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MicroRNA-338-3p inhibits the progression of bladder cancer through regulating ETS1 expression

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Abstract. – OBJECTIVE: MicroRNA-338-3p (miR-338-3p) was reported to influence the metastasis and development of several human cancers. However, in bladder cancer (BC), the special function of miR-338-3p remains unknown. Here, we aimed at exploring the miR-338-3p function in the progression of BC.

PATIENTS AND METHODS: miR-338-3p and ETS1 expressions were examined by quantitative Real-time polymerase chain reaction (qRT-PCR) in BC samples. Following that, transwell assays for cell migration and invasion were performed. And MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay for cell proliferation was conducted as well. Western blot was employed to examine the epithelial-mesenchymal transition (EMT) marker expressions. Finally, the relationship between miR-338-3p and E26 transformation specific-1 (ETS1) was verified by luciferase reporter assay.

RESULTS: The decreased miR-338-3p expression was examined in BC cells. Moreover, miR-338-3p upregulation repressed cell proliferation ability in BC. Next, miR-338-3p upregulation also depressed cell metastasis and EMT in BC cells. Furthermore, ETS1 was a direct target of miR-338-3p and inversely associated with its expression. And upregulation of ETS1 partially rescued the suppression of miR-338-3p for cell proliferation and metastasis in BC.

CONCLUSIONS: Upregulation of miR-338-3p inhibited the proliferation, metastasis and EMT in BC by suppressing ETS, showing that miR-338-3p might block the development of BC through regulating ETS1 expression.

Key Words:
Bladder cancer, miR-338-3p, EMT, Proliferation, Metastasis, ETS1.

Introduction

Bladder cancer (BC) is the most common malignancy in the urinary system, and its morbidity is higher than other tumors in the urological malignancy[1]. And BC is often occurred in the elderly over the age of 50[2]. Moreover, the high incidence of BC is usually associated with the increased age[3]. And the occurrence of BC is closely related to the diet, smoking and drinking water, indicating that preventing BC should firstly grab these sources[4]. In addition, early patients with BC may choose surgery based on their personal preference, but about 70% of patients will relapse after surgery[5]. Therefore, the key to improve prognosis is early diagnosis and treatment. Recently, abnormal expressions of microRNAs (miRNAs) have been identified in many human cancers, which is significantly associated with the tumor incidence and progression[6-8]. And as a key regulator, miRNA has been found to participate in several signaling pathways to affect the tumorigenesis of human cancers[9]. Especially, miR-338-3p was located on the seventh intron of apoptosis-associated tyrosine kinase (AATK) gene, which was identified to regulate AATK expression in rat neurons[10]. Moreover, miR-338-3p downregulation had been detected in hepatocellular carcinoma[11], glioblastoma[12], and non-small-cell lung cancer[13]. And some target genes of miR-338-3p had been also been partially examined, including SOX4[14], ALK5[15], and PKM2[16]. However, the special function of miR-338-3p for the

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progression of BC has not been investigated by now. E26 transformation specific-1 (ETS1) belonging to the ETS family of transcription factors was found to involve in cell metastasis[17]. And ETS-1 had been reported to regulate the expression of microRNA-126 in endothelial cells[18]. More importantly, it was found that ETS1 as a proto-oncogene was affected by miR-1[19], miR-144[20] and miR-377[21]. Moreover, ETS1 expression was found to associate with the progression of gastric cancer[22], hepatocellular carcinoma[23] and breast cancer[24]. Nevertheless, the relationship among miR-338-3p and ETS1 in BC is still unclear. In our research, the abnormal miR-338-3p expressions and the relationship with ETS1 were investigated in BC. Moreover, we emphasized the effects of miR-338-3p and ETS1 related to the progression of BC. Finally, the interaction between miR-338-3p and ETS1 was confirmed in BC.

Patients and Methods

Patients

Thirty-nine paired surgical BC specimens and adjacent tissue samples were acquired from the Second Hospital of Shandong University after receiving signature written informed consent. All BC patients received no treatment prior to the operation. Then, theses samples were frozen in liquid nitrogen and stored at -80° C refrigerator to be used in the further experiment. This study was approved by the Institutional Ethics Committee of the Second Hospital of Shandong University (Shandong, China).

Cell Culture and Transfection

The J82, 5637, T24 cell lines and SV-HUC-1 (normal bladder epithelial cell) were used for this experiment. These cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Next, these cells were seeded in Dulbecco's modified eagle medium (DMEM) (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and cultured at 37°C with 5% CO_2. The miR-338-3p mimic and inhibitor, ETS1 siRNA (si-ETS1), were purchased from Biomics Biotechnology Inc. (Nanjing, China); then, they were transferred into BC cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) based on the manufactures' protocols.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was applied to extract total RNA containing miRNA to quantitate miR-338-3p level in BC. qRT-PCR was carried out through the SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) on ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were applied as the control of miR-338-3p and ETS1. And their expressions were calculated using the $2^{-\Delta\Delta ct}$ method.

Dual Luciferase Assay

The wild or mutant type of 3'-UTR of ETS1 was cloned into the pmirGLO luciferase vector (Promega, Madison, WI, USA) to perform luciferase reporter experiments. Wild or mutant type of 3'-UTR of ETS1 and miR-338-3p mimics were transfected into HEK293T cells. Subsequently, the Dual Luciferase Assay System (Promega, Madison, WI, USA) was applied to analyze luciferase activity.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assay For Cell Proliferation

The MTT assay (Sigma-Aldrich, St. Louis, MO, USA) was applied to measure the proliferation of BC cells. Cells (4×10^3/well) were cultured onto 96-well plates in medium. The cells containing miR-338-3p mimics or inhibitor were incubated for 0-96 h. After incubation, the cells added with MTT were incubated for 4 h at 37°C. The absorbance at 490 nm (OD = 490 nm) was detected with a spectrophotometer.

