European Review for Medical and Pharmacological Sciences 2018; 22: 8298-8305

# MiR-490-3p inhibited the proliferation and metastasis of esophageal squamous cell carcinoma by targeting HMGA2

N.-N. KANG[1], S.-L. GE[2], R.-Q. ZHANG[1], Y.-L. HUANG[1], S.-D. LIU[2], K.-M. WU[1]

[1]Department of Thoracic Surgery, 1st Affiliated Hospital of Anhui Medical University, Hefei, China
[2]Department of Cardiovascular Surgery, 1st Affiliated Hospital of Anhui Medical University, Hefei, China

**Abstract. – OBJECTIVE: To identify the potential role of miR-490-3p in the development of esophageal squamous cell carcinoma (ESCC), and to explore the possible underlying mechanism.**

**PATIENTS AND METHODS: Human ESCC tissues and cancer-adjacent normal tissues were collected. The mRNA expression level of miR-490-3p was detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). On-line target gene prediction software was applied to screen high-mobility group AT-hook 2 (HMGA2). Subsequently, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), qRT-PCR, Western blotting, transwell and scratch-wound assays were conducted to analyze the effect of miR-490-3p on the biological function of the ESCC cell line (EC-109).**

**RESULTS: In our study, the mRNA expression level of miR-490-3p was remarkably reduced in ESCC tissues and cells. Molecular mechanism analysis confirmed that miR-490-3p could act on the 3'-UTR of HMGA2 and regulate its expression. Subsequent functional experiments indicated that decreased expression of HMGA2 resulting from the up-regulation of miR-490-3p could inhibit the proliferation, invasion, migration and epithelial-mesenchymal transition (EMT) of ESCC cells.**

**CONCLUSIONS: We discovered the inhibitory effect of miR-490-3p on ESCC by targeting HMGA2, and revealed that miR-490-3p could be a potential therapeutic target for ESCC.**

*Key Words:*
MiR-490-3p, Esophageal squamous cell carcinoma (ESCC), High-mobility group AT-hook 2 (HMGA2), Epithelial-mesenchymal transition (EMT).

## Introduction

Esophageal cancer is a very common malignant tumor in the digestive system. The morbidity and mortality rate of esophageal cancer ranks the eighth highest and the sixth highest among all malignancies in the world, respectively[1]. The incidence of esophageal cancer in China is especially high. In addition, more than 90% of esophageal cancer patients are esophageal squamous cell carcinoma (ESCC), which is characterized by high degree of malignancy, strong invasiveness, rapid progression and poor prognosis. In recent years, advances have been made in the diagnosis and treatment of ESCC. However, the prognosis of ESCC patients is still poor due to the indefinite proliferation, invasion and metastasis of ESCC cells. The overall five-year survival rate after surgery has remained at around 30-40% for a long time. Moreover, recurrence and metastasis are the main reasons leading to the treatment failure of ESCC[2]. Tumor metastasis is a complex process accumulated by multiple factors, multiple stages and multiple steps under gene regulation. Meanwhile, it's dominated by environmental factors and acted through gene regulation[3]. Although many genes correlated with the invasion and metastasis of ESCC have been discovered so far, a large number of genes involved in the progression of ESCC have not yet been widely studied. Moreover, the methods for early diagnosis and treatment are still scarce. Therefore, active and in-depth investigations of the regulatory mechanisms of the proliferation, invasion, and metastasis of ESCC, the identification of key regulatory factors and important signal regulatory networks during tumor metastasis, and the exploration of effective diagnosis and treatment targets for ESCC in the early stage, are of important practical significance for inhibiting the development and progression of ESCC and improving the patient's prognosis. Micro ribonucleic acids (miRNAs) are small ribonucleic acid (RNA) molecules that are involved


