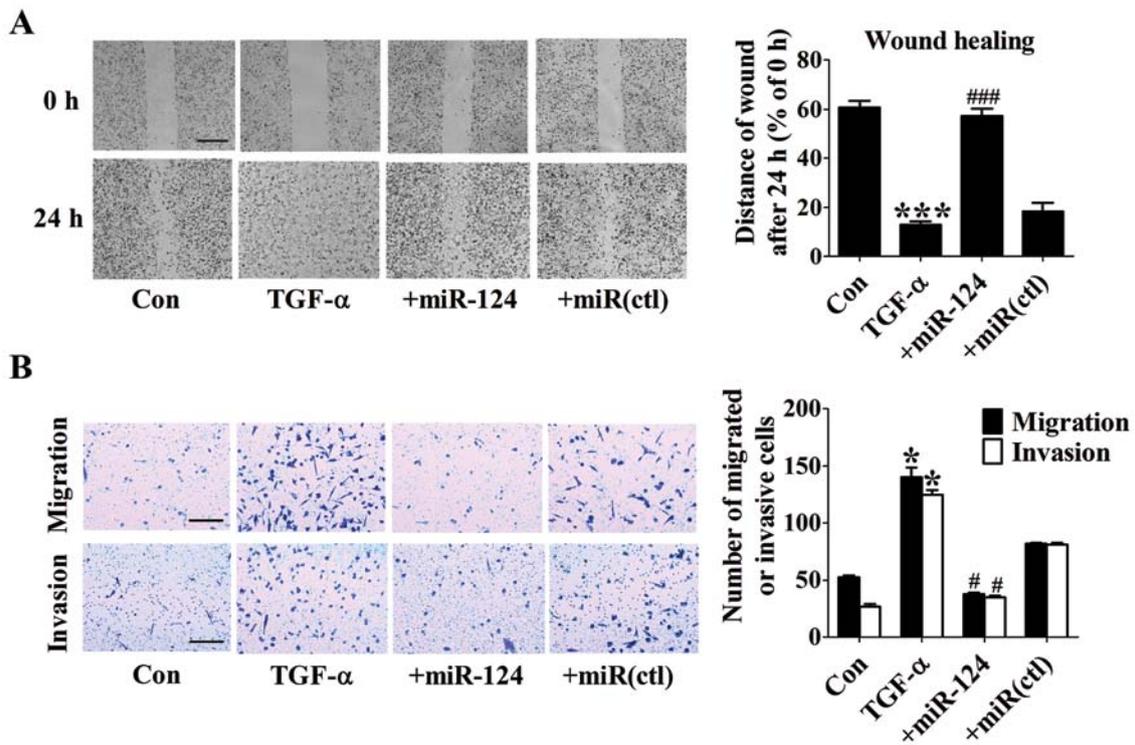


Figure 5. Overexpression of miR-124 inhibits migration and invasion of TGF- α treated DU145 cells. (A) Wound healing assay. Scale bar, 1,000 μ m. (B) Migration and invasion assay. Scale bar, 500 μ m. +miR-124, co-application of miR-124 mimics and TGF- α (50 ng/ml). +miR(ctl), co-application of miRNA negative control and TGF- α (50 ng/ml). n=3. *P<0.05; ***P<0.001 vs con. #P<0.05; ###P<0.001 vs TGF- α .

Discussion

The epidermal growth factor receptor (EGFR) and two of its ligands, epidermal growth factor (EGF) and TGF- α are frequently overexpressed and play a central role in the development of prostate cancer. Studies have reported that the progression of prostate cancer from androgen-dependence to androgen-independence involves a 'switch' in the synthesis from EGF to TGF- α . In the localized prostate cancer, EGF is more abundant than TGF- α . When the disease develops to advanced, metastatic stages, the expression of TGF- α increases and EGF decreases, indicating that TGF- α may play a more important role in cancer progression and metastasis. It is now recognized that carcinoma cells undergo a process called EMT to acquire migratory properties. Therefore, in this study, we determined if TGF- α is able to induce EMT in prostate cancer. Our data showed that treatment of DU145 cells with TGF- α induced a dramatic cell morphological alteration that is similar to EMT. Moreover, western blot results showed the expression of E-cadherin was downregulated and the expression of Vimentin was upregulated, which are consistent with the characteristics of EMT.

