

## Original Article

# miR-519 inhibits epithelial-mesenchymal transition and biologic behavior of gastric cancer cells by down-regulating FOXQ1

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**Abstract:** In recent years, a number of studies have shown that forkhead box Q1 (FOXQ1) plays an important role in the process of epithelial-mesenchymal transition (EMT) of tumors. The aim of this study is to investigate the biologic functions of FOXQ1 and miR-519 in gastric cancer. It was found that FOXQ1 was highly expressed in gastric cancer cells and tumor tissues, and promoted proliferation, migration, invasion, and EMT of gastric cancer cells. miR-519 was weakly expressed in both gastric cancer tissues and gastric cancer cells, up-regulation of miR-519 inhibited the biologic behavior of gastric cancer cells, while down-regulation of miR-519 showed the opposite results. Additionally, miR-519 directly targeted FOXQ1 and inhibited FOXQ1 mRNA and protein expression. Overexpression of FOXQ1 in gastric cancer cells reversed the inhibitory effect of miR-519 on cellular biologic behavior. The results of the present study suggest that the abnormal expression of miR-519 and FOXQ1 may be closely related to gastric cancer development, and miR-519 may play an important role in suppressing tumor related genes in gastric cancer by targeting and regulating FOXQ1.

**Keywords:** Gastric cancer, FOXQ1, miR-519, EMT

## Introduction

The invasion and metastasis of gastric cancer cells is a major driving factor behind the post-operative recurrence and advanced disease progression, as well as an important factor affecting the prognosis of patients [1-3]. It is currently unclear what factors contribute to the invasion and metastasis of gastric cancer, and the molecular mechanisms of action behind the invasion and metastasis of gastric cancer cells remain to be clarified.

Epithelial-mesenchymal transition (EMT) of tumor cells is closely related to the process of invasion and metastasis; namely, EMT induces the polarization of the cells, connection of tight intercellular junctions, and the gradual loss of adhesion connections. This allows the cells to migrate and invade tissues, and at the same time, produce a large number of extracellular matrix components, inhibit cell apoptosis, and transform into cells with the characteristics and morphology of interstitial cells [4, 5]. During

EMT, the expression of E-cadherin and keratin are downregulated, while the expression of N-cadherin and vimentin are up-regulated [6]. In recent years, several studies have shown that the transcription factor foxhead box (FOX) Q1 plays an important role in EMT [7]. The FOX protein is a large and versatile transcription factor, which is characterized by a highly conserved forkhead DNA domain. There are a large number of members of the FOX family with more than 100 confirmed family members, belonging to 17 sub-families. The biological functions of FOX family proteins involve embryonic development, cell cycle regulation, cell metabolism, biological aging, and immune regulation [8-10]. In recent years, many studies have shown that FOXQ1 is highly expressed in a variety of tumor tissues and cells, such as non-small cell lung cancer [11], breast cancer [12] and gastric cancer [13].

MicroRNAs (miRNAs) are a class of non-coding small RNA that bind to the target gene mRNA at either the coding or non-coding region, and reg-

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ulate gene expression by degrading the target mRNA or inhibiting its translation. Studies have reported that expression levels of certain miRNAs are correlated with the extent of tumorigenesis as well as tumor progression, invasion, and metastasis [14, 15]. Although various miRNAs have been found to be involved in the development of gastric cancer [16], the role of miR-519 in gastric cancer remains unclear. The purpose of this study was to analyze the role of miR-519 in gastric cancer by targeted regulation of FOXQ1 to provide a new direction for diagnosis and treatment.

### Materials and methods

#### *Human tissue samples*

Tissues were obtained from 134 patients (88 males and 46 females; mean age, 59.2±13.4 years) who were undergoing gastric cancer treatment at Changzheng Hospital, Second Military Medical University. Gastric cancer tissue and paracancerous tissue samples were provided by the General Surgery and Pathology Department, Changzheng Hospital, Second Military Medical University. Tissue specimens were stored at -80°C for subsequent analysis. All patients provided permission for the collection of their tissue samples and the experiments were approved by the Ethics Committee of the Changzheng Hospital, Second Military Medical University.

#### *Cell culture*

Gastric cancer cell lines (NCI-N87, MKN74, HGC-27 and AGS) and human normal gastric mucosal epithelial cells (GES1) were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The cells were maintained in DMEM supplemented with 10% fetal calf serum (Thermo Fisher Scientific, Inc.) and 100 U/ml penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO<sub>2</sub>.

#### *Cell transfection*

When cultured gastric cancer cell lines had reached >75% confluency, the cells were seeded onto six-well plates and cultured for 24 h. The cells were transfected then with FOXQ1 siRNA, miR-NC, miR-519 mimics, NC-inhibitor, miR-519 inhibitor and co-transfected with miR-

519 mimics plus FOXQ1. Cell transfection was performed using a reagent kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

#### *RT-qPCR*

Total RNA from tissues and cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. cDNA was synthesized using the cDNART kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) at 42°C. Reaction conditions are as follows: 95°C for 30 secs; 40 cycles of 95°C for 5 secs and 60°C for 30 secs. Relative expression levels of miRNA and other indicators were calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method, using U6 as an internal control for miR-519 and GAPDH as an internal control for FOXQ1. The primer sequences used are as follows: miR-519 forwards: 5'-TCCCTGCTGACCC-TCCC-3', miR-519 reverse: 5'-TCCCAAACGGTA-ACACTCTAAAGG-3'; FOXQ1 forwards: 5'-GTGAT-TTATTGCTATTGACCGATG-3', FOXQ1 reverse: 5'-GCCCAAGGAGACCACAGTTAGA-3'; U6 forwards: 5'-CTCCCTTGGGCAGCACA-3', U6 reverse: 5'-AACGCTTACCAATTTGCGT-3'; GAPDH forwards: 5'-CATCACCATCTCCAGGAGCG-3', GAPDH reverse: 5'-TAGCCTTGCCCACAGCCTTG-3'.