*Corresponding Author:* Shenglin Ge, MD; e-mail: zbvz92@163.com

in the regulation of gene expression. MiRNAs are endogenous non-coding single-stranded RNA molecules with about 21-25 nucleotides in length, which are ubiquitous in eukaryotes. MiRNAs can be completely or incompletely paired and combined with the 3'-untranslated region (3'-UTR) of target protein encoded gene messenger ribonucleic acid (mRNA) according to the principle of base pairing. Meanwhile, they may negatively regulate the expression of endogenous important genes at the post-transcriptional level, eventually participating in a series of important processes in life[4]. The expression of miRNA is time and tissue specific, and plays an important regulatory role in cell differentiation, proliferation, apoptosis, metabolism, cellular immune response and other biological processes[5]. The disorder of miRNA expression is involved in the development and progression of diseases, including inflammation and various malignancies[6]. As previously reported, miR-490-3p has exhibited its unique advantages in the diagnosis and treatment of a variety of malignant tumors, such as hepatocellular carcinoma[7], gastric cancer[8], breast cancer[9], colorectal cancer[10] and lung cancer[11]. However, few reports have focused on the function of miR-490-3p in the occurrence and development of ESCC as well as related molecular mechanism. In the present study, we investigated the role of miR-490-3p in the occurrence and development of ESCC by analyzing the expression of miR-490-3p and exploring the effect of miR-490-3p on biological behaviors of ESCC cells.

## Patients and Methods

### Esophageal Squamous Cell Carcinoma (ESCC) Cases and Cell Line

A total of 64 ESCC patients who underwent surgery in the First Affiliated Hospital of Anhui Medical University were pathologically confirmed as ESCC. Pre-operative chemotherapy or radiotherapy treatment was forbidden. The tissues were kept in a -80°C refrigerator. Relevant adjacent normal tissues were concerned by biological biopsy to make sure that they did not include ESCC cells. After all, Declaration of Helsinki should be mentioned and respected. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University. Informed consents were obtained from all participants before the study. The ESCC cell line (EC-109) and normal esophageal epithelial cell line (Het-1A) were purchased from the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640) medium (Gibco, Rockville, MD, USA) complemented with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 IU/mL penicillin (Gibco, Rockville, MD, USA), and were grown in a 37ºC, 5% $CO_2$ incubator.

### Luciferase Reporter Assay

By searching TargetScan, miRDB and microRNA websites, it was found that HMGA2 was a target gene of miR-490-3p. The binding sequence of miR-490-3p at the 3'-end of HMGA2 was mutated by using a point mutation kit (Agilent Technologies, Santa Clara, CA, USA). Next, the mutated HMGA2 (Mut-type) and non-mutant HMGA2 (WT-type) were connected with the pGL3-Basic luciferase reporter vector (Promega, Madison, WI, USA). PGL3-basic vector together with mutant HMGA2 were transfected into EC109 cells after lentivirus intervention on 24-well plates. Meanwhile, the same method was performed on the pGL3-Basic vector connection with the non-mutant HMGA2 according to the instructions of the Luciferase Reporter Gene Assay Kit (Yeasen, Shanghai, China). Finally, the luciferase activity was detected with a multi-function microplate reader.

### Cell Transfection

MiR-490-3p mimics and si-HMGA2 were synthesized and transfected to the ESCC cell line EC-109 to analyze the biological function of miR-490-3p. Three groups were individually established: including the NC group (negative control), the miR-490-3p mimics group (EC109 cells transfected with miR-490-3p mimics) and the mimics + HMGA2 group (EC109 cells transfected with miR-490-3p mimics and si-HMGA2). All the materials were purchased from RiboBio (Guangzhou, China). Cell transfection was performed according to the manufacturer's instructions of Lipofectamine RNAiMAX (Life Technologies, Gaithersburg, MD, USA).

### Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Analysis

The expression of miR-490-3p in EC109 cells was detected by quantitative reverse trancription-polymerase chain reaction (qRT-PCR). Total RNA was extracted by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the

manufacturer's instruction. SYBR green qRT-PCR assay (Thermo Fisher Scientific Waltham, MA, USA) was used to measure the mRNA expression of HMGA2. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control. TaqMan miRNA assay (Applied Biosystems, Foster City, CA, USA) was used to measure the level of miR-490-3p expression normalized to U6. Primers used in this study were as follows: HMGA2 F: ACCCAGGGGAAGACCCAAA; R: CCTCTTGGCCGTTTTTCTCCA. GAPDH F: CTGGGCTACACTGAGCACC; R: AAGTGGTCGTTGAGGGCAATG. MiR-490-3p: F: 5'-GCAAACAACCAUUCGGCUGUC-3', R: 5'-CGCAGGTCCGGAGTAGGT-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'.