Transwell Assays for Cell Migration and Invasion

Transwell chambers (8 μm pore size; Millipore, Billerica, MA, USA) were applied to evaluate the migratory and invasive ability of BC cells in 24-well plates. 4×10^4 BC cells without serum were put in the upper chamber on the non-coated membrane, and lower chamber filled with 10% fetal bovine serum (FBS) to induce BC cells to migrate or invade through the membrane. And the cells were put into the upper chamber with the coated membrane for invasion assay. Then, these cells were incubated for cell migration and invasion. These cells were stained with 0.1% crystal violet. A microscope was used for counting migrated and invaded cells.

Western Blot Analysis

The protein samples were obtained using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). The protein was separated through a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and incubated with 5% non-fat milk in polyvinylidene difluoride (PVDF) membranes at room temperature. Next, we incubated the membranes overnight at 4°C with rabbit polyclonal anti-EST1 (1:1000; Abcam, Cambridge, MA, USA), rabbit polyclonal anti-GAPDH antibody (1:1000; Abcam, Cambridge, MA, USA) and subsequently incubated with goat polyclonal anti-rabbit IgG secondary antibody (1:2000; Abcam, Cambridge, MA, USA). Then, protein expression levels were measured by ECL (ECL, Pierce, Rockford, IL, USA). In addition, the antibodies against Vimentin, E-cadherin and N-cadherin were obtained from Abcam (Cambridge, MA, USA).

Statistical Analysis

The data were analyzed by Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) and Graphpad Prism 6 (La Jolla, CA, USA). Clinicopathological parameters were compared using the χ^2-test, and difference was calculated according to Student's *t*-test. Significant difference was defined at $p < 0.05$.

Results

MiR-338-3p Expression was Examined in BC Cells

Here, the miR-338-3p expression levels were firstly measured in BC tissues *via* qRT-PCR. And miR-338-3p expressions were apparently declined in BC tissues (Figure 1A). Besides that, the same tendency of miR-338-3p expression was also observed in J82, 5637 and T24 cell lines (Figure 1B). Moreover, lower miR-338-3p expressions were identified in T24 cells selected for the following study. Furthermore, the association among miR-338-3p expression and their clinic-pathological characteristics in BC was explored in our research as well. Especially, low expressions of miR-338-3p were detected to be related to TNM stage ($p = 0.036$) and lymph node metastasis ($p = 0.007$, Table I).

MiR-338-3p Impaired the Proliferation of BC Cells

Then, we transfected miR-338-3p mimics or inhibitors into T24 cells to further explored its function in BC. And Figure 2A, 2B showed that miR-338-3p mimics reduced miR-338-3p level while miR-338-3p inhibitor enhanced miR-338-3p level. Besides that, the MTT assay revealed that miR-338-3p mimics repressed cell proliferation in BC (Figure 2C). Inversely, miR-338-3p downregulation promoted the proliferation in T24 cells (Figure 2D). Hence, miR-338-3p upregulation impaired the proliferation of BC cells.

MiR-338-3p Repressed Cell Metastasis in BC Cells

Next, the miR-338-3p function was investigated for cell metastasis through analyzing cell migration, invasion and epithelial-mesenchymal transition (EMT) in transfected T24 cells. Firstly, miR-338-3p mimics were found to prevent cell migration in T24 cells when miR-338-3p

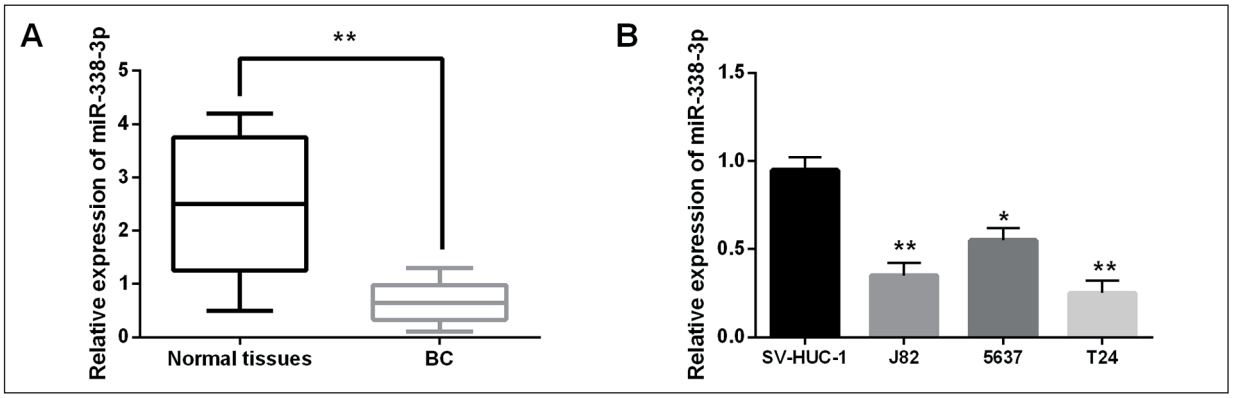

Figure 1. The expressions of miR-338-3p were examined in BC cells. ***A,*** The expressions of miR-338-3p were identified *via* qRT-PCR in BC tissues ***B,*** MiR-338-3p expressions in J82, 5637, T24 and SV-HUC-1 cell lines. *$p < 0.05$, **$p < 0.01$.

Table I. Relationship between miR-338-3p expression and their clinicopathological characteristics of bladder cancer patients.