#### *Western blot*

Cellular proteins were extracted using RIPA Lysis Buffer (C1053, Applygen Technologies Inc.). Protein concentration was determined using a BCA protein assays (Thermo Fisher Scientific, Inc.). 40 µg of protein was loaded into wells, separated by 12% SDS-PAGE, and transferred to a PVDF membrane at 200 mA for 3 h. The membranes were blocked with 5% non-fat dry milk powder for 2 h at 37°C. Primary antibodies against FOXQ1 (ab51340; 1:1,000) and rabbit anti-GAPDH (ab181602; 1:2,000; both Abcam) were incubated at 4°C overnight. The secondary antibody, horseradish peroxidase-labeled goat anti-rabbit (ab150077; 1:5,000, Abcam,) was incubated at room temperature for 1 h. An ECL chemiluminescence detection kit was used to visualize the protein bands.

#### *Luciferase reporter assay*

The TargetScan software (<http://www.targets-can.org/>) was used to predict the possible sites of action of miR-519 and FOXQ1. The miR-519

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binding site on the 3'-untranslated region (UTR) of FOXQ1 was amplified. Luciferase reporter plasmid [PsiCHECK2-FOXQ1-wild type (wt) or psiCHECK2-FOXQ1-mutant (Mut)], miR-519 mimics, miR-NC, miR-519 inhibitor or NC-inhibitor were co-transfected into AGS cells. Following transfection for 24 h, the luciferase activity was analyzed using a Dual-Luciferase Reporter Assay system (Promega Corporation) according to the manufacturer's recommendations.

### *Immunohistochemistry*

Tissue specimens were dehydrated, transparentized, and immersed in wax after being fixed in 4% paraformaldehyde for 48 h. Using a paraffin sectioning machine (RM2016, Leica), paraffin blocks were sliced into 5 µm serial sections, which were placed in oven at 65°C overnight. Hydrated tissue sections were treated with 3% H<sub>2</sub>O<sub>2</sub> to block endogenous peroxidase. Sections were subsequently acid-fixed using a pre-configured citrate buffer, and antigen retrieval was performed using the microwave heating procedure. Following antigen retrieval, blocking was performed with 5% normal goat serum (Thermo Fisher Scientific, Inc.) at room temperature for 30 min. Primary antibodies against FOXQ1 (ab51340; 1:1,000) and Ki67 (ab19723; 1:100; both Abcam) were incubated at 4°C overnight. The secondary antibody, horseradish peroxidase-labeled goat anti-rabbit (ab150077; 1:200; Abcam) was incubated at room temperature for 30 min. The sections were developed using a DAB Horseradish Peroxidase Color Development kit. Hematoxylin staining was performed at room temperature for 2 min, followed by dehydration and neutral resin packing. Positive images were observed with a vertical light microscope and counted.

### *Transwell migration assay*

Solutions of each cell group were prepared with 1×10<sup>6</sup> cells/ml, while transwells consisting of polycarbonate membranes (8-µm pore size) were placed within 24-well plates. A total of 100 µl cell suspension was loaded into the upper chamber and 600 µl 10% serum medium (Thermo Fisher Scientific, Inc.) was loaded into the lower chamber. After 24-48 h, the transwell was removed, and the cells in the upper compartment of the membrane were swabbed with a cotton swab. The cells in the lower chamber were stained using crystal violet for 20 min,

before observing and counting the cells under a vertical light microscope.

### *Transwell invasion assay*

Matrigel was incubated at 4°C overnight, and then diluted into medium at a ratio of 1:6. Transwells consisting of polycarbonate membranes were placed within 24-well plates. 50 µl matrigel diluent was added into the upper chamber, coating the filter membrane. This was incubated at 37°C for 4 h, to allow the matrigel solution to dry. The transwell assay was then performed as previously described.

### *CCK-8 assay*

Cells from all treatment groups were evenly spread into 96-well plates (100 µl/well) plate and cultured at 37°C incubator. Following transfection for 24 h as previously described, a CCK-8 kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used according to the manufacturer's instructions. Absorbance was then detected at 450 nm using an enzyme standard meter.

### *Flow cytometry*

After transfection for 48 h, the cells of each group were suspended, collected, and washed twice with saline before being fixed overnight at 4°C using 70% ethyl alcohol. 400 µl propidium iodide was added to each group at 37°C in the dark for 30 min. Finally, the proportion of cells at each stage in the cell cycle was detected using flow cytometry. The experiment was repeated three times.