### Western Blot Analysis

Radio-immunoprecipitation assay (RIPA) lysate (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was employed for total protein extraction in transfected cells. Briefly, the proteins were separated by using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Sigma-Aldrich, St. Louis, MO, USA), and were then transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland). Subsequently, the membranes were blocked with 5% milk and incubated with the following relevant antibodies at 4°C overnight: HMGA2, E-cadherin, N-cadherin, Vimentin and β-actin [diluted at 1:1000, Cell Signaling Technology (CST) Inc. Danvers, MA, USA]. The membranes were incubated with corresponding secondary antibodies (CST, Inc. Danvers, MA, USA) for 2 h at room temperature. Finally, enhanced chemiluminescence (ECL) (Thermo Fisher Scientific, Waltham, MA, USA) was used for band visualization. β-actin was used as an internal reference, and the relative changes in protein expression were detected.

### Cell Proliferation

When transfected cells grew to the logarithmic growth phase, they were collected and diluted into $1\times10^6$ cell suspension. Then, the cells were seeded into a 96-well cell culture plate ($5\times10^3$/100 μL per well). Wells only added with culture medium were used as blank controls. Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA). Briefly, 15 μL MTT reagent (500 μg/mL) were added into each well, followed by incubation for another 2 h. The absorbance was finally measured by using an enzyme-labeled spectrophotometer, and blank controls were used for zero setting.

### Cell Invasion and Migration

After 48 h of transfection, cell invasion ability was measured using a transwell chamber (Corning, Corning, NY, USA) with a pore size of 8 μm. Matrigel (BD, Franklin Lakes, NJ, USA) at a concentration of 1:9 was added in the upper chamber for detecting the invasion ability. Briefly, $2 \times 10^4$ cells were planted into the upper chamber with serum free medium, while the lower chamber was added with complete culture medium containing 10% fetal bovine serum (FBS) as a chemoattractant. After 2 days incubation, cells on the top of the membrane were wiped with a brush. Subsequently, the membrane was stained with 0.2% crystal violet and then drenched by 95% ethanol. The invading cells were recorded by an inverted microscope. Cell migration assay was performed by scratch-wound assay. EC109 cells were seeded in 6-well plates and allowed to reach confluence. After transfection, each well was scraped with 10 μL pipette tip to create a linear region devoid of cells. Subsequently, cells in each well were cultured with RPMI-1640 medium complemented with 2% FBS (both from Gibco, Rockville, MD, USA). Wound healing was monitored at 48 h after scraping. Five randomly selected fields of each well were chosen for analysis.

### Statistical Analysis

Student's *t*-test or *F*-test was used to compare difference among groups. All *p*-values were two-sided and $p < 0.05$ were considered statistically significant. Prism 6.02 software (La Jolla, CA, USA) was applied for statistical analysis.

## Results

### The Expression of miR-490-3p Was Decreased in ESCC

To examine the role of miR-490-3p in ESCC development, we detected the expression level of miR-490-3p in ESCC tissues and the adjacent normal tissues by qRT-PCR. The results showed that the expression of miR-490-3p in ESCC tissues was significantly lower than that of adjacent normal tissues (Figure 1A). Similar results were obtained in

