

Inhibition of epithelial-mesenchymal transition in gastric cancer cells by miR-711-mediated downregulation of CD44 expression

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Abstract. Gastric cancer is a common malignancy worldwide. The prognosis of early stage gastric cancer patients has significantly improved in recent years. However, in progressive stage gastric cancer patients, the prognosis remains relatively poor due to tumor metastases. In our previous study, we showed that the expression of miR-711 in gastric cancer tissues is low, and restoration of miR-711 inhibited the invasion and migration and the occurrence of epithelial-mesenchymal transition (EMT) in gastric cancer cells. Yet, the mechanisms involved in these processes remain unknown. In the present study, we demonstrated that miR-711-mediated downregulation of CD44 expression inhibited EMT of gastric cancer cells *in vitro* and *in vivo* by downregulating vimentin protein expression and upregulating E-cadherin protein expression through transfection, qRT-PCR and western blotting. Therefore, miR-711 may provide a promising target for EMT-related therapy for gastric cancer.

Introduction

Gastric cancer is a common malignancy of the digestive tract that is highly prevalent in Asia, particularly in China and Japan. Indeed, the incidence of gastric cancer is ranked

4th and 2nd in malignant cancer rankings in the world and in China, respectively, and worldwide, its mortality rate remains very high (1). Although the advancement in endoscopy has significantly improved the diagnosis and prognosis of early stage gastric cancer patients, at the time of definitive diagnosis, most patients already present with lymph node and distant metastases (2). Since tumor invasion and metastasis are critical factors that affect patient prognosis, understanding the mechanisms of tumor invasion and metastasis are of utmost importance.

Previous studies have shown that a key process, known as epithelial-mesenchymal transition (EMT), occurs during tumor invasion and metastasis. EMT refers to the loss of cell polarity, intercellular adhesion and junctions, increased migratory and invasive capacities in tumor epithelial cells (3). EMT is a complex process that is characterized by increased fibrous morphology and invasiveness of cells, reduced cell apoptosis and an increase in extracellular matrix content (4). In previous studies, EMT-induced primary tumor metastases have been reported to cause poor prognosis in various cancer (5-7). Therefore, EMT is an important mechanism underlying tumor invasion and metastasis, that in recent years has become an area of intensive research.

MicroRNAs (miRNAs) are non-coding RNAs that are approximately 20-25 nucleotides in length. miRNAs are involved in several biological processes, including development, cell proliferation, cell differentiation and cell apoptosis. In addition, miRNAs are associated with the development and progression of various cancers (8). The biological effects of miRNAs are mediated through the binding of miRNAs to the 3' UTR of target mRNAs via the seed regions (the 2nd to 7th base at the 5' end) and through Argonaute (Ago) protein-dependent degradation of mRNAs or blocking of mRNA translation (9). Zhang *et al* demonstrated that miR-181a is highly expressed in gastric cancer, and was found to promote gastric cancer cell proliferation, invasion and migration by binding to the 3' UTR of the anti-oncogene KLF6, thereby inhibiting KLF6

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expression (10). Moreover, Chen *et al* showed that miR-379-5p is expressed at a low level in liver cancer tissues and cells, and inhibited liver cancer cell migration, invasion and metastasis both *in vitro* and *in vivo*. Additional studies have demonstrated that miR-379-5p binds to the 3' UTR of focal adhesion kinase (FAK) to inhibit FAK expression (11). These findings have demonstrated that miRNAs play a critical role in tumor development, progression and metastasis. The recent development of miRNA microarray analysis of gastric cancer has facilitated the identification of gastric cancer development-, progression- and prognosis-related miRNAs, and includes members of the miR-200 family, miR-27, miR-373, miR-148, miR-129 and miR-711 (12-17). However, knowledge concerning these miRNAs only represent the tip of the iceberg, as the function of many miRNAs is still unknown.

In our previous study, we used miRNA microarray and identified miR-711 as an miRNA with low expression in gastric cancer. In addition, we confirmed that the expression of miR-711 in gastric cancer tissues was also low. Further investigation demonstrated that miR-711 inhibited gastric cancer cell invasion and migration, however the mechanism was unclear (17). In the present study, we demonstrated that miR-711 regulated EMT of gastric cancer cells by downregulating CD44 expression both *in vivo* and *in vitro*.

Materials and methods

Cell culture. Human gastric cancer cell lines MGC-803 and SGC-7901 provided by the Cancer Research Institute of the University of South China (Hengyang, China) were cultured in RPMI-1640 (Gibco; Thermo Fisher Scientific, Scoresby, VIC, Australia) culture medium, containing 10% fetal calf serum (FCS) in a 5% CO₂ constant-temperature incubator (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Transfection. SGC-7901 and MGC-803 human gastric cancer cells were seeded and cultured in 6-well plates for ~24 h. Once the cells reached 40-60% confluency, they were transfected with miR-711 mimics (Shanghai GenePharma, Co., Ltd., Shanghai, China), miR-711 inhibitor (Shanghai GenePharma, Co., Ltd.), or CD44 inhibitor (Shanghai GenePharma, Co., Ltd.) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Empty plasmid-transfected and non-transfected (blank) cells were used as controls. The 6-well plates were placed into the incubator, and after 6 h, the medium in each well was replaced with fresh 10% FCS-containing RPMI-1640 medium. The transfection efficiency was calculated at 48 h post-transfection, and cells with >60% transfection efficiency were used for subsequent experiments. The transfection efficiency = number of cells containing green fluorescence/a randomly observed visual field consisting of 100 cells using an optical microscope (Fig. 1).

Transwell invasion assay. After coating of the Transwell chamber with Matrigel, 500 μ l of 10% FCS-containing RPMI-1640 medium was added into the lower chamber, and 100 μ l of serum-free RPMI-1640 medium was added to the upper chamber. At 48 h post-transfection, the cells were digested with trypsin, prepared into a single-cell suspension,

seeded at a density of 1×10^5 cells/100 μ l into the upper chamber and cultured for 24 h. Cells were fixed with 4% formaldehyde, stained with crystal violet and observed under a light microscope (Olympus Corp., Tokyo, Japan) at a magnification of x100. Ten fields were randomly chosen and the number of transmigrated cells in each field was calculated by summing the Transwell cells. The assay was performed in triplicate and the mean \pm SD of the number of transmigrated cells was calculated.