### *Statistical analysis*

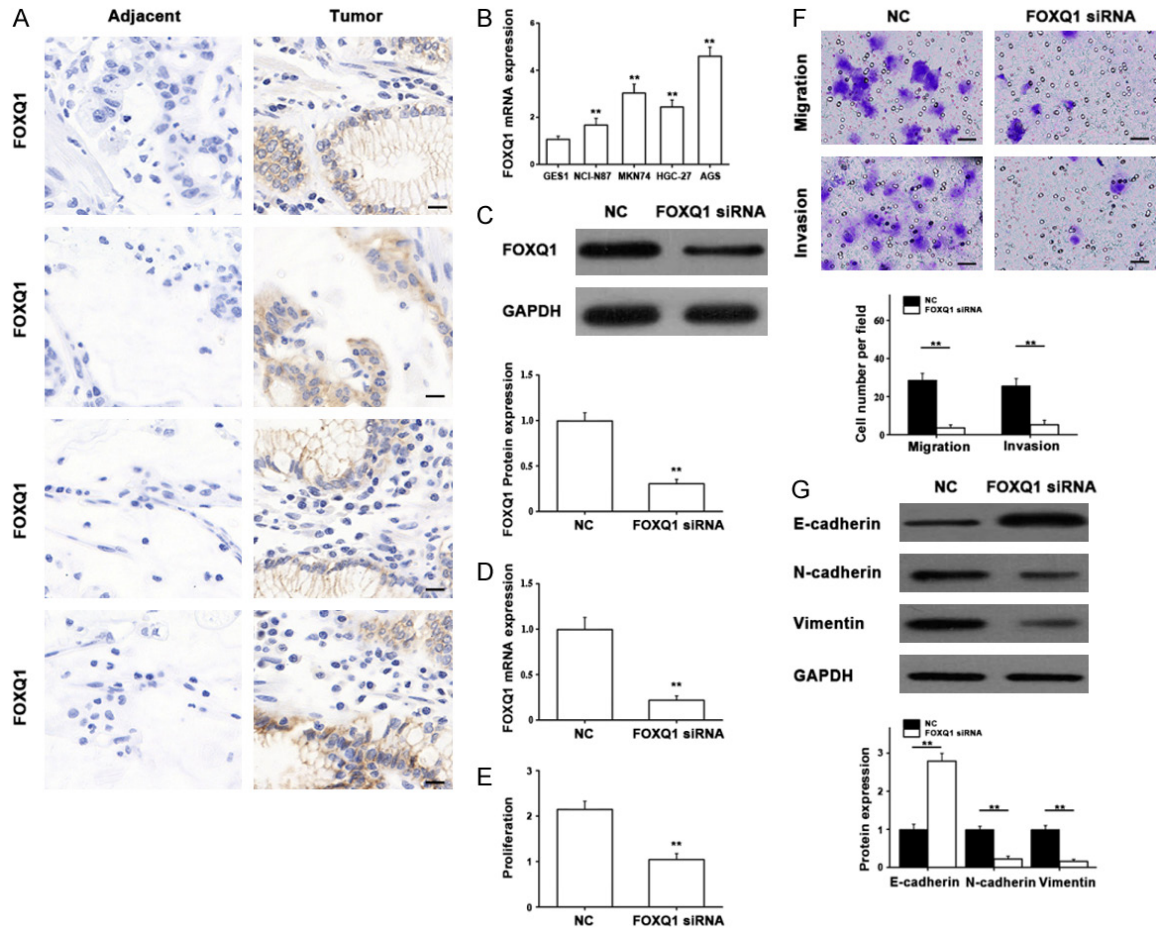
The statistical analysis was performed using SPSS 22.0 software (IBM Corp.). The results are presented as the mean ± SD. Student's t-tests were used to compare the mean values between the two groups. Comparisons among three or more groups were performed using one-way ANOVAs. P<0.05 was considered to indicate a significant difference.

## **Results**

### *Expression of FOXQ1 in gastric carcinoma cell lines and tissue samples*

FOXQ1 expression in gastric cancer was detected using immunohistochemistry. As shown as **Figure 1A**, FOXQ1 positive signal in tumor tis-

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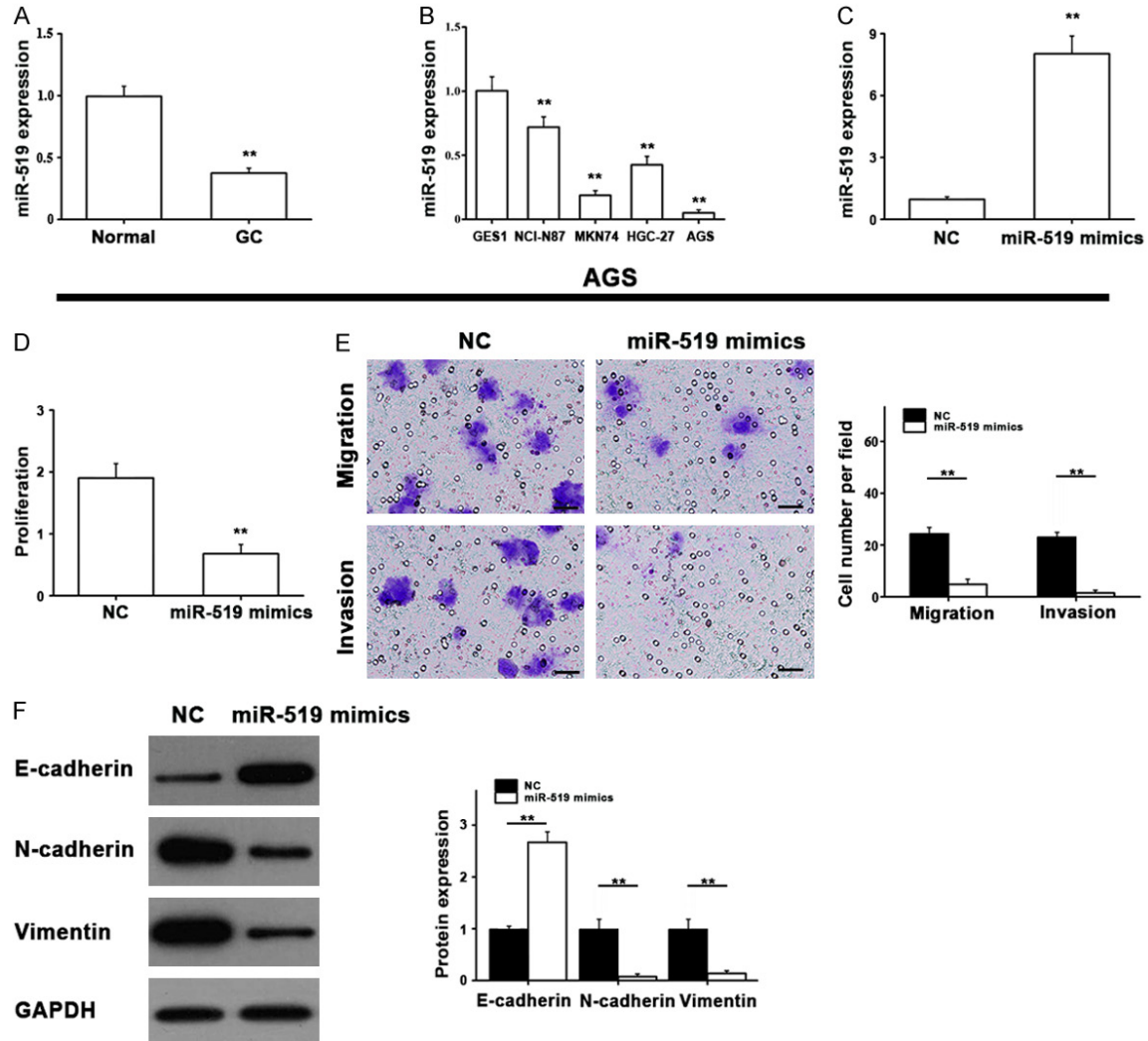