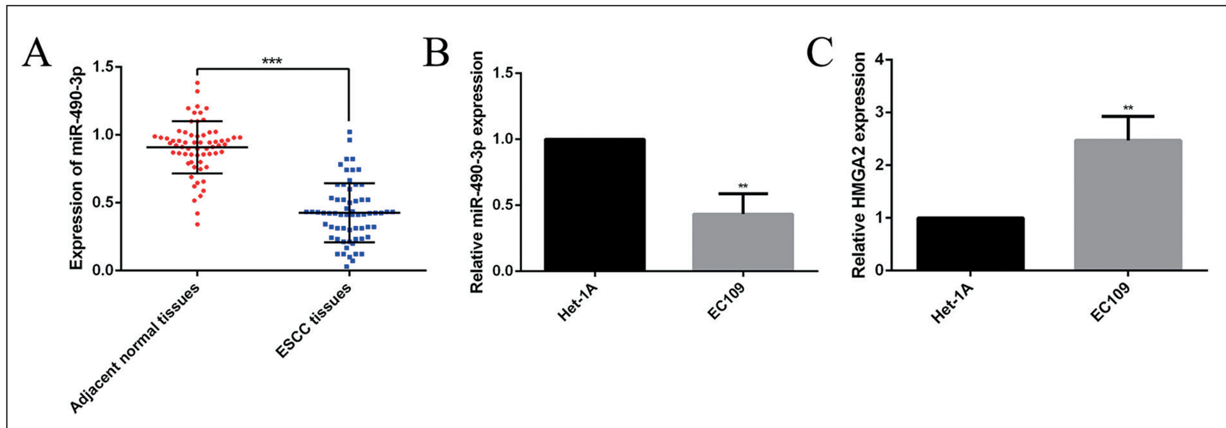


**Figure 1.** The expression of miR-490-3p and HMGA2 in ESCC tissues, corresponding adjacent normal tissues, ESCC cells and normal esophageal epithelial cells. **A,** Difference in the expression of miR-490-3p between ESCC tissues and corresponding adjacent normal tissues. (***$p < 0.001$ compared with adjacent normal tissues). **B,** and **C,** The expression of miR-490-3p and HMGA2 in the ESCC cell line (EC109) and normal esophageal epithelial cell line (Het-1A). (**$p < 0.01$ compared with Het-1A).

the cellular level (Figure 1B). However, the expression level of HMGA2 was significantly up-regulated in ESCC cells (Figure 1C). Taken together, we thought miR-490-3p might be correlated with HMGA2 during the progression of ESCC.

### HMGA2 Was a Direct Target of miR-490-3p

To elucidate the putative and possible targets of miR-490-3p, we checked three publicly available algorithms, including TargetScan, miRDB and microRNA. We found that HMGA2 was a supposed target of miR-490-3p (Figure 2A). Therefore, HMGA2 was implemented to our further studies. We then established relevant luciferase reporter vectors containing wild or mutant-type miR-490-3p seed sequences of the 3'UTR of HMGA2. Increased expression of miR-490-3p by mimics transfection resulted in the decreased luciferase activity of the wide-type HMGA2 3'UTR reporter gene. However, it had no effect on the mutant-type (Figure 2B), suggesting the expression of HMGA2 could be regulated by miRNA 490-3p.

### MiR-490-3p Suppressed Cell Proliferation

After cell transfection, we then performed MTT assay to detect the cell proliferation rate. MTT results suggested that the cell proliferation rate of EC109 cells was limited by miR-490-3p mimics transfection. In contrast, the increased cell growth of ESCC cells was found in the mimics+ HMGA2 group (Figure 3).

### MiR-490-3p Inhibited the Invasion and Migration of ESCC Cells

Migration and invasion are the two most key factors in the proliferation of cancer cells. Transwell assay revealed that the invasion ability of EC109 cells was restricted by miR-490-3p mimics transfection. Meanwhile, results of scratch-wound assay showed that cell migration was suppressed by the transfection of miR-490-

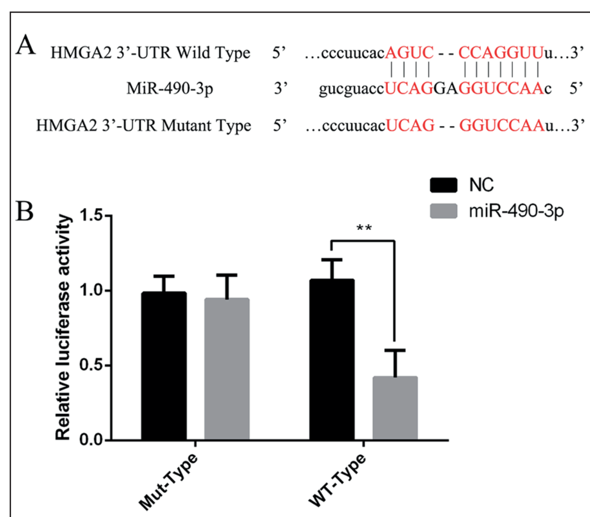


**Figure 2.** HMGA2 was a direct and functional target of miR-490-3p. EC109 cells were transfected with miR-490-3p mimics or inhibitor. **A,** Diagram of putative miR-490-3p binding sites of HMGA2. ***B,*** Relative activities of luciferase reporters (**$p < 0.01$).

3p. However, HMGA2 could reverse the effects of miR-490-3p on the migration and invasion of ESCC cells (Figure 4).