Characteristics	No. of cases (n = 39)	miR-338-3p		*p*-value
		High	Low	
Age (years)				0.075
≥ 60	24	7	17	
< 60	15	5	10	
Gender				0.818
Male	21	6	15	
Female	18	6	12	
Tumor size				0.139
< 3 cm	16	4	12	
≥ 3 cm	23	8	15	
TNM stage				0.036*
I + II	9	3	6	
III + IV	30	9	21	
Lymph node metastasis				0.007*
Yes	11	3	7	
No	28	9	20	
Tumor grade				0.215
Low	19	6	8	
High	20	6	19	

Statistical analyses were performed by the χ^2-test. *$p < 0.05$ was considered significant.

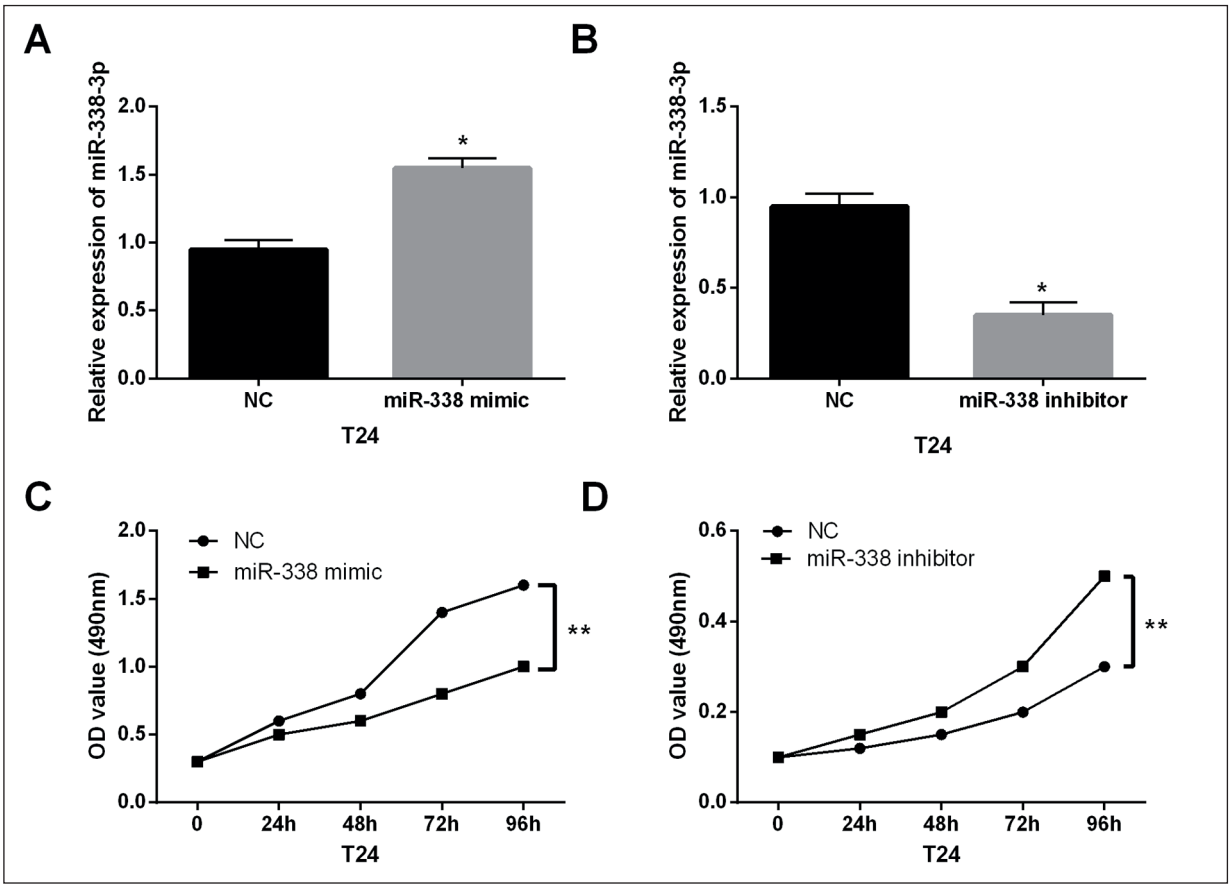

Figure 2. MiR-338-3p repressed cell proliferation in BC cells. ***A, B,*** The miR-338-3p expressions was examined in T24 cells contained miR-338-3p mimics or inhibitor *via* qRT-PCR ***C, D,*** The cell proliferation was measured in cells contained miR-338-3p mimics or inhibitor *via* MTT assay. **$p < 0.01$.