**Figure 1.** Expression of FOXQ1 in gastric carcinoma cell lines and tissue samples. A. The expression of FOXQ1 in gastric cancer was detected by immunohistochemistry. B. mRNA of FOXQ1 in gastric cancer cell lines NCI-N87, MKN74, HGC-27, and AGS and normal gastric mucosal epithelial cell lines GES1 was detected by RT-qPCR. C. Western blot assays were used to detect FOXQ1 protein expression in AGS cells. D. Expression of FOXQ1 mRNA in AGS cells was detected by RT-qPCR. E. The effect of FOXQ1 siRNA on proliferation of AGS cells was detected by CCK-8 assay. F. Transwell assays were used to detect the migratory and invasive ability of AGS cells. G. Western blot was used to detect the effect of FOXQ1 on epithelial-mesenchymal transformation in gastric carcinoma cells. \*\*P<0.01.

sues was markedly increased when compared with adjacent normal tissue samples. Furthermore, RT-qPCR results showed that the mRNA expression levels of FOXQ1 in the gastric cancer cell lines NCI-N87, MKN74, HGC-27 and AGS were significantly higher than that in normal gastric mucosal epithelial cell line, GES1 (Figure 1B). After FOXQ1 siRNA was successfully transfected into AGS cells, western blot assays showed that the expression level of FOXQ1 protein in cells transfected with FOXQ1 siRNA was significantly lower than that in the control group (Figure 1C). RT-qPCR results showed that the mRNA expression level of FOXQ1 was reduced when compared with that of the control group (Figure 1D). CCK-8 assays were used to detect the effect of FOXQ1 siRNA

on proliferation of gastric cancer cells. The results showed that the optical density (OD) values from the FOXQ1 siRNA transfection group were significantly lower than that of the control group (Figure 1E). Transwell assays were used to detect the effects of FOXQ1 siRNA on the migratory and invasive abilities of AGS cells. The results showed that after transfection with FOXQ1 siRNA, the invasive and migratory abilities of AGS cells were significantly inhibited (Figure 1F). Additionally, western blot was used to detect the effects of FOXQ1 on the epithelial mesenchymal transformation in gastric carcinoma cells. The results showed that the expression of the epithelial marker, E-cadherin, was significantly increased in AGS cells transfected with FOXQ1 siRNA, while the expression of the

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**Figure 2.** The regulatory role of overexpression of miR-519 in AGS cells. A. The expression of miR-519 in gastric cancer tumor tissues and adjacent normal tissues was detected by RT-qPCR. B. The expressions of miR-519 in the normal gastric mucosal epithelial cell line GES1 and gastric cancer cell lines (NCI-N87, MKN74, HGC-27 and AGS) were detected. C. miR-519 mimics were transfected into AGS cells. D. CCK-8 assay was used to detect the effect of miR-519 mimics on the proliferation of AGS cells. E. Transwell assays were used to detect the effects of miR-519 mimics on the migratory and invasive abilities of AGS cells. F. Western blot was used to detect the effect of miR-519 mimics on EMT. \*\*P<0.01.

interstitial cell markers, N-cadherin and vimentin, were decreased when compared with control group (Figure 1G). The above results indicated that abnormal expression level of FOXQ1 was closely related to gastric cancer development, and FOXQ1 may play an important role in promoting gastric cancer.

### *The regulatory role of overexpression of miR-519 in gastric cancer cells*

The expression level of miR-519 in gastric cancer tumor tissues and adjacent normal tissues

was detected by RT-qPCR, and the results demonstrated that miR-519 in gastric cancer tumor tissues was significantly lower than that in adjacent normal tissues (Figure 2A). The expressions of miR-519 in NCI-N87, MKN74, HGC-27 and AGS cells were significantly lower than that of GES1 cells (Figure 2B). To investigate the regulatory role of overexpressed miR-519 in gastric cancer cells, we transfected miR-519 mimics into AGS cells, RT-qPCR results showed that the expression of miR-519 was significantly higher than that of the control group (Figure 2C). CCK-8 assays were performed to investi-

gate the effect of miR-519 on the proliferation of gastric cancer cells. The results showed that cells transfected with miR-519 mimics exhibited a reduced rate of cell proliferation when compared with the control group (**Figure 2D**). Additionally, transwell assays showed that the migratory and invasive abilities were significantly suppressed in cells transfected with miR-519 mimics (**Figure 2E**). According to western blot, it was found that after transfection with miR-519 mimics, the protein expression of E-cadherin was significantly increased, while the expression of N-cadherin and vimentin were significantly decreased (**Figure 2F**). The above results demonstrate that abnormally low expression levels of miR-519 were correlated with gastric cancer. Up-regulating the expression of miR-519 may inhibit the proliferation, migration, invasion, and EMT of gastric cancer cells.