MicroRNAs are thought to play important roles in a wide variety of biological processes. Aberrant expression of miRNAs is often involved in the pathogenesis of human diseases, such as cardiovascular disease (22), infection (23) and tumor (24). Genomic profiling and expression studies found a global downregulation of miRNAs in many cancers. The expression of miR-124 was found to be downregulated in various tumors, including breast cancer (25), gastric cancer (26) and prostate cancer (27), indicating miR-124 may func-

tion as a negative regulator of cancer progression. A previous study reported that miR-124 inhibited proliferation of prostate cancer cells by targeting the androgen receptor (27). In this study, we found that the expression of miR-124 was reduced in TGF- α treated DU145 cells. To further validate the TGF- α -induced downregulation of miR-124 was not specific in prostate cancer cells, we treated different cell lines, including gastric cancer MKN-45 cells, breast cancer MDA-MB-231 cells, colon cancer HCT15 cells, ovarian teratocarcinoma PA1 cells, liver cancer HepG2 cells and Hu7 cells with TGF- α . Treatment of the above-mentioned cell lines with TGF- α all led to a decrease in the expression of miR-124. These results imply the possible role of miR-124 as a downstream effector of TGF- α in cancer cells.

Recent studies have reported that microRNAs, especially miR-200 family, acted as a key regulator of EMT. MiR-200 family and miR-205 were significantly suppressed in cells undergoing EMT and forced expression of miR-200 family was able to initiate MET, the reversed process of EMT, via downregulating transcription factors ZEB1 and SIP1 (28). Besides miR-200 family, miR-9 led to EMT-like transformation and increased cell invasive ability by directly targeting E-cadherin (19). Mir-30a was found to target Snail, a transcription repressor of E-cadherin, leading to EMT induction (29). Interestingly, microRNA could also affect the process of EMT by regulating expression of other microRNAs. As an example the miR-103/107 family, which directly targeted Dicer, resulting in decreased miR-200 levels and EMT occurrence (30). A previous study by Liang *et al* showed that miR-124 could regulate the process of EMT in breast cancer MDA-MB-231 cells (31). Since in this study, we have proven that TGF- α

was able to induce EMT and reduce miR-124 expression in DU145 cells, it is tempting to speculate that miR-124 may mediate TGF- α -induced EMT. Our results showed that transfection of DU145 cells with miR-124 before TGF- α treatment significantly increased the expression of epithelial marker E-cadherin and decreased the expression of mesenchymal marker Vimentin, suggesting that the reduction miR-124 contributed to TGF- α -induced EMT and re-expression of miR-124 could lead to the reversal of TGF- α -induced EMT.

Several transcription factors have been validated as pivotal inducers of EMT, including ZEB1 (32), ZEB2 (33), Twist (34), Snail (35), and Slug (36). Slug is a zinc-finger transcription factor known to repress endogenous E-cadherin expression by binding to E-box elements within the promoter. Upregulation of Slug has been shown to play an important role in the induction of EMT (37). Studies have confirmed the regulation of Slug by miR-124 using luciferase reporting assay (31,38). Liang *et al* (30) have proven that miR-124 could regulate EMT by targeting Slug in breast cancer cells. However, whether or not Slug could mediate the inhibitory effect of miR-124 in TGF- α -induced EMT is unknown. In this study, we found that Slug was significantly increased in response to TGF- α treatment and transfection of miR-124 inhibited Slug expression, suggesting Slug is a downstream effector of miR-124 in TGF- α -induced EMT.

Cancer cells that undergo EMT often have an increased migratory and invasive capacity (39), and the overexpression of miR-124 led to the reversal of TGF- α -induced EMT. In this study, our data indicated that transfection of DU145 cells with miR-124 significantly inhibited cell migration and invasion induced by TGF- α , indicating overexpression of miR-124 led to a less invasive cell phenotype, which was consistent with MET characteristics.