Scratch wound healing assay. At 48 h post-transfection, cells were digested, prepared into single-cell suspensions and seeded into 6-well plates. Once the cells reached 80% confluency, a horizontal line was scratched across each well using a pipette, and the wells were washed twice in phosphate-buffered saline (PBS). Cells were photographed at 0 and 48 h post-scratch using a light microscope (Olympus Corp.) to evaluate healing at the scratch site.

Dual-Luciferase reporter assay. Wild-type and mutated CD44 3' UTR vectors (Shanghai GeneChem, Co., Ltd., Shanghai China) were transfected into gastric cancer cells SGC-7901 that were transfected with miR-711 mimics or miR-711 NC (empty-transfected and used as a control) using the Dual-Glo™ Luciferase Assay System kit protocol (Promega, Madison, WI, USA). Firefly luminescence (M1) and *Renilla* luminescence (M2) were measured using a fluorescence detector of light signal by fluorescence microscope (Olympus Corp.).

Hematoxylin and eosin staining (H&E). Xenograft tumors were harvested and paraffin-embedded. Sections were cut and stained with hematoxylin and eosin (H&E). Cell nuclei were stained blue-purple by hematoxylin and the cytoplasm was stained pink with eosin. Tumor cell morphology and tumor cell number were determined at a magnification of x100 using a light microscope (Olympus Corp.).

Western blot analysis. Proteins were extracted from SGC-7901 and MGC-803 gastric cancer cells and mouse xenografts using methanesulfonyl fluoride (PMSF) (Beyotime Institute of Biotechnology, Haimen, China), and the protein concentration was determined using the bicinchoninic acid (BCA) assay (Beyotime Institute of Biotechnology). Target protein bands were cut from the 5% polyacrylamide gels and immersed in transfer buffer. Polyvinylidene fluoride (PVDF) membranes were soaked in methanol for 3-5 min and transferred to transfer buffer. Proteins (40 μ g/ μ l) were transferred to PVDF membranes and membranes were incubated in blocking buffer for 2 h at room temperature on a rocker at low speed. Membranes were initially incubated for 1 h at room temperature with anti-rabbit CD44 (bs-0521R), anti-rabbit E-cadherin (3195P) and anti-rabbit vimentin antibodies (5741P) at 1:500 dilution (Cell Signaling Technology, Danvers, MA, USA) on a shaker, and then placed overnight at 4°C. Next, PVDF membranes were washed and then incubated with goat anti-rabbit IgG secondary antibody (11040914) at 1:1,000 dilution (Cell Signaling Technology) for 1-2 h at room temperature on a shaker. Proteins were visualized using a gel imaging processing and analysis system (Uvitec Ltd., Cambridge, UK).

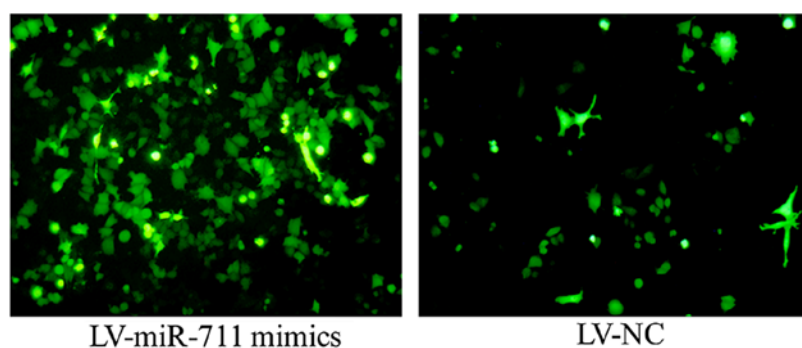


Figure 1. Efficiency of the miR-711 mimic transfection. Green fluorescent cells demonstrated that the transfection was successful. The transfection efficiency = number of cells containing green fluorescence/1 randomly observed visual field with 100 cells under an optical microscope.

Real-time quantitative polymerase chain reaction (real-time PCR). RNA was extracted according to the RNA kit instructions (Takara Bio, Inc., Otsu, Japan) and synthesized into cDNA using a Reverse Transcription kit (Takara Bio). Next, cDNA was amplified using a real-time PCR kit (Takara Bio) in a reaction mixture containing 10 μ l SYBR-Green qPCR Mix, 2 μ l miR-711 primers (Applied Biosystems; Life Technologies, Foster City, CA, USA), 1 μ l cDNA and 7 μ l dH₂O. Real-time PCR was performed at 95°C for 3 min, followed by 95°C for 10 sec, and 58°C for 30 sec for a total of 40 cycles.

Tumor xenografts in nude mice. The study was approved by The First Affiliated Hospital of the University of South China Institutional Ethics Committee (no. 201708). The 10 male Balb/c nude mice (4 weeks old) provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China (weight, 15.5 \pm 0.25 g), were bred and housed in the same temperature and humidity-controlled room on a 12-h light/dark cycle in laminar air flow room (LAFR). The mice were provided with autoclaved tap water and autoclaved standard laboratory chow *ad libitum*. The gastric cancer SGC-7901 cells were cultured and then the lentivirus carrying miR-711 mimics or miR-711 NC were transfected into gastric cancer SGC-7901 cells. SGC-7901 cells with stable miR-711 mimics, or miR-711 NC expression were cultured for subsequent use. Harvested SGC-7901 cells (1 \times 10⁷) were subcutaneously implanted into the bilateral axilla of the mice. After 1 week, tumor volumes were measured every 7 days and the corresponding volumes were calculated by multiplying the length by the width. The mice were sacrificed after 4 weeks by decapitation as dictated by the ethical guidelines, and tumor tissues were used for further research.

Measurement of xenograft tumor volume. The length (a) and width (b) of the tumors were measured weekly, and tumor volume (V) ($V = ab^2/2$) was calculated to create a growth curve of the xenograft tumors in the nude mice. Four weeks after tumor inoculation, the mice were euthanized, solid tumors were collected, and stored at -80°C for subsequent use.