### *The effect of down-regulation of miR-519 on gastric cancer cells*

To investigate the regulatory effect of down-regulating miR-519 on gastric cancer cells, AGS cells were transfected with the miR-519 inhibitor. According to RT-qPCR, after transfection, the expression of miR-519 was significantly lower than that of the control group (**Figure 3A**). CCK-8, transwell, and western blot assays were used to detect the proliferation, migration, invasion, and EMT changes to AGS cells. The results showed that after transfection with miR-519 inhibitor, the proliferative, migratory, and invasive abilities of AGS cells were significantly increased (**Figure 3B, 3C**). The expression of E-cadherin was significantly decreased, while the expression of N-cadherin and vimentin were significantly increased (**Figure 3D**).

### *Correlation between FOXQ1 and miR-519*

To investigate the potential targets of miR-519, FOXQ1 was screened for any predicted miRNA interacting sequences using TargetScan [17]. Interestingly, a conserved binding site for miR-519 was identified in the 3'-UTR of FOXQ1 (**Figure 4A**). To confirm whether FOXQ1 is the target gene of miR-519, we used double luciferase reporter gene assays for verification. PsiCHECK2-FOXQ1-wt or psiCHECK2-FOXQ1-Mut was co-transfected into AGS cells, together with miR-NC, miR-519 mimics, NC-inhibitor or miR-519 inhibitor. The results showed that miR-

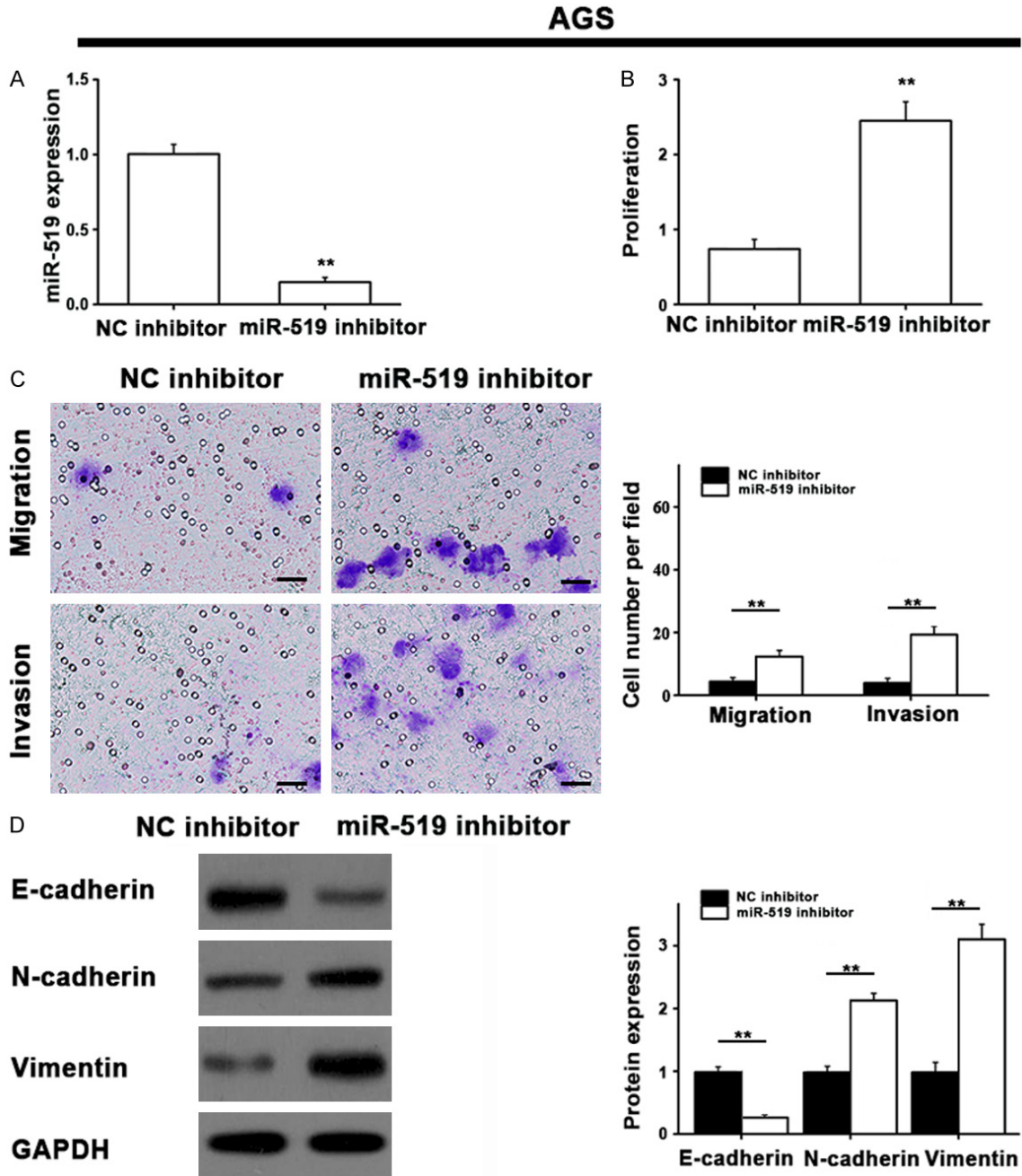
519 mimics significantly inhibited the luciferase activity of psiCHECK2-FOXQ1-wt, while transfection with miR-519 inhibitor produced the opposite effect. However, miR-519 mimics and miR-519 inhibitor did not affect the luciferase activity of the psiCHECK2-FOXQ1-Mut (**Figure 4B**). The effect of miR-519 on the expression of FOXQ1 was detected using RT-qPCR and western blot. The results showed that the mRNA and protein expression of FOXQ1 were significantly decreased after transfection of miR-519 mimics in AGS cells, while transfection with miR-519 inhibitor could up-regulate the expression of FOXQ1 (**Figure 4C, 4D**).