In conclusion, we found that TGF- α is able to induce EMT in DU145 cells, which may provide a reason for the EGFR ligand switch in the late stage of prostate cancer. Downregulation of miR-124 contributes to TGF- α -induced EMT and re-expression of miR-124 could reverse this process. In addition, miR-124 overexpression reduces cell migratory and invasive ability in TGF- α treated cells, the underlying mechanism involves the downregulation of Slug. These results provide evidence that miR-124 may be a potential therapeutic target for advanced prostate cancer.

Acknowledgements

This study was supported by grants from the National Youth Natural Science Foundation of China (no. 81101942).

References

- Baade PD, Youlten DR and Krnjacki LJ: International epidemiology of prostate cancer: geographical distribution and secular trends. *Mol Nutr Food Res* 53: 171-184, 2009.
- Siegel R, Ward E, Brawley O and Jemal A: Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 61: 212-236, 2011.
- Thiery JP, Acloque H, Huang RY and Nieto MA: Epithelial-mesenchymal transitions in development and disease. *Cell* 139: 871-890, 2009.
- Strutz F, Zeisberg M, Ziyadeh FN, *et al*: Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. *Kidney Int* 61: 1714-1728, 2002.
- Ikenouchi J, Matsuda M, Furuse M and Tsukita S: Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression of claudins/occludin by Snail. *J Cell Sci* 116: 1959-1967, 2003.
- Jorda M, Olmeda D, Vinyals A, *et al*: Upregulation of MMP-9 in MDCK epithelial cell line in response to expression of the Snail transcription factor. *J Cell Sci* 118: 3371-3385, 2005.
- Miyoshi A, Kitajima Y, Sumi K, *et al*: Snail and SIP1 increase cancer invasion by upregulating MMP family in hepatocellular carcinoma cells. *Br J Cancer* 90: 1265-1273, 2004.
- Kovacic JC, Mercader N, Torres M, Boehm M and Fuster V: Epithelial-to-mesenchymal and endothelial-to-mesenchymal transition: from cardiovascular development to disease. *Circulation* 125: 1795-1808, 2012.
- Kong D, Wang Z, Sarkar SH, *et al*: Platelet-derived growth factor-D overexpression contributes to epithelial-mesenchymal transition of PC3 prostate cancer cells. *Stem Cells* 26: 1425-1435, 2008.
- Valcourt U, Kowanetz M, Niimi H, Heldin CH and Moustakas A: TGF-beta and the Smad signaling pathway support transcriptional reprogramming during epithelial-mesenchymal cell transition. *Mol Biol Cell* 16: 1987-2002, 2005.
- Todaro GJ, Fryling C and De Larco JE: Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc Natl Acad Sci USA* 77: 5258-5262, 1980.
- Lippman ME: The development of biological therapies for breast cancer. *Science* 259: 631-632, 1993.
- Ching KZ, Ramsey E, Pettigrew N, D'Cunha R, Jason M and Dodd JG: Expression of mRNA for epidermal growth factor, transforming growth factor-alpha and their receptor in human prostate tissue and cell lines. *Mol Cell Biochem* 126: 151-158, 1993.
- Glynn-Jones E, Goddard L and Harper ME: Comparative analysis of mRNA and protein expression for epidermal growth factor receptor and ligands relative to the proliferative index in human prostate tissue. *Hum Pathol* 27: 688-694, 1996.
- Seth D, Shaw K, Jazayeri J and Leedman PJ: Complex post-transcriptional regulation of EGF-receptor expression by EGF and TGF-alpha in human prostate cancer cells. *Br J Cancer* 80: 657-669, 1999.
- DeHaan AM, Wolters NM, Keller ET and Ignatowski KM: EGFR ligand switch in late stage prostate cancer contributes to changes in cell signaling and bone remodeling. *Prostate* 69: 528-537, 2009.
- Scher HI, Sarkis A, Reuter V, *et al*: Changing pattern of expression of the epidermal growth factor receptor and transforming growth factor alpha in the progression of prostatic neoplasms. *Clin Cancer Res* 1: 545-550, 1995.
- Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
- Ma L, Young J, Prabhala H, *et al*: miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* 12: 247-256, 2010.
- Lin F, Lin P, Zhao D, *et al*: Sox2 targets cyclinE, p27 and survivin to regulate androgen-independent human prostate cancer cell proliferation and apoptosis. *Cell Prolif* 45: 207-216, 2012.
- Zhou F, Zhang L, Gong K, *et al*: LEF-1 activates the transcription of E2F1. *Biochem Biophys Res Commun* 365: 149-153, 2008.
- Zhou S, Liu Y, Prater K, Zheng Y and Cai L: Roles of microRNAs in pressure overload- and ischemia-related myocardial remodeling. *Life Sci* 93: 855-862, 2013.
- Chang Y, Dou Y, Bao H, *et al*: Multiple microRNAs targeted to internal ribosome entry site against foot-and-mouth disease virus infection in vitro and in vivo. *Virology* 11: 1, 2014.
- Luo Q, Wei C, Li X, *et al*: MicroRNA-195-5p is a potential diagnostic and therapeutic target for breast cancer. *Oncol Rep* 31: 1096-1102, 2014.
- Lv XB, Jiao Y, Qing Y, *et al*: miR-124 suppresses multiple steps of breast cancer metastasis by targeting a cohort of pro-metastatic genes in vitro. *Chin J Cancer* 30: 821-830, 2011.
- Xia J, Wu Z, Yu C, *et al*: miR-124 inhibits cell proliferation in gastric cancer through down-regulation of SPHK1. *J Pathol* 227: 470-480, 2012.
- Shi XB, Xue L, Ma AH, *et al*: Tumor suppressive miR-124 targets androgen receptor and inhibits proliferation of prostate cancer cells. *Oncogene* 32: 4130-4138, 2013.
- Gregory PA, Bert AG, Paterson EL, *et al*: The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 10: 593-601, 2008.