### MiR-490-3p Inhibited Epithelial-Mesenchymal Transition (EMT)

As expected, the expression level of *HMGA2* was decreased in EC109 cells after the transfection of miR-490-3p mimics. Meanwhile, results confirmed the regulatory effect of miR-490-3p on HMGA2 expression (Figure 5). EMT plays an important role in the metastasis of cancer cells. However, the exact role of miR-490-3p in the EMT of ESCC cells has not yet been reported. Therefore, in this study, we used Western blot to detect the protein expression of EMT markers in EC109 cells. The results demonstrated that the expression of the epithelial marker E-cadherin was notably increased, while the expression of the mesenchymal markers such as N-cadherin and Vimentin were significantly decreased after miR-490-3p overexpression. The above data suggested the inhibitory effect of miR-490-3p on EMT (Figure 5).

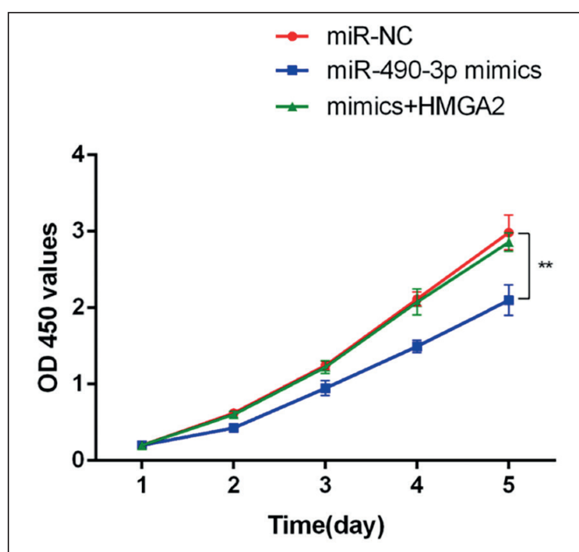


**Figure 3.** MiR-490-3p inhibited the proliferation of ESCC cells (** $p < 0.01$).

## Discussion

In malignant tumors, some miRNAs may serve as both tumor suppressor genes and on-