Cell morphology and cell number were determined by H&E staining. The expression of miR-711 was measured by RT-qPCR and CD44, E-cadherin and vimentin protein expression were examined by western blot analysis and immunohistochemistry.

Statistical analysis. All statistical analyses were performed using SPSS 18.0 statistical software (SPSS, Inc., Chicago, IL, USA). In this study, means and standard deviations were used for continuous variables. One-way ANOVA followed by Bonferroni was performed to test multiple variables between experimental groups, normal control groups and blank groups in Figs. 2-4. Student's t-test was performed in Figs. 5, and 7D and F. Independent samples non-parametric test was performed in xenograft tumor volume for non-normal variables. $P < 0.05$ was considered to indicate a statistically significant result.

Results

miR-711 inhibits gastric cancer cell invasion. The Transwell invasion assay showed that the number of transmigrated MGC-803 and SGC-7901 cells was significantly lower in the miR-711 mimics group compared to that noted in the miR-711 inhibitor ($P = 0.005$ and $P = 0.006$, respectively) and NC groups ($P = 0.02$ and $P = 0.03$, respectively), demonstrating that exogenous overexpression of miR-711 in SGC-7901 or MGC-803 gastric cancer cells diminished the invasiveness of these cells. Moreover, inhibition of miR-711 expression in MGC-803 or SGC-7901 cells significantly increased the number of transmigrated cells compared to the NC, indicating that miR-711 inhibition enhanced the invasiveness of these cells ($P = 0.01$ and $P = 0.02$, respectively). There was no difference between the NC and blank group ($P = 0.96$ and $P = 0.63$) (Fig. 2).

miR-711 inhibits gastric cancer cell migration. The scratch wound healing assay revealed that cancer cell migration distance and 'scratch' healing abilities were significantly reduced in the miR-711 mimics group (7.08 \pm 0.73) when compared to the miR-711 NC (32.52 \pm 1.73) and blank groups (32.15 \pm 1.55). These findings indicated that exogenous overexpression of miR-711 significantly inhibited the migratory ability of MGC-803 or SGC-7901 cells ($P = 0.004$, $P = 0.001$, $P = 0.003$ and $P = 0.001$, respectively). In addition, inhibition of miR-711 expression enhanced the migration of MGC-803 or SGC-7901 cells, and the 'scratch' healing abilities were significantly higher in the miR-711 inhibitor group (49.03 \pm 1.59) compared to that noted in the NC group (32.52 \pm 1.73) ($P = 0.02$ and $P = 0.007$). The NC group was not significantly different comparing with the blank group ($P = 0.28$ and $P = 0.87$) (Fig. 3).

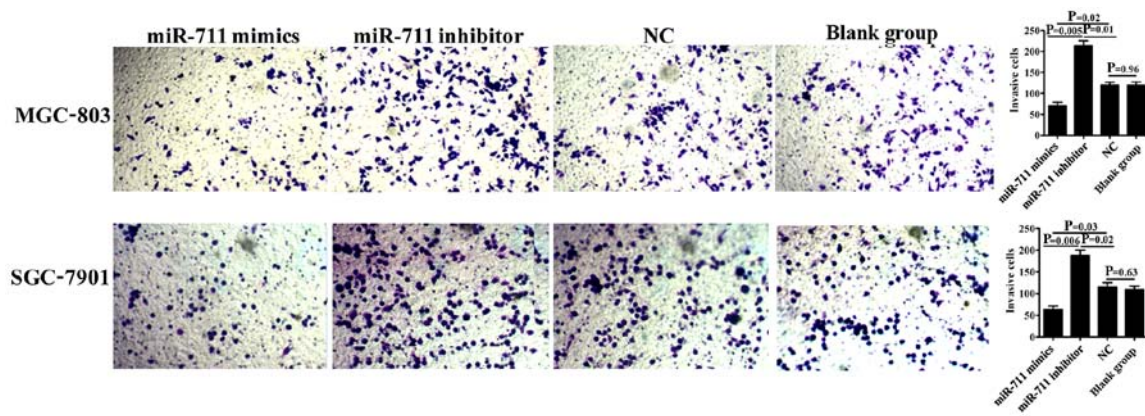


Figure 2. miR-711 inhibits gastric cancer cell invasion. (Upper panel) Exogenous overexpression of miR-711 in MGC-803 gastric cancer cells diminished the invasiveness of these cells compared to the NC and miR-711 inhibitor groups ($P=0.02$ and $P=0.005$). Moreover, inhibition of miR-711 expression in MGC803 cells significantly increased the number of transmigrated cells compared to the NC group ($P=0.01$). There was no difference between the NC and blank group ($P=0.96$). (Lower panel) At the same time, in SGC-7901 cells, exogenous overexpression of miR-711 inhibited the invasiveness of these cells compared to the NC and miR-711 inhibitor group ($P=0.03$ and $P=0.006$). Moreover, inhibition of miR-711 expression significantly promoted the number of transmigrated cells compared to the NC group ($P=0.02$). There was no significant difference between the NC and blank group ($P=0.63$).