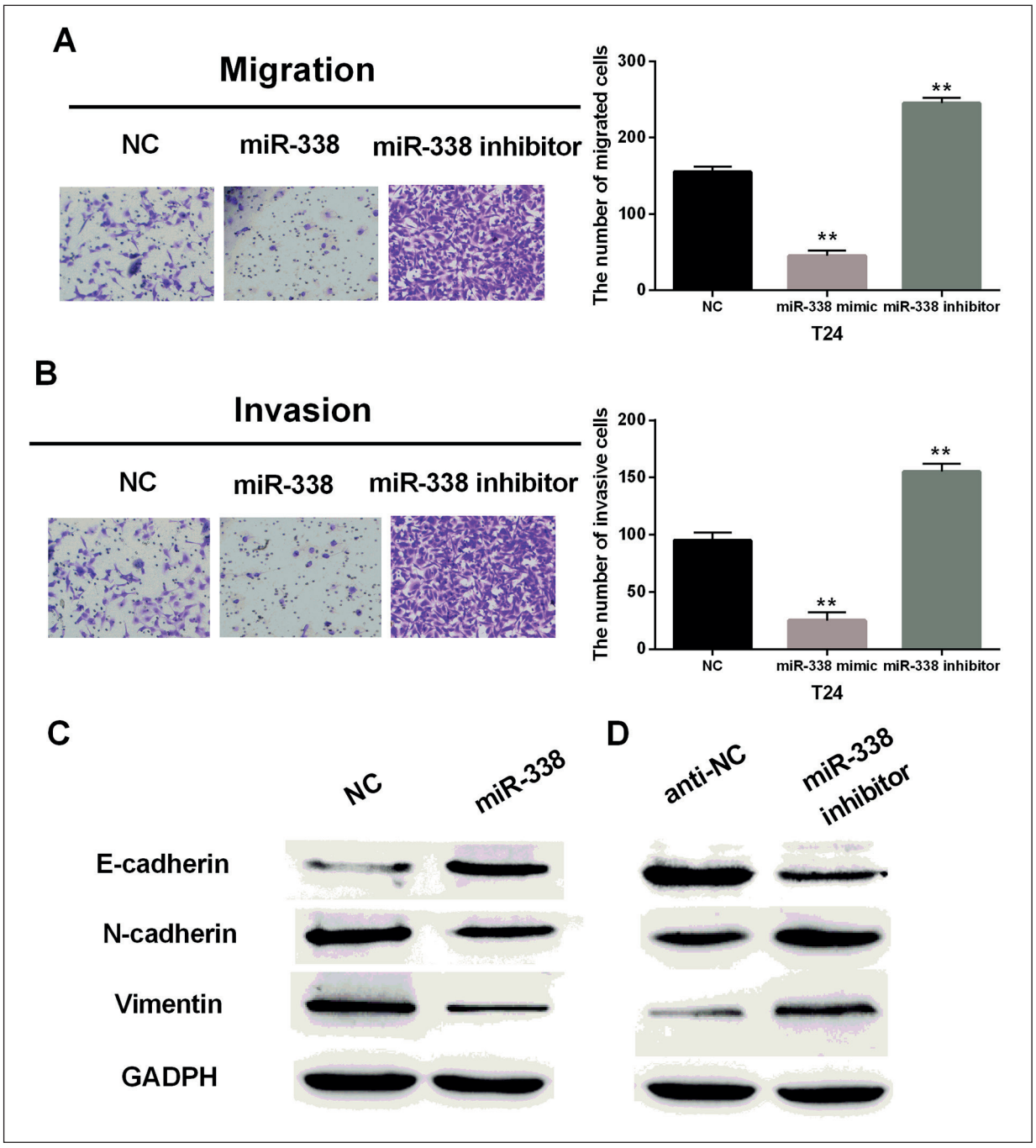

Figure 3. MiR-338-3p suppressed cell metastasis in BC. ***A, B,*** Cell migration and invasion analysis in T24 cells with miR-338-3p mimics or inhibitor was detected by transwell assay. ***C, D,*** Western blot analysis of E-cadherin, Vimentin and N-cadherin in T24 cells contained miR-338-3p mimics or inhibitor. $^{**}p < 0.01$.

inhibitor improved its ability (Figure 3A). And the same results were also detected for cell invasion in T24 cells as shown in Figure 3B. Furthermore, the results of EMT revealed that miR-338-3p overexpression promoted E-cadherin expression and repressed N-cadherin and Vimentin expressions (Figure 3C). Conversely, the silence of miR-338-3p reduced E-cadherin expression and promoted N-cadherin and Vimentin expressions (Figure 3D). Taken together, miR-338-3p upregulation inhibited cell metastasis and EMT in BC.

ETS1 Was a Target Gene of miR-338-3p in BC

Furthermore, ETS1 was predicted as a target of miR-338-3p as the prediction of TargetScan (http://www.targetscan.org/vert_71/). The binding site among miR-338-3p and ETS1 was shown in Figure 4A. In order to verify the above prediction, Dual Luciferase Assay was conduct. The results suggested miR-338-3p mimics declined the luciferase activity of wt-ETS1 and had no effect on that of mutant type of ETS1 (Figure 4B). Besides that, the negative association among miR-338-3p and ETS1 was also found in this study ($p < 0.001$, $R^2 = 0.5513$, Figure 4C). Furthermore, the ETS1 expression was detected in T24 cells containing miR-338-3p mimics or inhibitor. The miR-338-3p overexpression blocked ETS1 expression whereas miR-338-3p downregulation promoted ETS1 expression (Figure 4D, 4E). Collectively, we considered miR-338-3p directly targeted ETS1 and inversely associated with its expression.

The Function of ETS1 was Identified in the Progression of BC

Afterwards, the ETS1 siRNA was transfected into T24 cells to explore its function in BC. And si-ETS1 significantly decreased the expression of ETS1 (Figure 5A). Functionally,