### *miR-519 inhibited proliferation, invasion, migration, and EMT of gastric cancer cells by negatively regulating FOXQ1*

To verify that miR-519 regulated proliferation, invasion, migration and EMT inhibition of gastric cancer cells through FOXQ1, AGS cells were transfected with miR-519 mimics or miR-519 mimics plus the FOXQ1 gene lacking the 3'-UTR (FOXQ1-no UTR). Cells transfected with miR-519 mimics exhibited reduced protein expression of FOXQ1 when compared with the control. Compared with the miR-519 mimics group, the FOXQ1 protein expression was significantly increased in the miR-519 mimics plus FOXQ1-no UTR group (**Figure 5A**). CCK-8 assays showed that OD value in the miR-519 mimics plus FOXQ1-no UTR group was significantly higher than that in the miR-519 mimics group (**Figure 5B**). Transwell assays showed that the number of cells passing through the compartment membrane in the miR-519 mimics plus FOXQ1-no UTR group was significantly higher than that in the miR-519 mimics group (**Figure 5C**). Additionally, western blot assays showed that compared with the miR-519 mimics group, the expression of E-cadherin in the miR-519 mimics plus FOXQ1-no UTR group decreased significantly, while the expression of the N-cadherin and vimentin increased significantly (**Figure 5D**). The above results suggest that miR-519 inhibited the proliferation, invasion, migration, and EMT of gastric cancer cells by negatively regulating the expression of FOXQ1.

## Discussion

In recent years, studies have shown that FOXQ1 is highly expressed in various tumor tissue types and cells, such as non-small cell lung

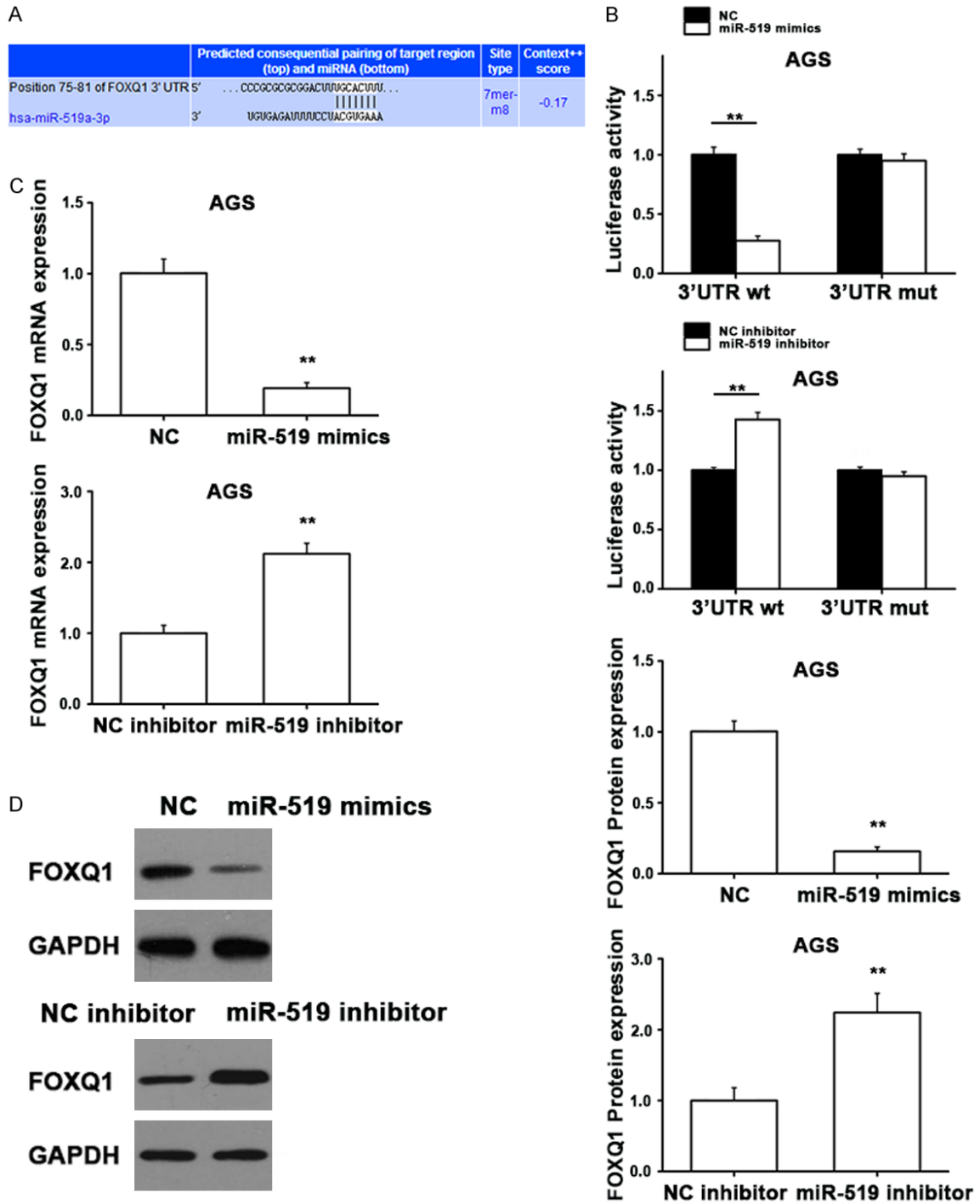


**Figure 3.** Effect of down-regulation of miR-519 on AGS cells. A. miR-519 inhibitor was transfected into AGS cells. B. The effect of miR-519 inhibitor on proliferation of AGS cells was detected by CCK-8 assay. C. Transwell assays were used to detect the effect of the miR-519 inhibitor on the migratory and invasive abilities of AGS cells. D. Western blot was used to detect the effect of the miR-519 inhibitor on EMT. \*\*P<0.01.