29. Kumarswamy R, Mudduluru G, Ceppi P, *et al*: MicroRNA-30a inhibits epithelial-to-mesenchymal transition by targeting Snail and is downregulated in non-small cell lung cancer. *Int J Cancer* 130: 2044-2053, 2012.
30. Martello G, Rosato A, Ferrari F, *et al*: A MicroRNA targeting dicer for metastasis control. *Cell* 141: 1195-1207, 2010.
31. Liang YJ, Wang QY, Zhou CX, *et al*: MiR-124 targets Slug to regulate epithelial-mesenchymal transition and metastasis of breast cancer. *Carcinogenesis* 34: 713-722, 2013.
32. Eger A, Aigner K, Sonderegger S, *et al*: DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. *Oncogene* 24: 2375-2385, 2005.
33. Comijn J, Berx G, Vermassen P, *et al*: The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell* 7: 1267-1278, 2001.
34. Yang J, Mani SA, Donaher JL, *et al*: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117: 927-939, 2004.
35. Cano A, Perez-Moreno MA, Rodrigo I, *et al*: The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2: 76-83, 2000.
36. Hajra KM, Chen DY and Fearon ER: The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res* 62: 1613-1618, 2002.
37. Wen FC, Chang TW, Tseng YL, Lee JC and Chang MC: hRAD9 functions as a tumor suppressor by inducing p21-dependent senescence and suppressing epithelial-mesenchymal transition through inhibition of Slug transcription. *Carcinogenesis*: Feb 5, 2014 (Epub ahead of print).
38. Xia H, Cheung WK, Ng SS, *et al*: Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. *J Biol Chem* 287: 9962-9971, 2012.
39. Tamagawa S, Beder LB, Hotomi M, *et al*: Role of miR-200c/miR-141 in the regulation of epithelial-mesenchymal transition and migration in head and neck squamous cell carcinoma. *Int J Mol Med* 33: 879-886, 2014.