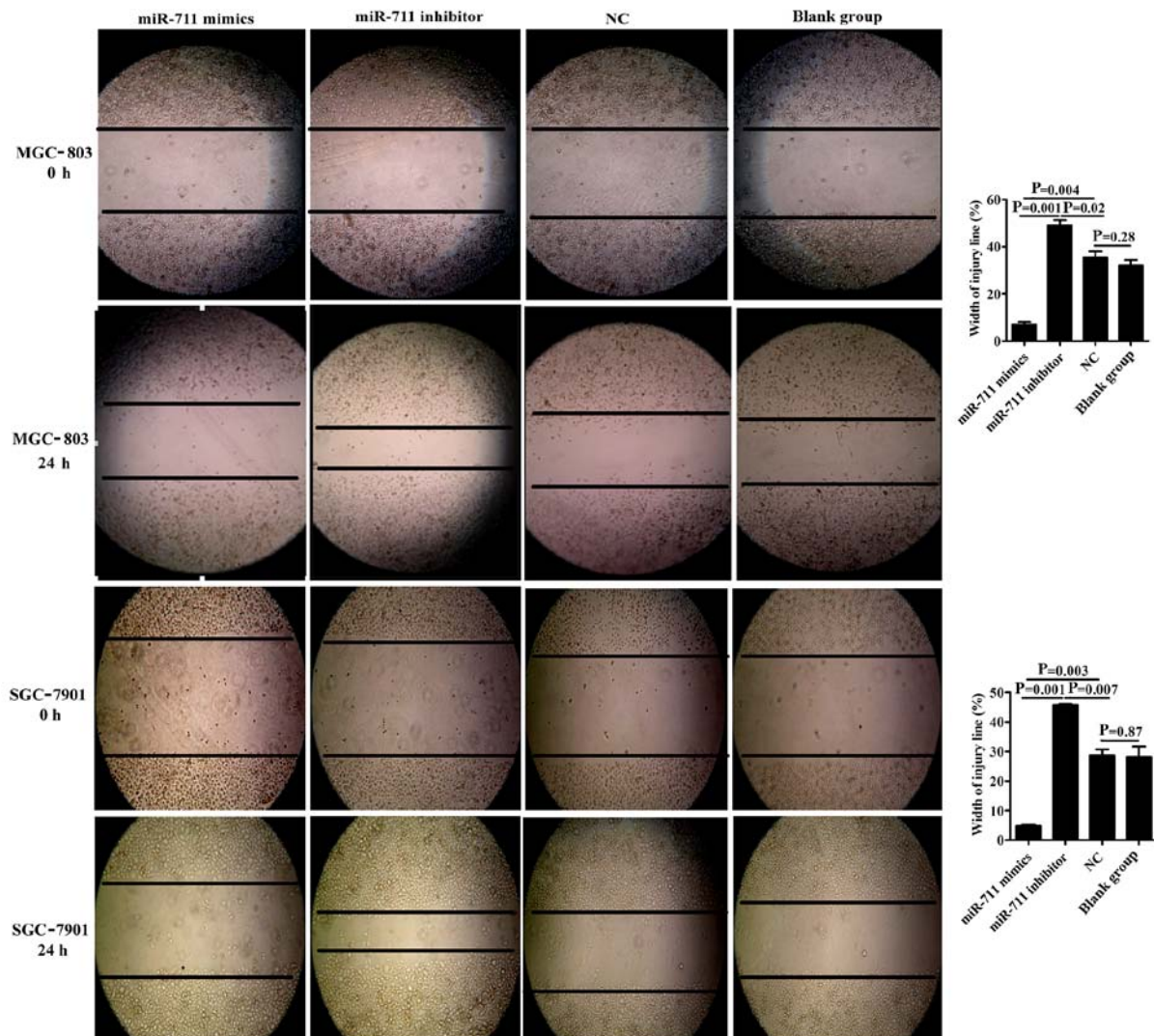


Figure 3. miR-711 inhibits gastric cancer cell migration. (Upper panels) Significantly reduced migration ability was noted in the miR-711 mimics group when compared to the NC and miR-711 inhibitor groups in the MGC-803 cells ($P=0.004$ and $P=0.001$). In addition, inhibition of miR-711 expression enhanced the migration of MGC-803 cells compared to NC group ($P=0.02$). There was no difference between the NC and blank group ($P=0.28$). (Lower panels) Meanwhile, the results also showed that overexpression of miR-711 inhibited SGC-7901 cell migration compared with the NC and miR-711 inhibitor groups ($P=0.003$ and $P=0.001$). In addition, inhibition of miR-711 in SGC-7901 cells promoted cell migration compared with the NC group ($P=0.007$). There was no significant difference between the NC and blank group ($P=0.87$).