cancer, breast cancer, gastric cancer, colorectal cancer, liver cancer, bladder cancer, ovarian cancer and glioblastoma [18, 19]. Epithelial-mesenchymal transition (EMT) promotes cell infiltration, migration, and metastasis, as well as leads to cell cycle escape and apoptosis. EMT is an important event in the process of

invasion and metastasis of epithelial tumor cells [20]. At present, it is believed that transcription factors such as Snail, Slug, Zeb1, ZEB2/SIP1, Twist and E49 induce the EMT [21]. These proteins are able to bind to the E-BOX element of E-cadherin, leading to transcriptional inactivation of E-cadherin. In non-small cell

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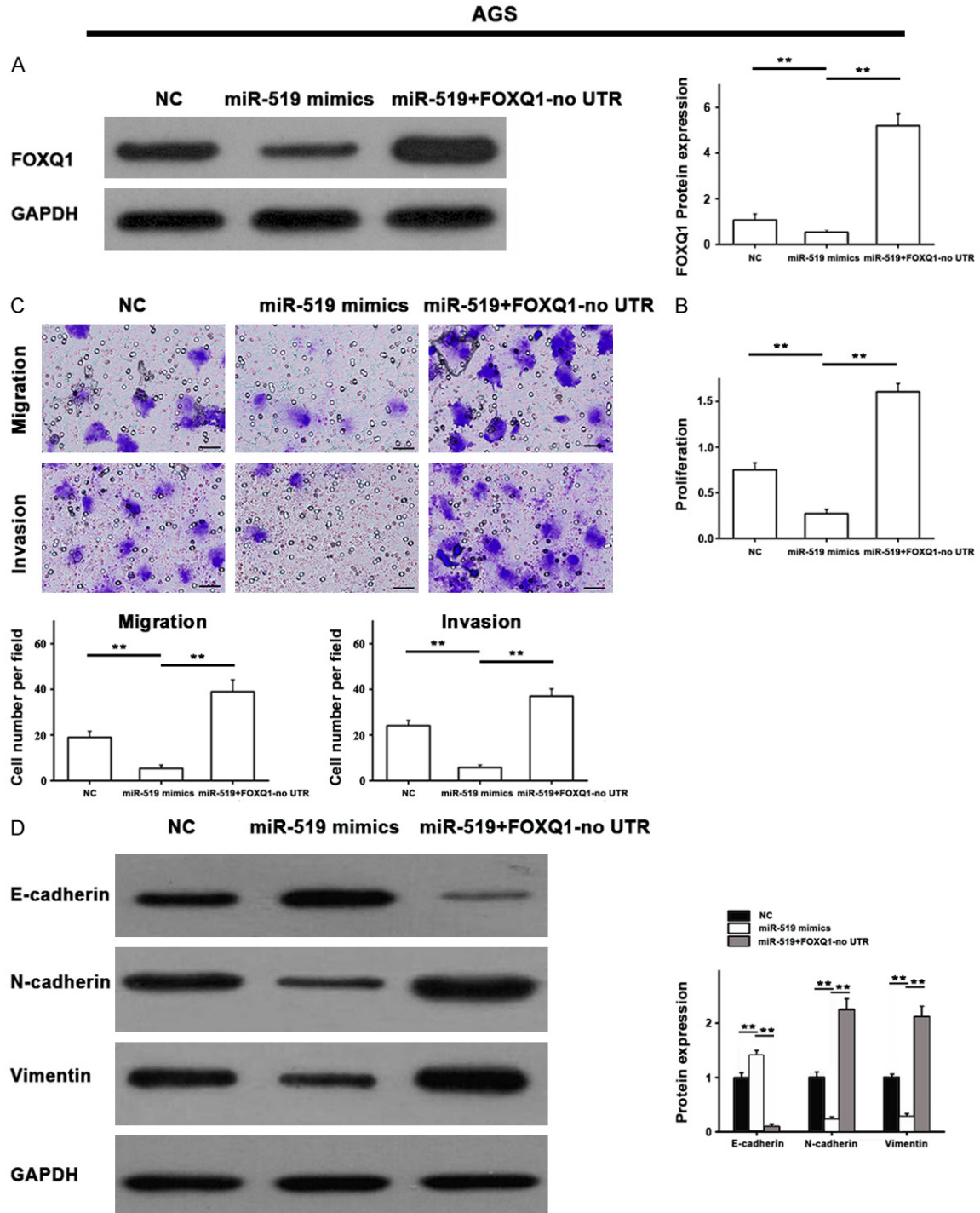
**Figure 4.** Correlation between FOXQ1 and miR-519. A. TargetScan was used to predict the possible binding of miRNA to FOXQ1. B. Double luciferase reporter gene assay. C. Expression of FOXQ1 mRNA in AGS cells was detected using RT-qPCR. D. Western blot was used to detect the FOXQ1 protein expression in AGS cells. \*\*P<0.01.

lung cancer, Feng *et al.* [11] found that high expression of FOXQ1 was negatively correlated with the expression of the epithelial marker, E-cadherin, and positively correlated with the expression of the mesenchymal phenotypic

markers, vimentin and S100A4. In colorectal cancer, cDNA microarray results showed that overexpression of FOXQ1 increased the expression of vascular endothelial growth factor A (VEGFA), WNT3A, R-spondin 2, and CD31.