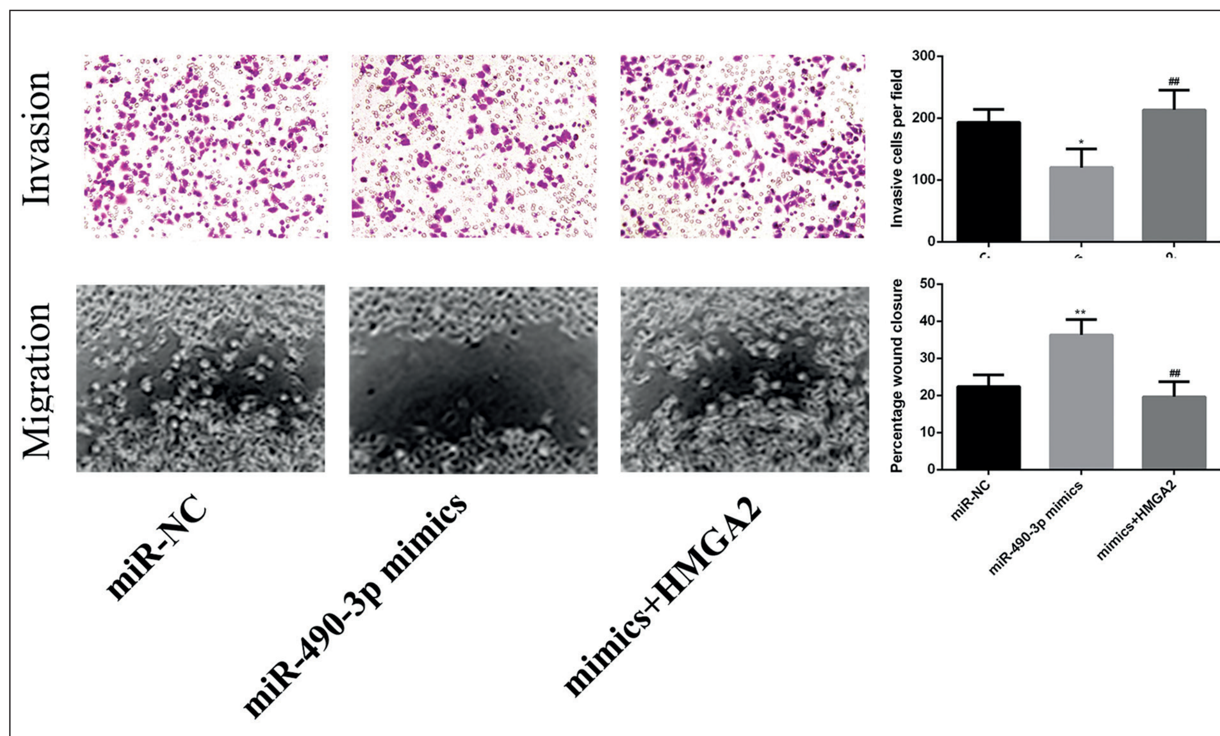


**Figure 4.** MiR-490-3p/HMGA2 axis inhibited the invasion and migration of ESCC cells. HMGA2 overexpression attenuated the suppression effect of miR-490-3p on EC109 cells. ***A,*** The invasion ability detected by transwell assay. ***B,*** The migration ability detected by scratch-wound assay. (* $p < 0.05$, ** $p < 0.01$ *vs.* the NC group; ## $p < 0.01$ *vs.* the mimics group).

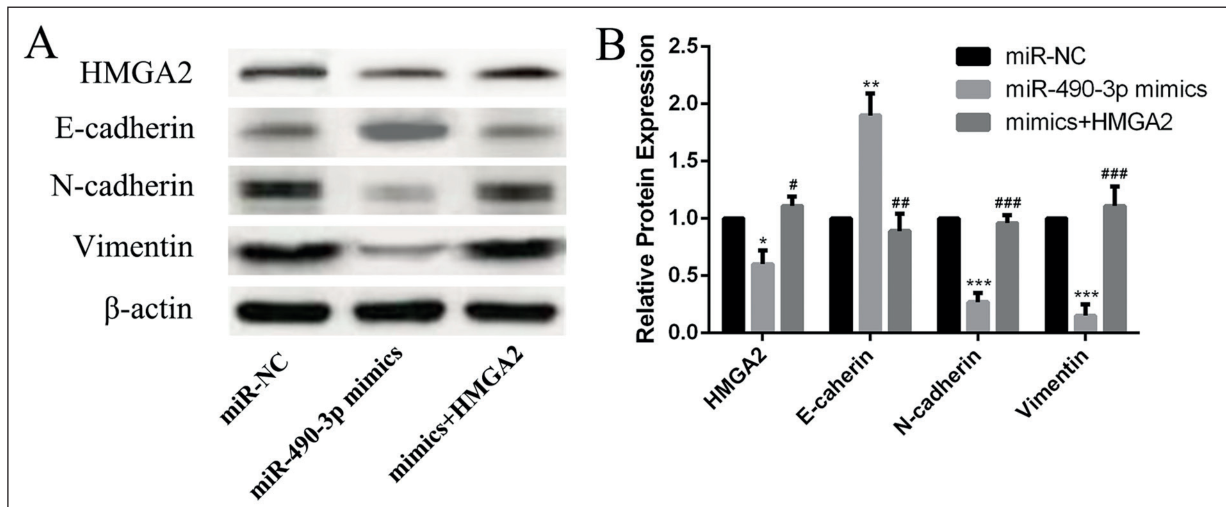


**Figure 5.** The decreased expression of HMGA2 resulted from miR-490-3p up-regulation could reduce the EMT of ESCC cells. **A,** The expression of HMGA2 and EMT markers was detected by Western blot after transfection with mimics or si-HMGA2. **B,** Quantitative analysis of the proteins. Data were presented as means ± standard deviations. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* the NC group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs.* the mimics group).