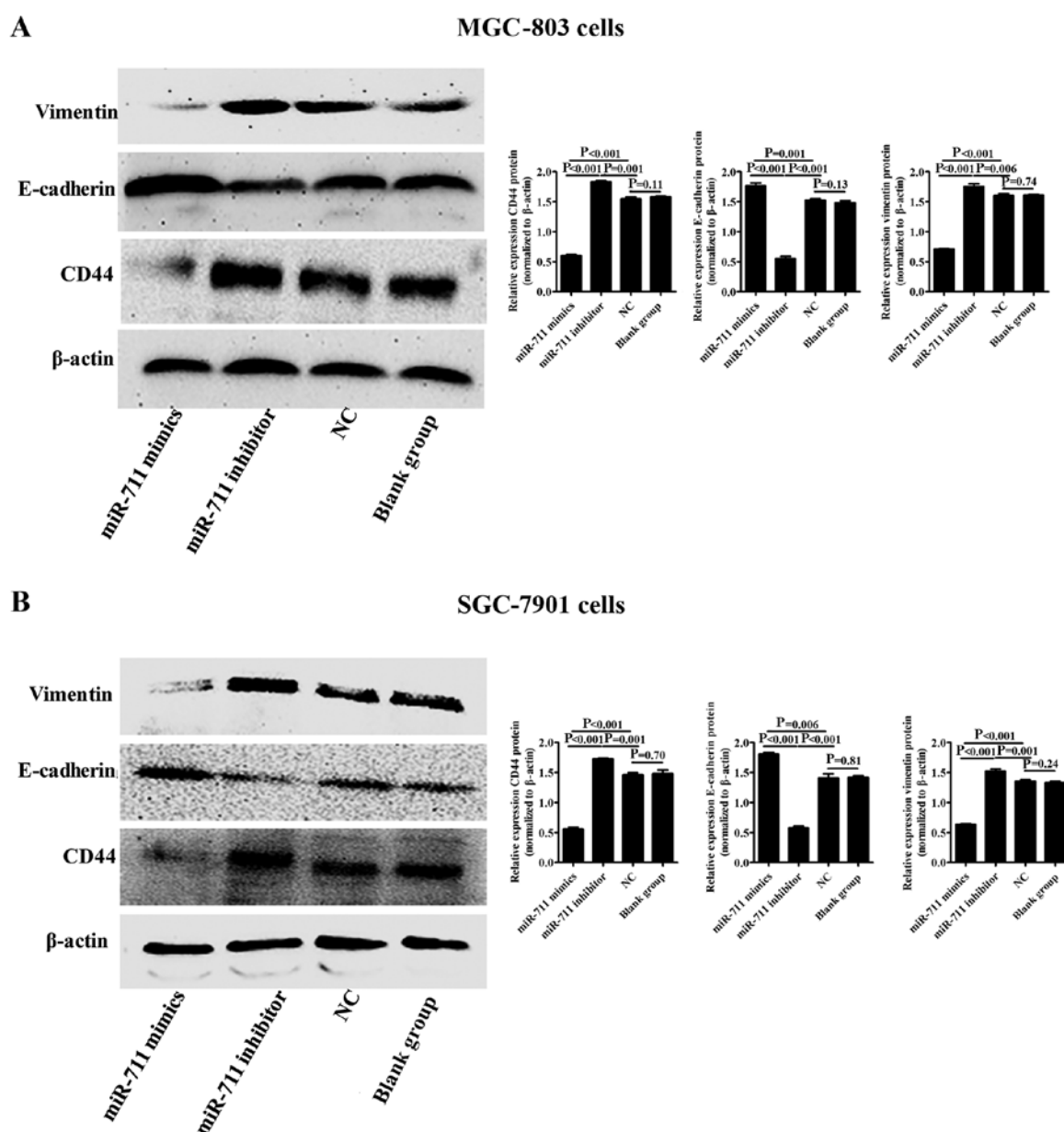


Figure 4. miR-711 regulates CD44, E-cadherin and vimentin expression. (A) In MGC-803 cells, exogenous miR-711 downregulated the CD44 and vimentin protein expression ($P < 0.001$) and upregulated E-cadherin protein ($P = 0.001$ and $P < 0.001$), compared with the NC and miR-711 inhibitor groups, while inhibition of miR-711 upregulated the expression of CD44 and vimentin protein expression ($P = 0.001$ and $P = 0.006$), and inversely, downregulated E-cadherin protein ($P < 0.001$). (B) In SGC-7901 cells, overexpression of miR-711 decreased CD44 and vimentin protein expression ($P < 0.001$ and $P < 0.001$) and upregulated E-cadherin protein ($P < 0.001$). However, inhibition of miR-711 upregulated the expression of CD44 and vimentin protein ($P < 0.001$ and $P < 0.001$) and downregulated E-cadherin protein ($P < 0.001$).

miR-711 regulates CD44, E-cadherin and vimentin expression. The expression of CD44, E-cadherin and vimentin in MGC-803 or SGC-7901 cells with exogenous miR-711 overexpression or inhibition was evaluated using western blot analysis. Compared with the miR-711 NC and blank groups, CD44 and vimentin protein expression was downregulated, whereas E-cadherin protein expression was upregulated in the MGC-803 or SGC-7901 cells with miR-711 overexpression ($P < 0.001$ and $P < 0.001$) (Fig. 4A and B). However, CD44 and vimentin protein expression were upregulated, and E-cadherin protein expression was downregulated when miR-711 was inhibited in the MGC-803 or SGC-7901 cells ($P < 0.001$ and $P < 0.001$) (Fig. 4A and B).

CD44 regulates E-cadherin and vimentin expression. Since CD44 has been shown to be highly expressed in gastric cancer tissues and cells, we blocked CD44 expression in MGC-803 or SGC-7901 cells and evaluated E-cadherin and vimentin expression in these cell types using western blot analysis. We found that inhibition of CD44 expression in MGC-803 or SGC-7901 cells significantly downregulated vimentin protein expression ($P < 0.001$ and $P < 0.001$) and upregulated E-cadherin protein expression ($P = 0.003$ and $P < 0.001$) when compared to the NC group (Fig. 5A and B).

miR-711 specifically binds to CD44. Based on the TargetScan prediction that miR-711 specifically binds

