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Original Article

miR-124 inhibits the proliferation, migration and invasion of esophageal squamous cell carcinoma by downregulating STATS

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Abstract: Objective: To investigate the expression, role and the underlying mechanism of miR-124 in esophageal squamous cell carcinoma (ESCC). Methods: A total of 25 pairs of ESCC and adjacent tissues were collected for analysis. The human normal esophageal epithelial cell line (Het-1A) and human esophageal cancer cell lines (EC-1) was cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). Cells were transfected with miR-124 mimics and inhibitors. A series of assays, including Cell-Counting-Kit-8 (CCK-8), Transwell migration and invasion assays, real-time quantitative polymerase chain reaction (RT-qPCR), enzyme-linked immunosorbent assay (ELISA) and luciferase reporter gene assay, were performed to detect the effects of miR-124 on ESCC cells. Spearman's correlation analysis was used to evaluate the relationship of miR-124 expression and signal transducer and activator of transcription-3 (STAT3) expression. Results: miR-124 was significantly downregulated in ESCC tissues and cells. Overexpression of miR-124 inhibited the proliferation, migration and invasion of ESCC cells in vitro, while inhibition of miR-124 promoted these processes (all $P<0.05$). miR-124 was found to specifically bind to the 3'-untranslated region (3'UTR) of STAT3. The expression level of STAT3 was obviously higher in tumor tissues and cells compared to normal adjacent tissues and cells (all $P<0.05$). Moreover, miR-124 expression was negatively associated with STAT3 levels. Restoring STAT3 expression in ESCC cells transfected with miR-124 mimics partially reversed the inhibitory effects of miR-124 mimics on cell proliferation, migration, and invasion. Conversely, inhibiting STAT3 expression in ESCC cells transfected with miR-124 inhibitors partially abolished the promoting effects of miR-124 inhibitors on the proliferation, migration and invasion of ESCC cells. Conclusion: miR-124 inhibits the proliferation, migration and invasion of ESCC cells by downregulating STATS, indicating that miR-124 may serve as a molecular candidate for treating ESCC.

Keywords: Esophageal squamous cell carcinoma, miR-124, STAT3, proliferation, migration and invasion

Introduction

Esophageal carcinogenesis is a complex, multistage process involving the dysregulation of various genes [1]. Increasing evidence have shown that epigenetics plays a crucial role in the development of malignant tumors, and the reversibility of epigenetic modifications offers novel therapeutic strategies for cancer treatment [2]. Recent studies have highlighted the involvement of microRNAs (miRNAs) in the regulation of tumor epigenetics [3]. During tumor progression, a variety of differentially expressed miRNAs bind to the 3'-untranslated region (3'UTR) of target gene mRNAs, typically through complete or partial base pairing. This binding inhibits protein translation at the post-transcriptional level, thus modulating the expression of the target gene and its associated downstream genes. These changes regulate critical cellular processes such as cell proliferation, differentiation, development, and apoptosis. Notably, a single miRNA can act on multiple downstream genes, and multiple miRNAs can also regulate the same target gene. This multipathway or signaling pathway regulation creates a complex miRNAs-mediated regulatory network of [4]. Therefore, identifying and characterizing tumor-associated miRNAs is essential for understanding the mechanism of tumorigenesis, and manipulating miRNA expression to inhibit the proliferation and migration of tumor

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cells holds promise as a new pathway for cancer treatment.