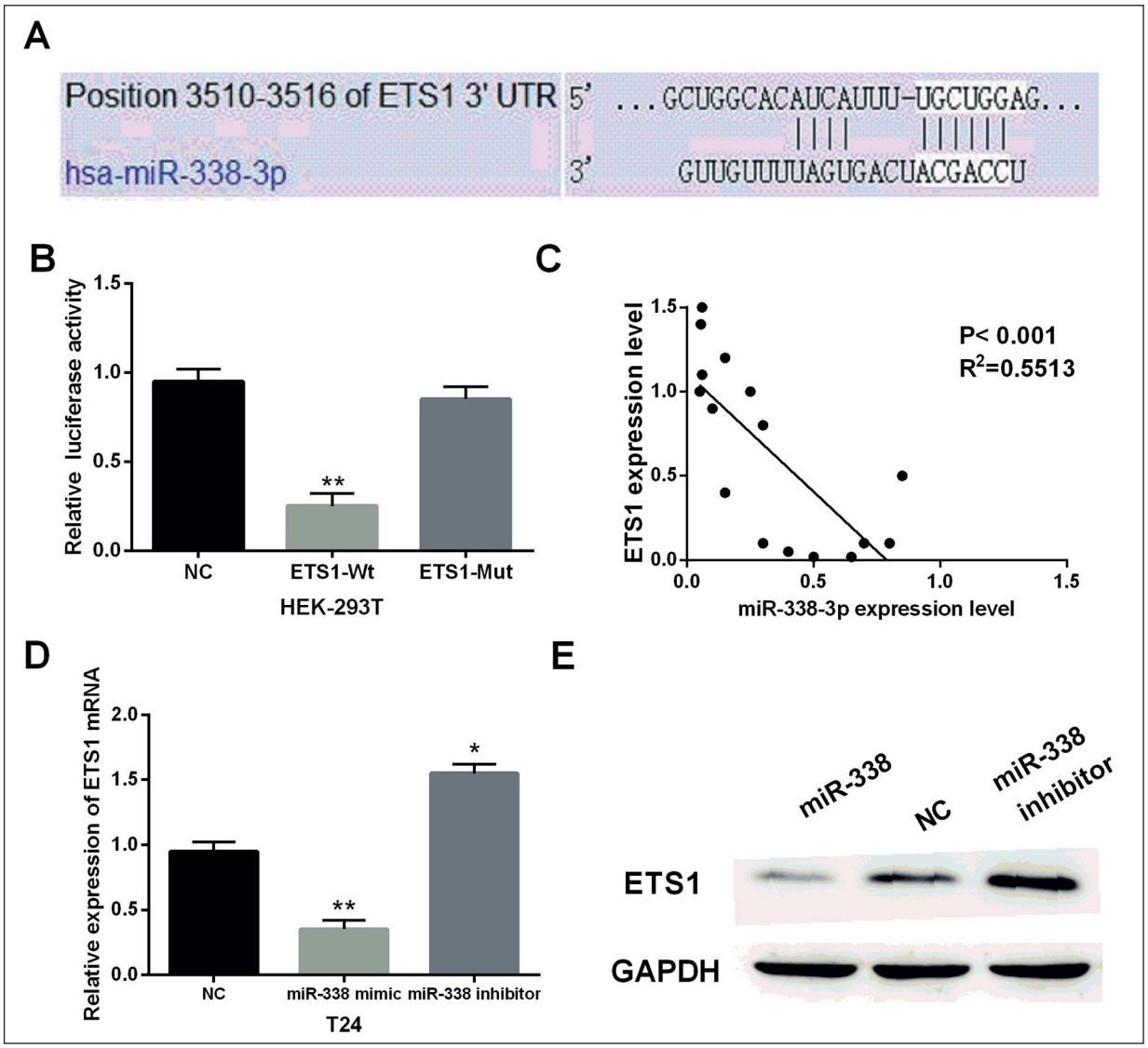

Figure 4. MiR-338-3p directly targeted ETS1 in BC. ***A,*** The binding site of miR-388-3p on the 3'-UTR of ETS1. ***B,*** Luciferase reporter assays. ***C,*** The association between miR-388-3p and ETS1. ***D, E,*** The expressions of ETS1 were analyzed in cells containing miR-388-3p mimics or inhibitor **$p < 0.01$.