cogenes. They may bind to the 3'-UTR of target genes, and regulate the occurrence, development and prognosis of tumors[12]. Therefore, discovering and determining the functional targeting of miRNAs is of great significance in clarifying the biological functions of corresponding miRNAs. In our study, bioinformatics software was used to analyze the genome structure. Meanwhile, it was found that HMGA2 might be a target gene of miR-490-3p based on the principle of complementary pairing. According to the results of luciferase reporter assay, miR-490-3p mimics significantly down-regulated the luciferase activity of the wild-type (WT) HMGA2-3'UTR rather than the mutant-type (Mut) HMGA2-3'UTR. To sum up, these results speculated that HMGA2 might be a downstream target gene of miR-490-3p. HMGA2 is a non-histone chromatin-associated protein that is widely existed in the nucleus of eukaryotic organisms. Meanwhile, HMGA2 is named for its high migration rate in polyacrylamide gel electrophoresis. HMGA2 is widely involved in various physiological functions of cells, inducing the regulation of gene transcription, integrating retrovirus into the chromosome, inducing transformation and promoting the activation of cancer cells[13-15]. At present, there is more attention to the role of HMGA2 in cancer. HMGA2 has been reported to be abnormally expressed in a variety of malignancies, and is closely related to tumor proliferation, apoptosis, invasion and metastasis, chemotherapy resistance and poor prognosis[16-19]. However, few reports have investigated the expression and biological function of HMGA2 in ESCC. Moreover, the underlying potential molecular regulatory mechanism has not fully been elucidated. At present, the role of EMT in the invasion and metastasis of malignant tumors has become a research hotspot. Most of the researches on HMGA2 promoting tumor invasion and metastasis have focused on HMGA2-related EMT. It's known to all that EMT is a key starting point for cells to gain the movement and migration ability. After EMT in tumor cells, the invasion and metastasis abilities may be stronger, and metastasis may occur more easily. The main features of EMT include the loss of intercellular connecting substances, the disappearance of intercellular polar complexes and the changes in the structure of extracellular matrices[20]. Meanwhile, EMT plays an extremely important role in the biological processes such as the formation, invasion, metastasis, and tumor self-renewal of malignant cells. It's also recognized as a marker step of tumor progression[21]. Down-regulated expression of adhesion factor E-cadherin on the surface of epithelial cells and up-regulated expression of N-cadherin, Vimentin and other interstitial proteins are often used as symbols of the occurrence of EMT[22]. Current researches[23-26] have confirmed that EMT plays an extremely important role in mediating the invasion and metastasis of various malignancies.

Therefore, it is of great significance to explore key factors and the possible regulatory mechanism of EMT, and to study the invasion and metastasis of malignant tumors for seeking key molecular targets for effective treatment, especially tumor cell metastasis. In our work, after up-regulating the expression of miR-490-3p, the proliferation, migration and invasion of ESCC cells were significantly decreased. Furthermore, when miR-490-3p was overexpressed in ESCC cells, the activation of EMT-associated proteins were decreased. However, when the expression of HMGA2 was up-regulated, the inhibitory effect of miR-490-3p was counteracted. The above results all indicated the inhibitory effect of miR-490-3p on the functional ability of ESCC cells by targeting HMGA2 expression.

## Conclusions

We preliminarily revealed the molecular mechanism of miR-490-3p in inhibiting the proliferation, migration, invasion and EMT of ESCC cells by target regulating HMGA2 expression. Meanwhile, our study provided an important theoretical basis for studying the pathogenesis of ESCC and an important reference value for further developing bio targeted gene therapy for esophageal cancer.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