miR-124 is a miRNA that has been found to be abundantly expressed in the nervous system, where it plays a role in processes such as growth, apoptosis, autophagy in neuronal cells. In addition, miR-124 is closely related to the occurrence and development of a variety of malignant tumors. Several studies have shown that miR-124 expression is down-regulated in gastric cancer cells, where it inhibits cell proliferation, migration and invasion by targeting the expression of Notch1 protein [5]. In prostate cancer cells, miR-124 suppresses tumor cell proliferation by targeting enhancer of zeste homolog-2 (EZH2) and Src [6]. In breast cancer, miR-124 is lowly expressed, and its overexpression inhibits the proliferation, migration and invasion of breast cancer cells by regulating the expression of Ets-1 [7]. Sun et al. found that miR-124 can inhibit the growth of colorectal cancer cells both *in vitro* and *in vivo* by targeting key enzymes in the pentose phosphate pathway [8]. Wan et al. found that miR-124 suppresses cell motility and angiogenesis in cervical cancer cells by suppressing the expression of amotL1, functioning as an oncogene [9]. In addition, miR-124 has been shown to inhibit tumor progression in osteosarcoma [10].

These studies collectively highlight that miR-124 plays an important regulatory role in the proliferation, migration, and epithelial-mesenchymal transition (EMT) of various malignant tumors. However, the expression and role of miR-124 in esophageal cancer remain underexplored both domestically and internationally. To clarify the specific regulatory function and mechanism of miR-124 in the development of esophageal cancer, this study explored the effects of miR-124 on the biological functions of esophageal cancer cells at the cellular level. Furthermore, bioinformatics tools were used to predict and validate the possible downstream target genes of miR-124, providing an experimental basis for considering miR-124 as a new therapeutic target for esophageal cancer.

Materials and methods

Samples collection

In this retrospective study, 25 paired samples of esophageal squamous cell carcinoma (ESCC) and adjacent noncancerous tissues were collected from patients undergoing surgery at Weifang People's Hospital, The First Affiliated Hospital, Shandong Second Medical University. The Ethics Committees of Weifang People's Hospital, The First Affiliated Hospital, Shandong Second Medical University approved this study (Approval No. 2019-037).

Inclusion criteria: ① A diagnosis of ESCC confirmed by histological examination; ② Adjacent noncancerous tissues were located at least 6 cm away from the cancer tissues; ③ No previous anti-tumor treatment, such as radiotherapy and chemotherapy; ④ The samples were stored in liquid nitrogen; ⑤ Complete clinical data.

Exclusion criteria: ① Presence of other concurrent primary tumors; ② Presence of surgical contraindications such as cardiopulmonary insufficiency; ③ A history of severe venous thrombosis or pulmonary embolism; ④ Patients had the infective diseases.

Cell transfection

Cells (5×10^5/well) were seeded in 6-well cultured plates and incubated overnight at 37°C until they reached 50%-60% confluency. The culture medium was then replaced with serum-free medium. miR-124 mimics (5'-UAAGGCACGCGGUGAAUGCC-3') (Beyotime Biotech Inc, China), miR-124 mimics control (5'-UUGUACUACACAAAAGUACUG-3') (Beyotime Biotech Inc, China), miR-124 inhibitor (5'-CGUGUUCACAGCGGACCUUGAU-3') (Beyotime Biotech Inc, China), and miR-124 inhibitor control (5'-CAGUACUUUUGUGUAGUA CAA-3') (Beyotime Biotech Inc, China) were transfected into cells for 6 h using Lipofectamine (Invitrogen, USA), following manufacturer's protocol. STAT3 cDNA (NM_139276) was cloned into the pcDNA3.1 vector (Takara Bio, Inc., Japan) according to the manufacturer's instructions. Then, miR-124 mimics and pcDNA3.1-STAT3 vector (NM_139276, Beyotime Biotech Inc, China) or miR-124 inhibitor and pcDNA3.1-shRNA-STAT3 (Sense: 5'-GATCCGAGTCAAGGAGACCATGCAATTCAAGAGATTGCATGTCTCCTTGACTCTTTTTT GG-3' and anti-sense, 5'-AGCTTTTCCAAAAAAGAGTCAAGGAGACATGCAATCTCTTGA ATTGCATGTCTCCTTGACTCG-3') (Beyotime Biotech Inc, China) were co-transfected into cells. Further, the cells were cultured in medium containing