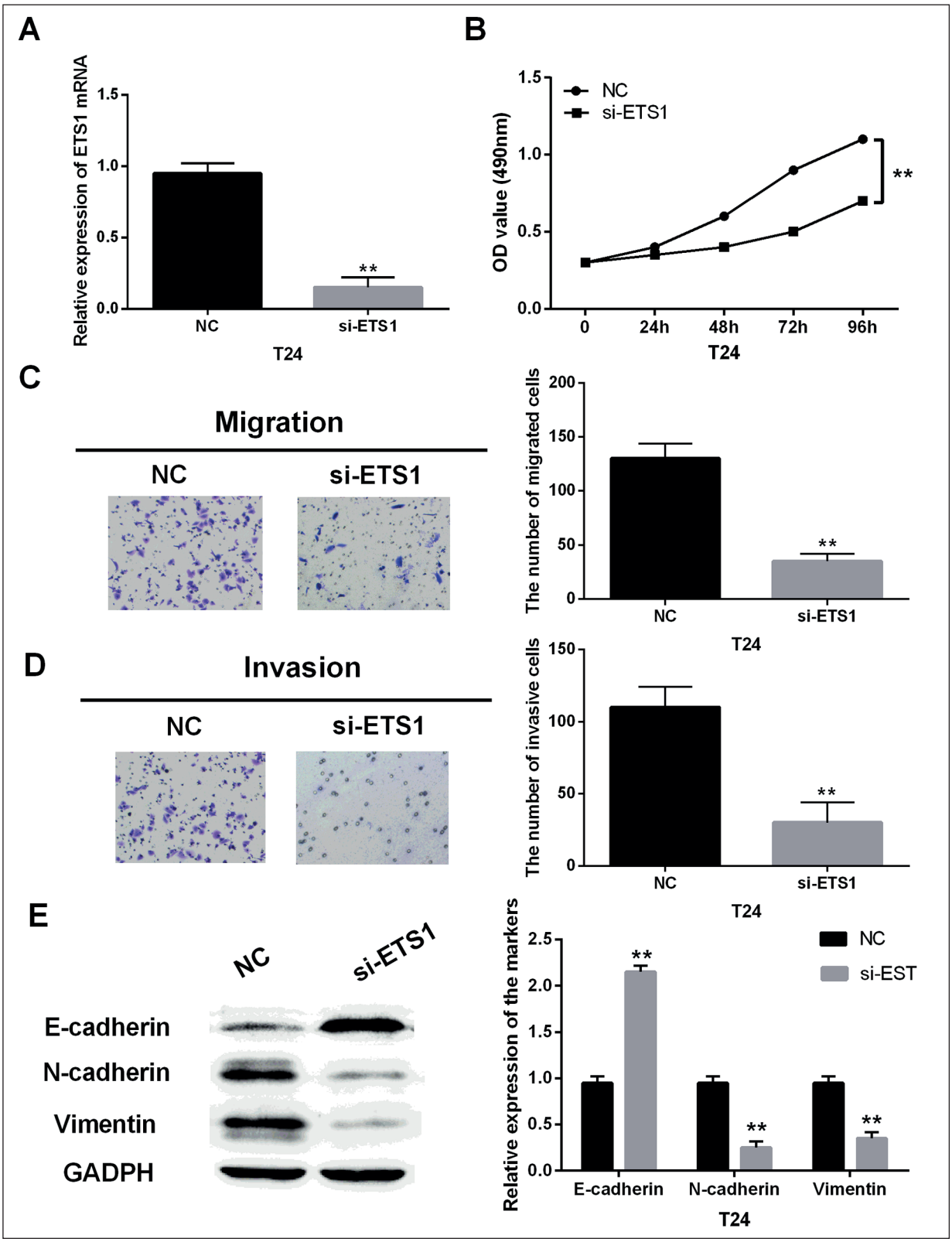

Figure 5. The function of ETS1 was identified in the progression of BC. ***A,*** The expression of ETS1 was measured in cells containing ETS1 siRNA. ***B,*** The cell proliferation was measured in cells with si-EST1 *via* MTT. ***C, D,*** Cell migration and invasion in T24 cells with si-EST1 were detected *via* transwell assay. ***E,*** Western blot analysis of E-cadherin, Vimentin and N-cadherin in T24 cells contained si-EST1. $^{**}p < 0.01$.

the ability of cell proliferation was impeded by si-ETS1 in T24 cells (Figure 5B). Similarly, the inhibitory effect of si-ETS1 was also examined for cell migration and invasion (Figure 5C, 5D). Besides that, si-ETS1 enhanced E-cadherin level and repressed N-cadherin and Vimentin expressions in T24 cells (Figure 5E). In conclusion, ETS1 silence obstructed the progression of BC through suppressing cell proliferation and metastasis.

Upregulation of ETS1 Partially Rescued the Suppression of miR-338-3p in BC

Finally, we transfected miR-338-3p mimics and ETS1 vector into T24 cells to further confirmed their relationship. Interestingly, the expression of ETS1 was not changed in cells containing miR-338-3p mimics and ETS1 vector while decreased in cells only containing miR-338-3p mimics (Figure 6A). More importantly, we found that ETS1 overexpression partially rescued the suppression