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**Figure 5.** miR-519 inhibits proliferation, invasion, migration, and EMT of AGS cells by negatively regulating FOXQ1. A. Western blot was used to detect FOXQ1 protein expression. B. CCK-8 assays were used to detect the proliferation of AGS cells. C. Transwell assays were used to detect the invasive and migratory ability of AGS cells. D. Western blot was used to detect the expression of proteins related to EMT in AGS cells. \*\*P<0.01.

Mechanistic studies found that FOXQ1 increased tumor angiogenesis by directly targeting VEGFA, while FOXQ1 resisted apoptosis and promoted the formation of tumors and the

growth of colorectal cancer by trans-activating the expression of P21 [22]. Zhang *et al.* showed that FOXQ1 binds to the E-box of E-cadherin promoter in breast cancer, leading to the re-

duced expression of E-cadherin and to EMT [23]. Xia *et al.* [24] demonstrated that FOXQ1 induced EMT in liver cancer by trans-activating ZEB2 expression in combination with ZEB2 promoter. At the same time, by inducing the expression of versicanV1, the invasion of macrophages was recruited, which promoted the metastasis of liver cancer cells [24]. In bladder cancer, high expression levels of FOXQ1 were negatively correlated with the expression of E-cadherin and positively correlated with the expression of TGF- $\beta$ 1 and vimentin. Interference with the expression levels of FOXQ1 significantly reduced the invasion and metastasis of highly metastatic bladder cancer cells and reversed EMT in tumor cells [25]. In this present study, it was found that FOXQ1 was expressed at abnormally high levels in gastric cancer cell lines and tumor tissues, suggesting that it may be correlated with the development of gastric cancer. Furthermore, it was found that the use of FOXQ1 siRNA to interfere with the expression of FOXQ1 in AGS cells inhibited the proliferation, migration, invasion, and EMT of the cells. This suggests that FOXQ1 may promote the development of gastric cancer types.

Many studies have shown that miRNAs interfere with mRNA translation to down-regulate the expression of target genes [26, 27]. miRNAs exert influence in a variety of tumor types, affecting the growth and invasion of tumor cells through multiple regulatory pathways [28]. For example, miR-185-3p regulates invasion and metastasis of nasopharyngeal carcinoma cells by mediating WNT2B *in vitro* [29]. Down-regulating the expression of miR-193b affects the growth of colon cancer cells through the TGF- $\beta$  and Smad3 signaling pathways [30]. Studies have found that miR-519 is abnormally expressed in a variety of tumors and may play specific roles in these tumor types. For example, miR-519 is highly expressed in preeclampsia, and its expression inhibits the invasion and migration of trophoblast cells [31]. In cervical cancer, miR-519 promotes the metastasis and progression of tumor cells by targeting Smad7 [32]. miR-519 inhibits cell growth and proliferation by inhibiting the expression of MKi67 in hepatocellular carcinoma cells [33]. Nevertheless, the expression levels and biological effects of miR-519 in gastric cancer remain unclear. In this present study, it was found that miR-519 was expressed at a low level in both

gastric cancer cells and tumor tissues, suggesting that abnormal expression of miR-519 may be related to gastric cancer. To further investigate the effect of miR-519 on the biological behavior of gastric cancer cells, miR-519 mimics or miR-519 inhibitor were transfected into AGS cells. It was found that after transfection of miR-519 mimics, the proliferative, migratory and invasive ability of AGS cells were significantly decreased. The expression of E-cadherin was increased, N-cadherin and vimentin were significantly decreased. On the other hand, transfection with miR-519 inhibitor showed the opposite results. These results suggest that miR-519 may have a suppressive effect in gastric cancer.

A previous study has confirmed the important role of the miRNA-FOXQ1 axis in tumor growth and metastasis in other tumors [34]. Peng *et al.* [35] found that the expression levels of miR-124 were low in nasopharyngeal carcinoma cells and tissues, while the expression of FOXQ1 was increased, demonstrating a negative correlation. Up-regulation of miR-124 inhibits tumor growth and metastasis by inhibiting the expression of FOXQ1 [35]. Zhang *et al.* [36] found that miR-422a was down-regulated in osteosarcoma, and overexpression of miR-422a inhibited the growth, invasion, and metastasis of osteosarcoma cells. TargetScan predicted that FOXQ1 is a potential target gene of miR-519, and in this present study, it was confirmed that miR-519 could bind to 3'-UTR of FOXQ1. Furthermore, transfection of miR-519 mimics in AGS cells inhibited the expression of FOXQ1 at both the mRNA and protein levels, whereas transfection of miR-519 inhibitor produced the opposite results. This indicated that miR-519 may target and regulate FOXQ1. Subsequently, miR-519 mimics plus FOXQ1-no UTR were co-transfected into AGS cells, and we found that the overexpression of FOXQ1 partially reversed the effects of miR-519 on the proliferation, invasion, migration and EMT of gastric cancer cells. This suggested that miR-519 may regulate the biologic behavior of gastric cancer cells by targeting FOXQ1.

### Conclusions

In conclusion, this study revealed the role of miR-519 in gastric cancer and its related mechanism. Experimental data showed that abnormally low expression of miR-519 was correlated

with gastric cancer, and miR-519 may have a tumor-inhibiting effect in gastric cancer. Thus FOXQ1 may be the key target of miR-519 in regulating the biologic behavior of gastric cancer cells.

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### Disclosure of conflict of interest

None.

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