Table 1. Primer sequences used in this study

Gene	Primers
miR-124	F: 5'-TGCCGTCGACAACGTGGTG-3' R: 5'-CCAGTCGATCATATGAGGT-3'
U6	F: 5'-ACCCATACACCATATAT-3' R: 5'-TCAGGAGGAGACAGGAGTTTATAT-3'
GAPDH	F: 5'-TGCGATTACGCTTTCGATGCAGC-3' R: 5'-CCAGCAGGCAGATGCAGGT-3'
STAT3	F: 5'-AGAGCCAGGAGCACCCTGAA-3' R: 5'-GGTCAATGGTATTGCTGCAGGTC-3'

F: Forward primer; R: Reverse primer.

10% FBS for 48 h. Finally, the cells in different groups were harvested at various time points for the subsequent experiments.

Cell cultures

The human normal esophageal epithelial cell line (Het-1A) and human esophageal cancer cell lines (EC-1) were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Cells were incubated at 37°C in a 5% CO_2 incubator using Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) containing 10% fetal bovine serum (BFS; Gibco, USA) and 1% Penicillin-Streptomycin reagents (Solarbio, China).

Cell-Counting-Kit-8 (CCK-8) analysis

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay, as described in previous studies [11]. Briefly, 5×10^3 cells/well were transfected with miR-124 mimics, inhibitors, or miR-124 mimics + STAT3 vector and seeded in 96-well plates. The viability of EC-1 cells was measured at various time points (0 d, 1 d, 2 d, 3 d and 4 d). After incubation, 10 μL of CCK-8 solution was added to each well and the cells were incubated for 2 h at 37°C and 5% CO_2. The optical density (OD) values were measured at 450 nm using a microplate reader, and a growth curve was drawn.

Transwell migration and invasion assays

For the migration assay, 100 μl of serum-free medium containing 1×10^6 cells/mL was placed in the upper chamber of a Transwell insert. The lower chamber was filled with 500 μl of DMEM containing 20% FBS, serving as chemo-attractants. After an 18-hour incubation, cells that migrated to the lower surface of the membrane were fixed with methanol for 30 min and stained with 1% crystal violet solution. Non-migrated cells were then removed using swabs. The stained cells were observed under a light microscope (Nikon, Tokyo, Japan) at 200 × magnification, and five random fields were selected for cell counting.

For the invasion assay, Transwell inserts were pre-coated with 40 μl of working Matrigel. The following procedures followed the same protocol as the migration assay. After culture, the cells that invaded the lower chamber were quantified using the same methods applied in the migration assay.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from EC-1 and Het-1A cells, human esophageal cancer tissues and paracancerous tissues using a one-step RNA extraction procedure. The extracted RNA was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit and RNase Inhibitor Kits (Thermo Fisher Scientific, USA). The relative expression levels of miR-124, STAT3, GAPDH and U6 were detected with using FastFire qPCR PreMix (SYBR Green, Tiangen, China) in a ProFlex™ PCR system (Thermo Fisher). The primers used in the study are listed in **Table 1**. PCR products were as follows: initial degeneration at 95°C for 15 s, followed by 45 cycles of denaturation at 95°C for 5 seconds, annealing at 60°C for 20 seconds. The expression levels of STAT3 mRNAs were normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the expression of miR-124 was normalized to U6. The relative expression levels of targeted genes were calculated by the 2-ΔΔCt method.

ELISA analysis

The level of STAT3 protein in cancer tissues and cell lines was quantified using enzyme-linked immunosorbent assay (ELISA). STAT3 ELISA Kits (Lot number: ab264629, Abcam, USA) were used strictly following the operating instructions on the Kits.