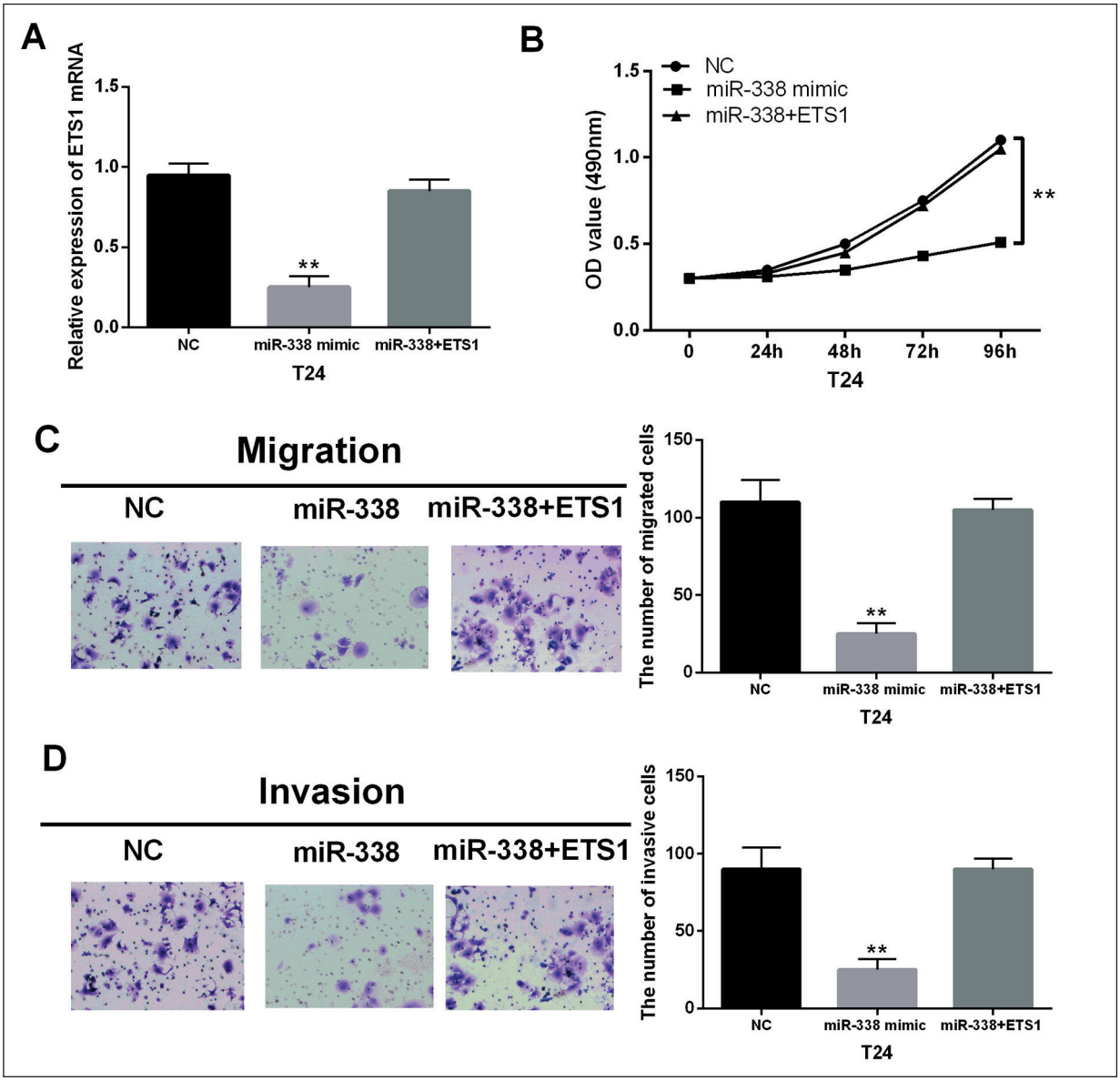

Figure 6. Upregulation of ETS1 partially rescued the suppression of miR-338-3p for cell proliferation and metastasis. ***A,*** The EST1 expression was measured in cells containing EST1 vector and miR-338-3p. ***B,*** The proliferation was measured in cells with EST1 vector and miR-338-3p *via* MTT. ***C, D,*** The migration and invasion in cells containing EST1 vector and miR-338-3p were measured by transwell assay. $^{**}p < 0.01$.

of miR-338-3p for the proliferation of T24 cells (Figure 6B). Besides that, the same results were also detected for cell migration and invasion in T24 cells (Figure 6C, 6D). In brief, upregulation of ETS1 partially rescued the suppression of cell proliferation and metastasis induced by miR-338-3p in BC.

Discussion

In recent years, many miRNAs had been found to express aberrantly and involve in the progression of BC, such as miR-23b, miR-24, miR-186 and miR-429[25-28]. In current research, miR-338-3p downregulation was firstly demonstrated in BC. And miR-338-3p upregulation was observed to suppress cell proliferation, metastasis and EMT in BC. These findings indicated that miR-338-3p influenced the tumorigenesis and development of BC. Moreover, Liang et al[29] reported the diagnostic significance and potential function of miR-338 in hepatocellular carcinoma. And the miR-338-3p downregulation was also observed in non-small cell lung cancer, which was negatively related to TNM and lymph node metastasis[30], indicating that miR-338-3p could be used as a biomarker to diagnose human cancers. Consistently, miR-338-3p expressions were also reduced in BC, which was related to TNM stage and lymph node metastasis. In addition, Liu et al[31] revealed that miR-338 repressed cell proliferation through regulating CTBP2 in glioma. In our study, we also found the same function of miR-338-3p for the proliferation of BC cells. Besides that, it was reported thT miR-338-3p repressed cell EMT and metastasis in hepatocellular carcinoma[32]. In the meantime, the same miR-338-3p function for EMT and metastasis was also examined in this study. Furthermore, miR-338-3p was verified to directly target ETS1 and inversely associated with its expression in this study. And upregulation of ETS1 partially rescued the suppression of miR-338-3p in the development of BC. It had been demonstrated miR-9 suppressed cell metastasis and proliferation *via* targeting ETS1 in gastric cancer[33]. Similarly, we also found that miR-338-3p suppressed cell proliferation and metastasis in BC through suppressing ETS1. Additionally, Zhang et al[20] reported that abnormal expression of ETS1 was closely related to EMT in laryngeal squamous cell carcinoma. Consistent with previous study, we revealed the ETS1 silence could inhibit EMT in BC. Combined with all these findings, miR-338-3p was speculated to prevent the progression of BC through repressing ETS1 expression.

Conclusions

We revealed that miR-338-3p downregulation was detected in BC, which was related to the lymph node metastasis and TNM stage. Moreover, upregulation of miR-338-3p repressed the proliferation, metastasis and EMT of BC through directly inhibiting ETS1. We hoped that miR-338-3p could function as a new biomarker to diagnose and treat BC patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Metts MC, Metts JC, Milito SJ, Thomas CJ. Bladder cancer: a review of diagnosis and management. J Natl Med Assoc 2000; 92: 285-294.
2) Griffiths TR. Current perspectives in bladder cancer management. Int J Clin Pract 2013; 67: 435-448.
3) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
4) Bulbul MA, Husseini N, Houjaij A. Superficial bladder cancer epidemiology, diagnosis and management. J Med Liban 2005; 53: 107-113.
5) Shen Z, Shen T, Wientjes MG, O'Donnell MA, Au JL. Intravesical treatments of bladder cancer: review. Pharm Res 2008; 25: 1500-1510.
6) Yuan L, Yuan P, Yuan H, Wang Z, Run Z, Chen G, Zhao P, Xu B. miR-542-3p inhibits colorectal cancer cell proliferation, migration and invasion by targeting OTUB1. Am J Cancer Res 2017; 7: 159-172.
7) Zhang G, Liu Z, Xu H, Yang Q. miR-409-3p suppresses breast cancer cell growth and invasion by targeting Akt1. Biochem Biophys Res Commun 2016; 469: 189-195.
8) Tian ZG, Zhuang Y, Jin Z, Zhou F, Zhu LF, Shen PC. MicroRNA-337-5p participates in the development and progression of osteosarcoma via ERBB, MAPK and VEGF pathways. Eur Rev Med Pharmacol Sci 2018; 22: 5460-5470.
9) Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013; 14: 475-488.
10) Kos A, Olde LN, Wieczorek ML, Glennon JC, Martens GJ, Kolk SM, Aschrafi A. A potential regulato-

ry role for intronic microRNA-338-3p for its host gene encoding apoptosis-associated tyrosine kinase. PLoS One 2012; 7: e31022.

11) Zhang T, Liu W, Zeng XC, Jiang N, Fu BS, Guo Y, Yi HM, Li H, Zhang Q, Chen WJ, Chen GH. Down-regulation of microRNA-338-3p promoted angiogenesis in hepatocellular carcinoma. Biomed Pharmacother 2016; 84: 583-591.