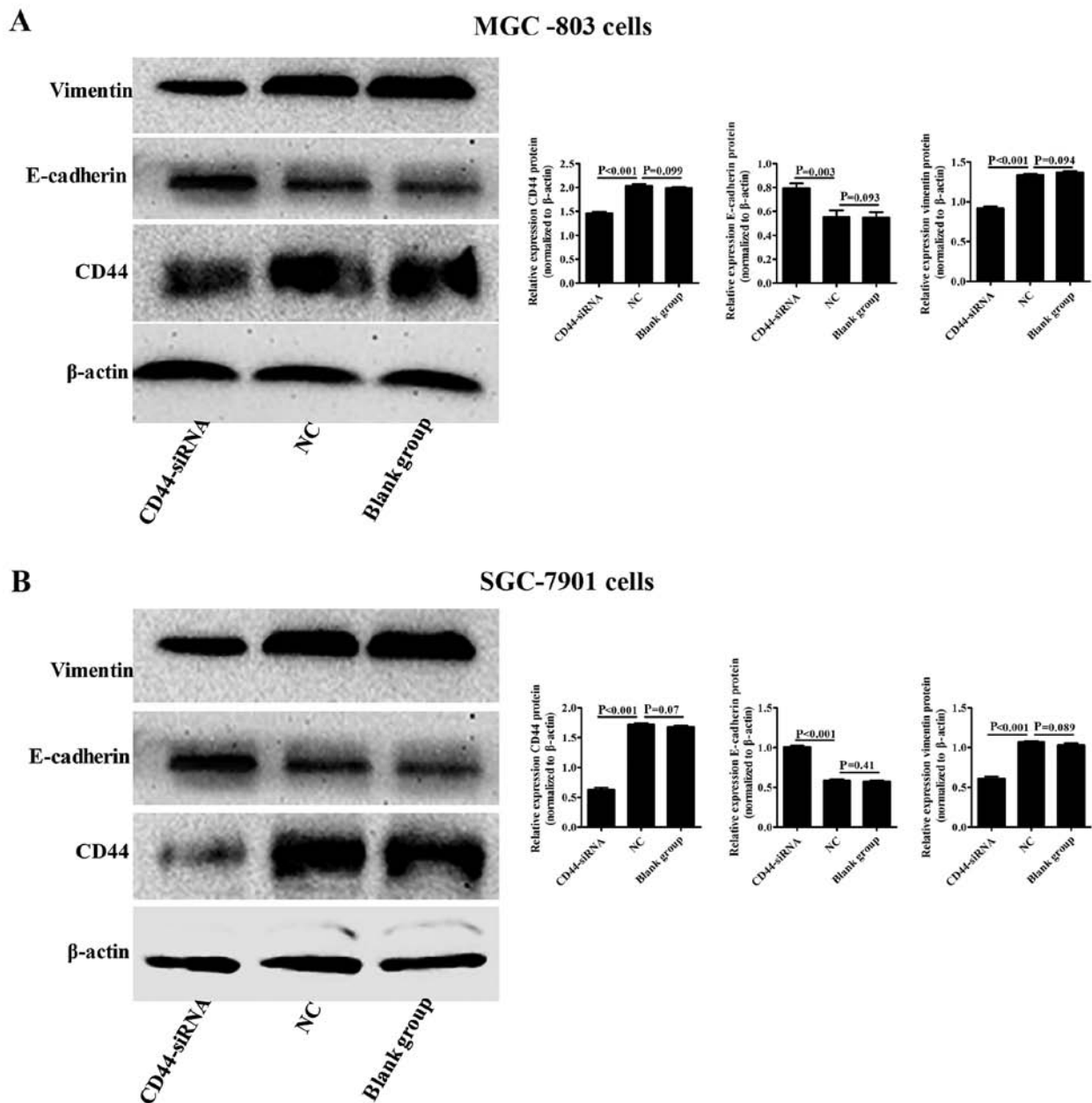


Figure 5. CD44 regulates E-cadherin and vimentin expression. (A) In MGC-803 cells, inhibition of CD44 reduced vimentin protein ($P < 0.001$), but, increased E-cadherin protein ($P = 0.003$). (B) The results showed that downregulation of CD44 in SGC-7901 cells, also downregulated vimentin protein ($P < 0.001$), however, upregulated E-cadherin protein ($P < 0.001$).

to CD44 3' UTR (Fig. 6A), we constructed CD44 3' UTR-wild (wild-type) and CD44 3'-UTR-mut (mutant) vectors and co-transfected SGC-7901 cells with either vectors and miR-711 mimics. Dual-Luciferase reporter assays revealed that in the CD44 3' UTR-wild group, luciferase activity was significantly reduced compared to the CD44 3' UTR-mut group ($P = 0.009$) (Fig. 6B).

miR-711-mediated upregulation of CD44 inhibits EMT in gastric cancer *in vivo*. SGC-7901 gastric cancer cells with stable miR-711 mimics (overexpression) or miR-711 NC were grafted into nude mice under the armpit, and nude mice were euthanized 4 weeks post-grafting. Tumor formation assay indicated that the xenograft tumor volume was significantly smaller in the miR-711 mimics group compared to that in the miR-711 NC group ($P < 0.001$) at day 28 (Fig. 7A-C). Moreover,

qRT-PCR revealed that miR-711 expression was significantly higher in the xenograft tumors with miR-711 overexpression when compared to the NC group ($P = 0.0008$) (Fig. 7D). Immunohistochemistry using H&E staining showed that, at each magnification used, the number of tumor cells was significantly lower in the miR-711 mimics group when compared to the miR-711 NC group ($P < 0.05$) (Fig. 7E). Furthermore, western blot analysis demonstrated that CD44 and vimentin protein expression levels were significantly downregulated, and E-cadherin protein expression was significantly upregulated in the xenograft tumors in the miR-711 mimics group compared to those in the miR-711 NC group ($P = 0.015$, $P = 0.035$ and $P = 0.028$, respectively) (Fig. 7F). Therefore, it was discovered that miR-711 can inhibit cell invasion and migration *in vitro*, and inhibit cell proliferation and xenograft tumor growth *in vivo*.

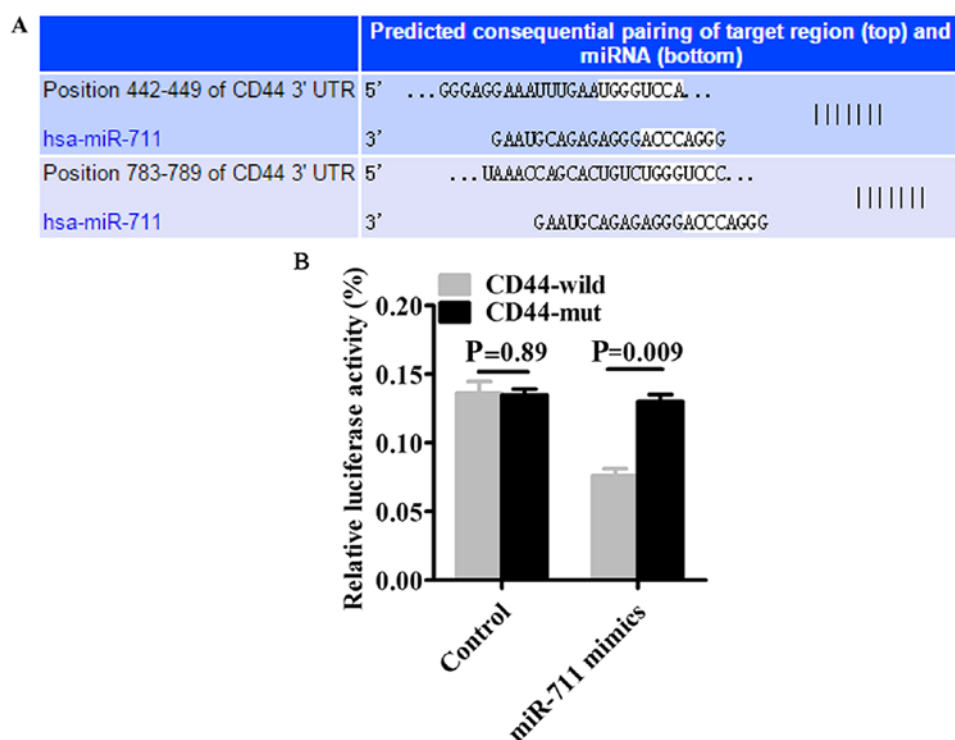


Figure 6. miR-711 specifically binds to CD44. (A) miR-711 was found to specifically bind to CD44 3' UTR by using bioinformatic analysis. (B) SGC-7901 cells were co-transfected with miR-711 mimics or control, *Renilla* luciferase vector pRL-SV40, and full length CD44 3' UTR luciferase reporter for 48 h. Both firefly luciferase signals were normalized with the *Renilla* luciferase signals. The results revealed that in the CD44 3' UTR-wild group, luciferase activity was significantly reduced compared to the CD44 3' UTR-mut group ($P=0.009$).