Dual-luciferase reporter gene assay

To verify whether miR-124 targets the STAT3 gene, potential transcription factor binding

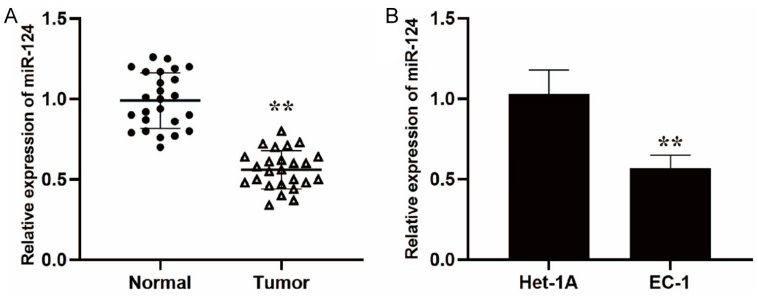

Figure 1. Expression of miR-124 in tissue sample and cells. A. miR-124 expression in tumor and para-cancerous tissues. B. miR-124 expression in normal esophageal epithelial cell line (Het-1A) and human esophageal cancer cell line (EC-1). **P<0.01 vs normal or Het-1A.

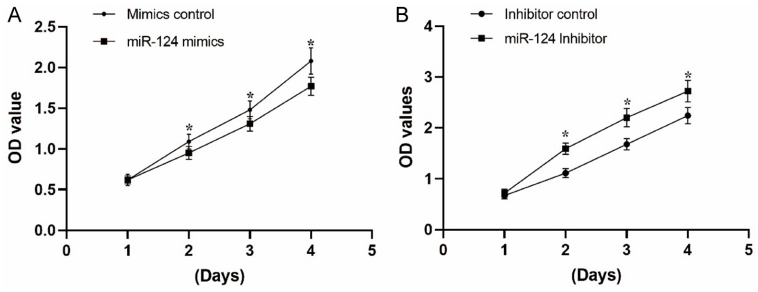

Figure 2. Effects of miR-124 expression on cell viability detected by CCK-8 assay. A. The effects of miR-124 mimics on cell viability. B. The effects of miR-124 inhibitor on cell viability. *P<0.05 vs Mimics control or Inhibitor control.

sites within the promoter region were predicted through bioinformatics analysis. A luciferase reporter gene containing either the wild-type or mutant-type 3'UTR of the STAT3 gene was constructed, and miR-124 with a plasmid containing the STAT3 target fragment were co-transfected into cells. The detailed protocol was as follows: (1) Cells in good condition were digested with trypsin and developed into the single-cell suspension, followed by cell counting. The appropriate volume of medium was added to a 24-well plate, and cells seeded at a density of 2×10^4/well. The cells were cultured at 37°C in a 5% CO_2 incubator, and the medium was replaced when the cell confluence reached about 50%. (2) Cellfectin II Reagent (Thermo Fisher Scientific Company, USA) was diluted with medium in advance, and the appropriate volume of Cellfectin II Reagent was added to each well, followed by a 5-min incubation at room temperature. (3) miR-124 mimics/inhibitors and 0.5 μg of the plasmid were then added to each well, incubated for 5 min. The dilutions were then mixed and incubated for 20 min at room temperature. (4) Following transfection, proteins were isolated, and luciferase activity was measured by introducing a luciferase substrate. Finally, the relative fluorescence intensities were determined.

Statistical analysis

Data were analyzed using SPSS 23.0 software. The measurement data were present as mean ± standard deviation (SD). Comparisons between the two groups were performed using the independent t-test. For comparisons among three or more groups, one-way analysis of variance (ANOVA) with Bonferroni post hoc test was applied. Spearman analysis was used to evaluate the relationship between miR-124 expression and STAT3 expression. P<0.05 was defined as statistically significant.

Results

miR-124 expression in tumor and cell lines

The relative expression level of miR-124 in adjacent non-cancerous tissues was 1.01± 0.14, while that in esophageal cancer tissues was significantly higher at 0.49±0.09 (P= 0.006). Moreover, in EC-1 cell lines, the relative expression level of miR-124 was 0.57±0.08, which was significantly lower than that in Het-1A cell lines (1.03±0.15; P=0.008), as shown in **Figure 1**.