## References

1) Procter DS. Oesophageal carcinoma. S Afr Med J 1973; 47: 348-351.
2) Davies AR, Pillai A, Sinha P, Sandhu H, Adeniran A, Mattsson F, Choudhury A, Forshaw MJ, Gossage JA, Lagergren J, Allum WH, Mason RC. Factors associated with early recurrence and death after esophagectomy for cancer. J Surg Oncol 2014; 109: 459-464.
3) Matejcic M, Iqbal PM. Gene-environment interactions in esophageal cancer. Crit Rev Clin Lab Sci 2015; 52: 211-231.
4) Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M, Calin GA, Ivan M. A microRNA signature of hypoxia. Mol Cell Biol 2007; 27: 1859-1867.
5) Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011; 12: 861-874.
6) Ranjha R, Paul J. Micro-RNAs in inflammatory diseases and as a link between inflammation and cancer. Inflamm Res 2013; 62: 343-355.
7) Zhang LY, Liu M, Li X, Tang H. MiR-490-3p modulates cell growth and epithelial to mesenchymal transition of hepatocellular carcinoma cells by targeting endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3). J Biol Chem 2013; 288: 4035-4047.
8) Ou M, Li L, Zheng WC. Reduced miR-490-3p expression is associated with poor prognosis of Helicobacter pylori induced gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 3384-3388.
9) Jia Z, Liu Y, Gao Q, Han Y, Zhang G, Xu S, Cheng K, Zou W. MiR-490-3p inhibits the growth and invasiveness in triple-negative breast cancer by repressing the expression of TNKS2. Gene 2016; 593: 41-47.
10) Khan AH, Anees M, Arshad M, Muhammad YS, Iqbal M, Yousaf S. Effects of illuminance and nutrients on bacterial photo-physiology of hydrocarbon degradation. Sci Total Environ 2016; 557-558: 705-711.
11) Gu H, Yang T, Fu S, Chen X, Guo L, Ni Y. MicroRNA-490-3p inhibits proliferation of A549 lung cancer cells by targeting CCND1. Biochem Biophys Res Commun 2014; 444: 104-108.
12) de Planell-Saguer M, Rodicio MC. Analytical aspects of microRNA in diagnostics: a review. Anal Chim Acta 2011; 699: 134-152.
13) Fedele M, Fusco A. HMGA and cancer. Biochim Biophys Acta 2010; 1799: 48-54.
14) Morishita A, Zaidi MR, Mitoro A, Sankarasharma D, Szabolcs M, Okada Y, D'Armiento J, Chada K. HMGA2 is a driver of tumor metastasis. Cancer Res 2013; 73: 4289-4299.
15) Natarajan S, Hombach-Klonisch S, Droge P, Klonisch T. HMGA2 inhibits apoptosis through interaction with ATR-CHK1 signaling complex in human cancer cells. Neoplasia 2013; 15: 263-280.
16) D'Angelo D, Esposito F, Fusco A. Epigenetic mechanisms leading to overexpression of HMGA proteins in human pituitary adenomas. Front Med (Lausanne) 2015; 2: 39.
17) Wu A, Wu K, Li J, Mo Y, Lin Y, Wang Y, Shen X, Li S, Li L, Yang Z. Let-7a inhibits migration, invasion and epithelial-mesenchymal transition by targeting HMGA2 in nasopharyngeal carcinoma. J Transl Med 2015; 13: 105.
18) Xiang Q, Tang H, Yu J, Yin J, Yang X, Lei X. MicroRNA-98 sensitizes cisplatin-resistant human lung adenocarcinoma cells by up-regulation of HMGA2. Pharmazie 2013; 68: 274-281.
19) Yamazaki H, Mori T, Yazawa M, Maeshima AM, Matsumoto F, Yoshimoto S, Ota Y, Kaneko A, Tsuda H, Kanai Y. Stem cell self-renewal factors Bmi1 and HMGA2 in head and neck squamous cell carcinoma: clues for diagnosis. Lab Invest 2013; 93: 1331-1338.

20) Celia-Terrassa T, Meca-Cortes O, Mateo F, Martinez DPA, Rubio N, Arnal-Estape A, Ell BJ, Bermudo R, Diaz A, Guerra-Rebollo M, Lozano JJ, Estaras C, Ulloa C, Alvarez-Simon D, Mila J, Vilella R, Paciucci R, Martinez-Balbas M, de Herreros AG, Gomis RR, Kang Y, Blanco J, Fernandez PL, Thomson TM. Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. J Clin Invest 2012; 122: 1849-1868.
21) Kharbanda A, Rajabi H, Jin C, Alam M, Wong KK, Kufe D. MUC1-C confers EMT and KRAS independence in mutant KRAS lung cancer cells. Oncotarget 2014; 5: 8893-8905.
22) Liao G, Wang M, Ou Y, Zhao Y. IGF-1-induced epithelial-mesenchymal transition in MCF-7 cells is mediated by MUC1. Cell Signal 2014; 26: 2131-2137.
23) Cichon MA, Nelson CM, Radisky DC. Regulation of epithelial-mesenchymal transition in breast cancer cells by cell contact and adhesion. Cancer Inform 2015; 14: 1-13.
24) Jiang JH, Liu C, Cheng H, Lu Y, Qin Y, Xu YF, Xu J, Long J, Liu L, Ni QX, Yu XJ. Epithelial-mesenchymal transition in pancreatic cancer: Is it a clinically significant factor? Biochim Biophys Acta 2015; 1855: 43-49.
25) Liu Z, Zhang Y, Xu Z. UNC119 promotes the growth and migration of hepatocellular carcinoma via Wnt/beta/catenin and TGF/beta-EMT signaling pathways. J BUON 2018; 23: 188-192.
26) Zhang W, Shi X, Peng Y, Wu M, Zhang P, Xie R, Wu Y, Yan Q, Liu S, Wang J. HIF-1alpha promotes Epithelial-Mesenchymal transition and metastasis through direct regulation of ZEB1 in colorectal cancer. PLoS One 2015; 10: e129603.