12) Lei D, Zhang F, Yao D, Xiong N, Jiang X, Zhao H. MiR-338-5p suppresses proliferation, migration, invasion, and promote apoptosis of glioblastoma cells by directly targeting EFEMP1. Biomed Pharmacother 2017; 89: 957-965.

13) Zhang G, Zheng H, Zhang G, Cheng R, Lu C, Guo Y, Zhao G. MicroRNA-338-3p suppresses cell proliferation and induces apoptosis of non-small-cell lung cancer by targeting sphingosine kinase 2. Cancer Cell Int 2017; 17: 46.

14) Tong Z, Meng X, Wang J, Wang L. MicroRNA-338-3p targets SOX4 and inhibits cell proliferation and invasion of renal cell carcinoma. Exp Ther Med 2017; 14: 5200-5206.

15) Zhang X, Wang C, Li H, Niu X, Liu X, Pei D, Guo X, Xu X, Li Y. miR-338-3p inhibits the invasion of renal cell carcinoma by downregulation of ALK5. Oncotarget 2017; 8: 64106-64113.

16) Zhang Y, Shi B, Chen J, Hu L, Zhao C. MiR-338-3p targets pyruvate kinase M2 and affects cell proliferation and metabolism of ovarian cancer. Am J Transl Res 2016; 8: 3266-3273.

17) Oda N, Abe M, Sato Y. ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin beta3. J Cell Physiol 1999; 178: 121-132.

18) Harris TA, Yamakuchi M, Kondo M, Oettgen P, Lowenstein CJ. Ets-1 and Ets-2 regulate the expression of microRNA-126 in endothelial cells. Arterioscler Thromb Vasc Biol 2010; 30: 1990-1997.

19) Wei W, Hu Z, Fu H, Tie Y, Zhang H, Wu Y, Zheng X. MicroRNA-1 and microRNA-499 downregulate the expression of the ets1 proto-oncogene in HepG2 cells. Oncol Rep 2012; 28: 701-706.

20) Zhang SY, Lu ZM, Lin YF, Chen LS, Luo XN, Song XH, Chen SH, Wu YL. miR-144-3p, a tumor suppressive microRNA targeting ETS-1 in laryngeal squamous cell carcinoma. Oncotarget 2016; 7: 11637-11650.

21) Wang R, Ma Y, Yu D, Zhao J, Ma P. miR-377 functions as a tumor suppressor in human clear cell renal cell carcinoma by targeting ETS1. Biomed Pharmacother 2015; 70: 64-71.

22) Zheng L, Pu J, Qi T, Qi M, Li D, Xiang X, Huang K, Tong Q. miRNA-145 targets v-ets erythroblastosis virus E26 oncogene homolog 1 to suppress the invasion, metastasis, and angiogenesis of gastric cancer cells. Mol Cancer Res 2013; 11: 182-193.

23) Ma N, Chen F, Shen SL, Chen W, Chen LZ, Su Q, Zhang LJ, Bi J, Zeng WT, Li W, Huang XH, Wang Q. MicroRNA-129-5p inhibits hepatocellular carcinoma cell metastasis and invasion via targeting ETS1. Biochem Biophys Res Commun 2015; 461: 618-623.

24) Zhang Y, Yan LX, Wu QN, Du ZM, Chen J, Liao DZ, Huang MY, Hou JH, Wu QL, Zeng MS, Huang WL, Zeng YX, Shao JY. miR-125b is methylated and functions as a tumor suppressor by regulating the ETS1 proto-oncogene in human invasive breast cancer. Cancer Res 2011; 71: 3552-3562.

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

26) Zhang S, Zhang C, Liu W, Zheng W, Zhang Y, Wang S, Huang D, Liu X, Bai Z. MicroRNA-24 upregulation inhibits proliferation, metastasis and induces apoptosis in bladder cancer cells by targeting CARMA3. Int J Oncol 2015; 47: 1351-1360.

27) Yao K, He L, Gan Y, Zeng Q, Dai Y, Tan J. MiR-186 suppresses the growth and metastasis of bladder cancer by targeting NSBP1. Diagn Pathol 2015; 10: 146.

28) Wu CL, Ho JY, Chou SC, Yu DS. MiR-429 reverses epithelial-mesenchymal transition by restoring E-cadherin expression in bladder cancer. Oncotarget 2016; 7: 26593-26603.

29) Liang L, Gao L, Zou XP, Huang ML, Chen G, Li JJ, Cai XY. Diagnostic significance and potential function of miR-338-5p in hepatocellular carcinoma: a bioinformatics study with microarray and RNA sequencing data. Mol Med Rep 2018; 17: 2297-2312.

30) Zhang P, Shao G, Lin X, Liu Y, Yang Z. MiR-338-3p inhibits the growth and invasion of non-small cell lung cancer cells by targeting IRS2. Am J Cancer Res 2017; 7: 53-63.

31) Liu DZ, Zhao H, Zou QG, Ma QJ. MiR-338 suppresses cell proliferation and invasion by targeting CTBP2 in glioma. Cancer Biomark 2017; 20: 289-297.

32) Chen JS, Liang LL, Xu HX, Chen F, Shen SL, Chen W, Chen LZ, Su Q, Zhang LJ, Bi J, Zeng WT, Li W, Ma N, Huang XH. miR-338-3p inhibits epithelial-mesenchymal transition and metastasis in hepatocellular carcinoma cells. Oncotarget 2017; 8: 71418-71429.

33) Zheng L, Qi T, Yang D, Qi M, Li D, Xiang X, Huang K, Tong Q. microRNA-9 suppresses the proliferation, invasion and metastasis of gastric cancer cells through targeting cyclin D1 and Ets1. PLoS One 2013; 8: e55719.