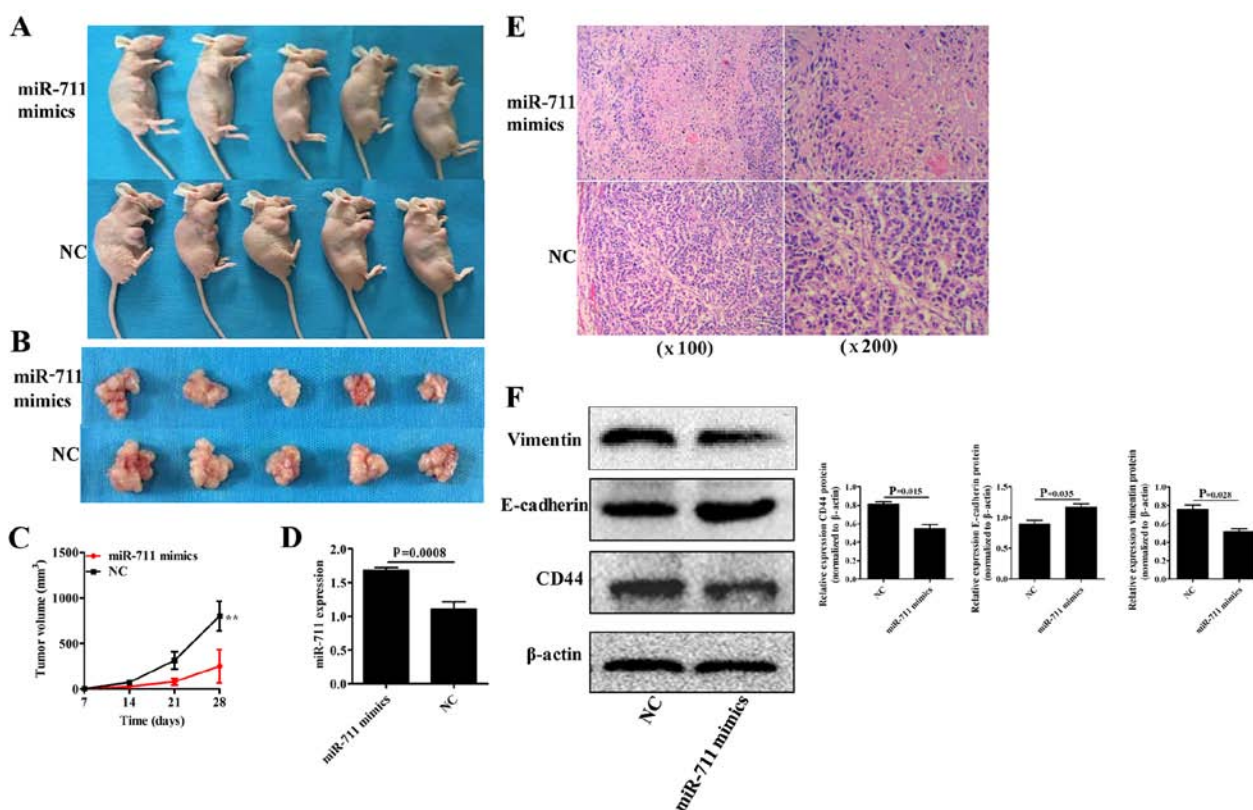


Figure 7. miR-711-mediated downregulation of CD44 inhibits EMT in gastric cancer *in vivo*. (A-C) Tumor formation assay indicated that the xenograft tumor volume was significantly smaller in the miR-711 mimics group compared to the NC group ($P<0.001$). (D) qRT-PCR revealed that miR-711 expression was significantly higher in xenograft tumors with miR-711 overexpression when compared to the NC group ($P=0.0008$). (E) H&E staining showed that the number of tumor cells was significantly increased in the miR-711 mimics group compared to the NC group ($P<0.05$). (F) Western blot analysis demonstrated that CD44 and vimentin protein expression was significantly downregulated, and E-cadherin protein expression was significantly upregulated in xenograft tumors in the miR-711 mimics group compared to those in the NC group ($P=0.015$, $P=0.035$ and $P=0.028$, respectively).

Discussion

Over 90% of cancer-related deaths are associated with tumor epithelial-mesenchymal transition (EMT). Regulation of tumor cells by EMT-related proteins during tumor development and progression results in weakened intercellular adhesion, varying degrees of mesenchymal phenotypes, and enhanced mobility and invasiveness. This allows cells to cross the basement membrane with the help of proteases in the surrounding microenvironment, and results in local infiltration and distant metastasis. Tumor cells that arrive at the site of metastatic lesions can reverse EMT and re-transform into tumor epithelial cells, which then proliferate to form distant metastatic lesions and promote tumor progression (18-21). Several factors induce or regulate the biological processes of EMT, such as the tumor microenvironment, cytokines, signaling kinases and transcription factors. Moreover, changes in EMT-related proteins including the expression of epithelial cell adhesion proteins, cell morphology-related proteins and mesenchymal cell-related proteins, such as E-cadherin and vimentin have also been reported (22-25). In fact, several studies have demonstrated that the biological processes of EMT play a critical role in the development, progression, invasion and migration.