Effects of miR-124 expression on cell viability

As shown in **Figure 2**, CCK-8 analysis showed that, from the second day of culture, cell viability in the miR-124 mimics group was significantly lower than that in the mimics control group (all P<0.05). Conversely, cell viability in the miR-124 inhibitor group was obviously higher than that in the inhibitor control group (all P<0.05).

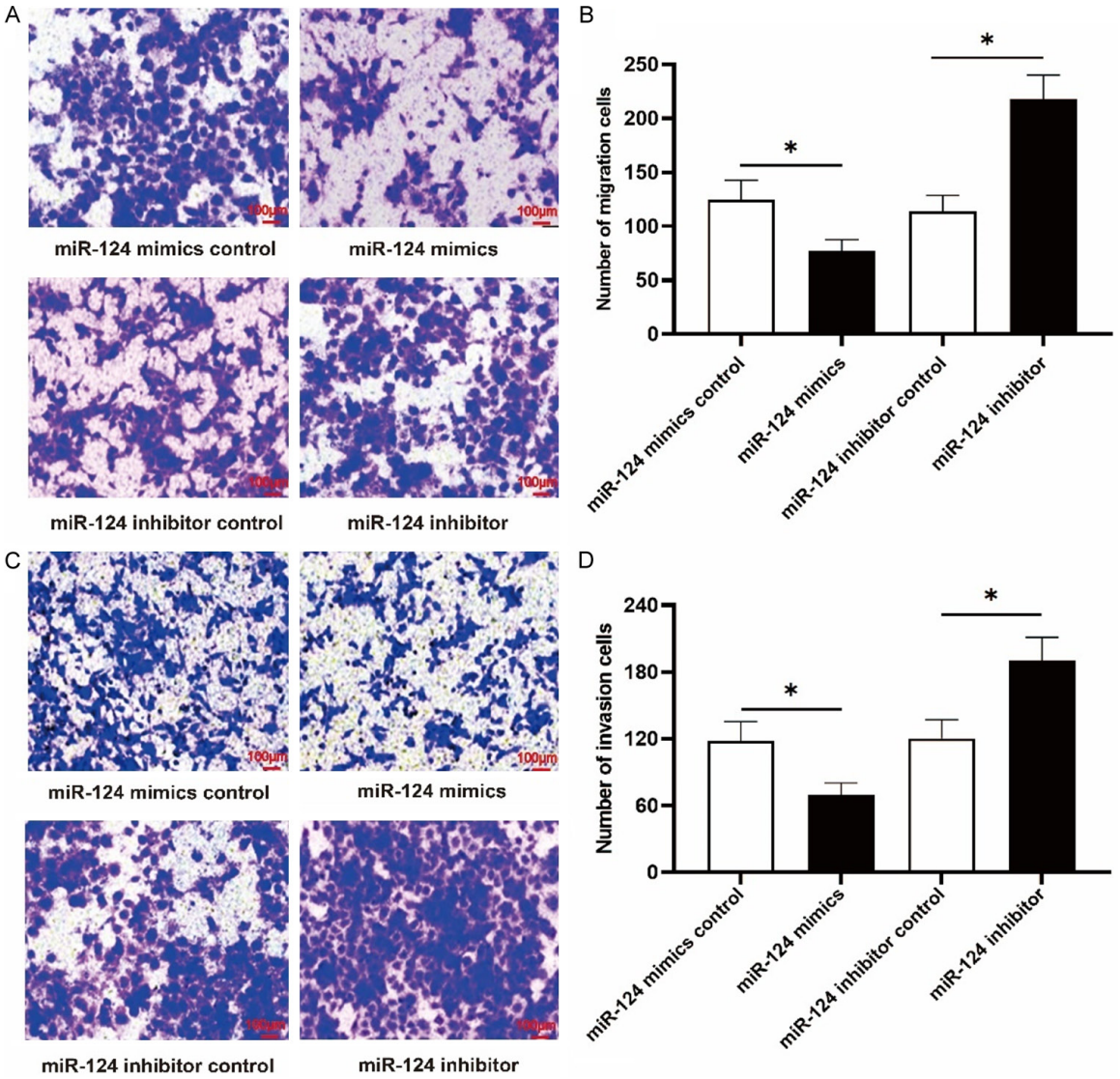

Figure 3. Effects of miR-124 expression on cell migration and invasion detected by Transwell assays. A. The representative images of Transwell migration assays (×200). B. Quantified results of migrated cells after transfection with miR-12 mimics/inhibitors. C. The representative images of Transwell invasion assays (×200). D. Quantified results of invaded cells after transfection with miR-12 mimics/inhibitors. *P<0.05 vs Mimics control or Inhibitor control.

Effects of miR-124 expression on cell migration and invasion

As shown in **Figure 3**, migration and invasion analyses showed that, in contrast to the control group, the average number of migrated (P=0.027) and invaded cells (P=0.031) in the miR-124 mimics group was significantly reduced, while the average number of migrated (P=0.021) and invaded cells (P=0.034) in the miR-124 inhibitor group was obviously increased compared to the control group.

Target relationship between miR-124 and STAT3 validated by Dual luciferase reporter gene assay

To clarify the possible regulatory mechanism of miR-124, downstream target genes of miR-124 were predicted using the TargetScan and miRanda databases. It was found that the 3'UTR of the STAT3 gene has a binding site for miR-124, as shown in **Figure 4A**. Dual luciferase assay results showed that luciferase activity from the wild-type group was significantly

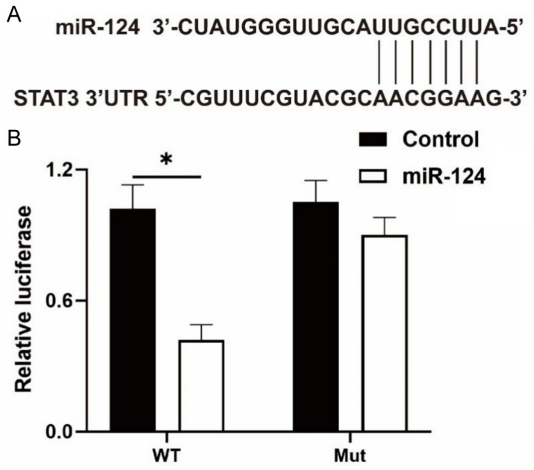

Figure 4. Target relationship between miR-124 and STAT3 validated by Dual luciferase assay. A. The bioinformatics analysis. B. The results of Dual luciferase assay. *P<0.05 vs control.

decreased after transfection of miR-124 (P= 0.029), while luciferase activity from the mutant-type group was not significantly different from the control (P=0.112), as shown in **Figure 4B**. These results indicate that miR-124 can specifically bind to the 3'UTR of STAT3.

STAT3 expression in tumor tissues

As shown in **Figure 5A**, **5B**, qRT-PCR results showed that the expression level of STAT3 mRNA in tumor tissues was significantly higher than in normal tissues (P<0.001). Moreover, ELISA revealed that the STAT3 protein level in tumor tissue was 431.50±32.89 pg/mL, significantly higher than that in normal tissues (225.40±24.50 pg/mL) (P<0.001). As shown in **Figure 5C**, Spearman correlation analysis indicated a negative relationship between miR-124 expression and STAT3 expression (P=0.001).

Effects of miR-124 mimics + STAT3 or miR-124 inhibitor + shRNA-STAT3 on cell proliferation

As shown in **Figure 6**, CCK-8 analysis showed that, at day 3 and 4 after culture, cell viability in the miR-124 mimics + STAT3 group was obviously higher than that in miR-124 mimics group (all P<0.05), while the cell viability in miR-124 inhibitor + shRNA-STAT3 was significantly lower than that in miR-124 inhibitor group (all P<0.05).