EMT is often accompanied by an increase in cancer stem cells (CSCs), and is therefore believed to be one of the inducing factors for CSC formation (26). Previous studies have suggested that prostate CSCs may be the source of prostate cancer and that they have an important role in the development and progression of prostate cancer. Prostate CSCs can be identified by several unique markers, including CD133, CD44, BCRP-1/ABG2 and telomerase (27,28). Ishimoto *et al* found that CD44 is an important marker for CSCs (29). In another study, it was demonstrated that the use of an anti-CD44 monoclonal antibody eliminated acute myeloid leukemia (AML) CSCs (30). CD44 is a multifunctional cell surface adhesion receptor that plays a key role in the invasion and metastasis of multiple cancers. *In vitro* and *in vivo* studies have revealed that miR-647 may inhibit gastric cancer metastasis via inhibition of CD44 expression (31). In a study by Lee *et al*, it was reported that downregulation of CD44 expression in HCT116 colon cancer cells inhibited cancer cell proliferation, migration and invasion and promoted cell apoptosis (32). Moreover, it was found that exogenous asporin bound to the CD44 receptor on pancreatic cancer cells to downregulate epithelial phenotype-related proteins and upregulate mesenchymal phenotype-related proteins, which induced tumor cell EMT, and thereby promoted tumor cell invasion and migration (33).

At present, studies on the biological roles of miR-711 are limited. In a previous study, it was shown that pioglitazone inhibited collagen-I synthesis and post-infarction myocardial fibrosis by upregulating miR-711 expression in cardiomyocytes (34). miR-711-mediated upregulation of adiponectin (ApN) inhibited Toll-like receptor 4 (TLR4) signaling and thereby suppressed inflammation during myositis (35). In a recent study by Hu *et al*, a new role of miR-711 in cancer was revealed. Specifically, the authors showed that high miR-711 expression in breast cancer tissues was a risk factor for breast cancer patients, and that high miR-711 expression promoted breast cancer cell proliferation, invasion and migration *in vitro*,

suggesting that miR-711 served as an oncogene (36). However, Waseem *et al* found that lower miR-711 expression in prostate cancer resulted in a higher Gleason score, greater malignancy, and higher cancer metastatic rate, indicating that miR-711 was an anti-oncogene (37). Because of the inconsistent findings, the biological functions of miR-711 remain unclear. In our previous study, we showed that the expression of miR-711 in gastric cancer tissues and cells was low, and that exogenous miR-711 expression inhibited gastric cancer cell invasion and migration by an unidentified mechanism (17). In this study, exogenous miR-711 overexpression in SGC-7901 or MGC-803 gastric cancer cells inhibited cancer cell invasion and migration. In contrast, inhibition of miR-711 expression promoted gastric cancer invasion and migration, indicating that miR-711 inhibited gastric cancer progression. Moreover, exogenous miR-711 overexpression can inhibit xenograft tumor growth *in vivo* and may therefore be an anti-oncogene. It was discovered that the process of EMT is dispensable for metastasis, and caused chemo-resistance in lung and pancreatic cancer (38,39). In this study, EMT contributed to metastasis in gastric cancer. Since bioinformatics shows that miR-711 specifically binds to the 3' UTR of CD44, we speculated that the biological roles of miR-711 may be mediated through CD44. To test this hypothesis, we performed western blot analysis and confirmed that exogenous miR-711 overexpression inhibited CD44 and vimentin protein expression and promoted E-cadherin protein expression. In contrast, inhibition of miR-711 expression promoted CD44 and vimentin protein expression and inhibited E-cadherin protein expression. These findings indirectly demonstrated that miR-711 may inhibit EMT in gastric cancer by regulating CD44, vimentin and E-cadherin expression. In a previous study, CD44 was shown to be highly expressed in gastric cancer tissues and cells, and was involved in the regulation of gastric cancer metastasis (40). We inhibited CD44 expression in SGC-7901 and MGC-803 gastric cancer cells and demonstrated that CD44 inhibition downregulated vimentin protein expression and upregulated E-cadherin protein expression. Therefore, we believed that miR-711 may inhibit EMT in gastric cancer by downregulating CD44 expression, which in turn inhibited vimentin expression and promoted E-cadherin expression. Further investigation using dual-fluorescence assays revealed that the relative luciferase activity was significantly lower in miR-711 mimics-transfected CD44 3' UTR-wt SGC-7901 cells compared to miR-711 mimics-transfected CD44 3' UTR-mut SGC-7901 cells, thereby indicating that miR-711 specifically bound to the CD44 3' UTR. It further demonstrated that miR-711 inhibited EMT in gastric cancer via inhibition of CD44 3' UTR. To further confirm our hypothesis, we cultured and grafted SGC-7901 cells with stable miR-711 overexpression into nude mice, and showed that exogenous miR-711 overexpression significantly inhibited xenograft tumor growth in the miR-711 mimics group compared to the NC group, demonstrating that miR-711 inhibited xenograft tumor growth in nude mice. In addition, H&E staining showed that the number of tumor cells was significantly reduced in the miR-711 mimics group compared to that in the NC group, indicating that miR-711 indeed inhibited tumor growth. qRT-PCR confirmed that miR-711 expression was significantly elevated in the miR-711 mimics group when compared to the NC group. Moreover,

western blot analyses demonstrated that exogenous miR-711 overexpression inhibited EMT in gastric cancer by inhibiting CD44 and vimentin protein expression and by promoting E-cadherin protein. In summary, this study was the first to show that miR-711-mediated inhibition of CD44 expression inhibited EMT of gastric cancer cells *in vitro* and *in vivo* via downregulation of vimentin expression and upregulation of E-cadherin expression. Thus, our findings provide novel insights into the development of miR-711-based targeted therapy for EMT in gastric cancer.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

WSX, AJL and DFL conceived and designed the study. YPT, YZC, WBD and JC completed the experiments and collected the data. WWZ, YPT, YZC, WBD and JC conducted the statistical analysis. DFL wrote the manuscript. AJL, WWZ and WSX revised the manuscript. AJL and DFL obtained funding. All authors approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The study was approved by The First Affiliated Hospital of the University of South China Institutional Ethics Committee (no. 201708).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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