Effects of miR-124 mimics + STAT3 or miR-124 inhibitor + shRNA-STAT3 on cell migration and invasion

As shown in **Figure 7**, cell migration and invasion analysis showed that, in contrast to miR-124 mimics group, the average number of migrated (P=0.031) and invaded cells (P= 0.022) in miR-124 mimics + STAT3 group were obviously increased. Conversely, compared to the miR-124 inhibitor group, the average number of migrated (P=0.029) and invaded cells (0.018) in miR-124 inhibitor + shRNA-STAT3 were significantly decreased.

Discussion

The differential expression of miRNAs has been observed in various human malignancies, where they act as oncogenes or suppressors to play important regulatory roles. In esophageal cancer, miRNAs are similarly involved in key processes in its development and progression [12]. Investigating the regulatory mechanisms of miRNAs in esophageal cancer is conducive to the development of new molecular targets for the treatment of esophageal cancer. In recent years, miR-124 has been found to be down-regulated in several solid tumors, including gastric cancer, colorectal cancer, breast cancer, and hepatocellular carcinoma, where it exhibits antitumor effects [13]. Li et al. found that miR-124 targets STAT3 to inhibit the proliferation and invasion process of NSCLC [14]. miR-124 expression is significantly reduced in nasopharyngeal carcinoma, and its overexpression suppresses the proliferation and invasion of nasopharyngeal carcinoma cells [15]. Further investigation revealed that miR-124 targets the Wnt/p-catenin pathway, thus inhibiting the adhesion and motility of nasopharyngeal cancer cells [16]. The expression of miR-124 varies across different tumors, exerting different regulatory effects. It was found that miR-124 itself can also be used as a potential indicator for therapeutic efficacy and prognosis of tumor patients [17]. The results of this study suggested that miR-124 was significantly down-regulated in esophageal cancer tissues compared with normal tissues. Additionally, miR-124 expression was significantly down-regulated in the esophageal cancer cell line EC-1 compared to normal esophageal mucosal cells.

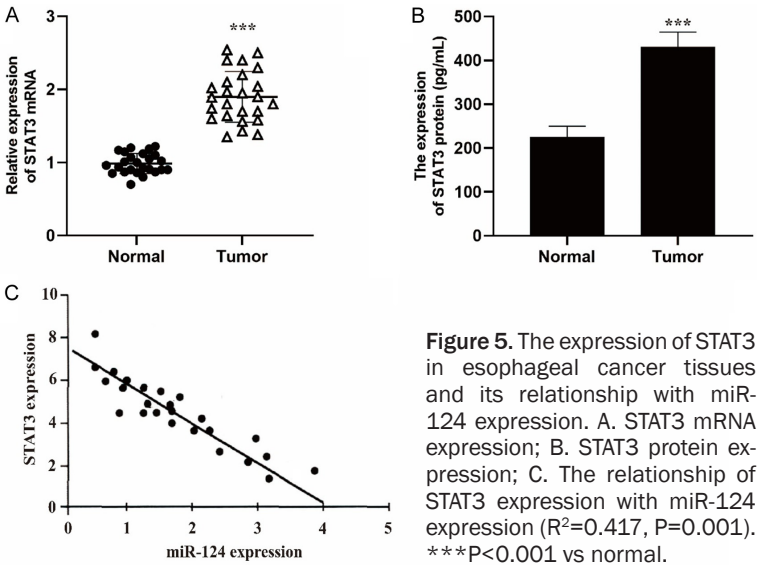

Figure 5. The expression of STAT3 in esophageal cancer tissues and its relationship with miR-124 expression. A. STAT3 mRNA expression; B. STAT3 protein expression; C. The relationship of STAT3 expression with miR-124 expression (R^2=0.417, P=0.001). ***P<0.001 vs normal.

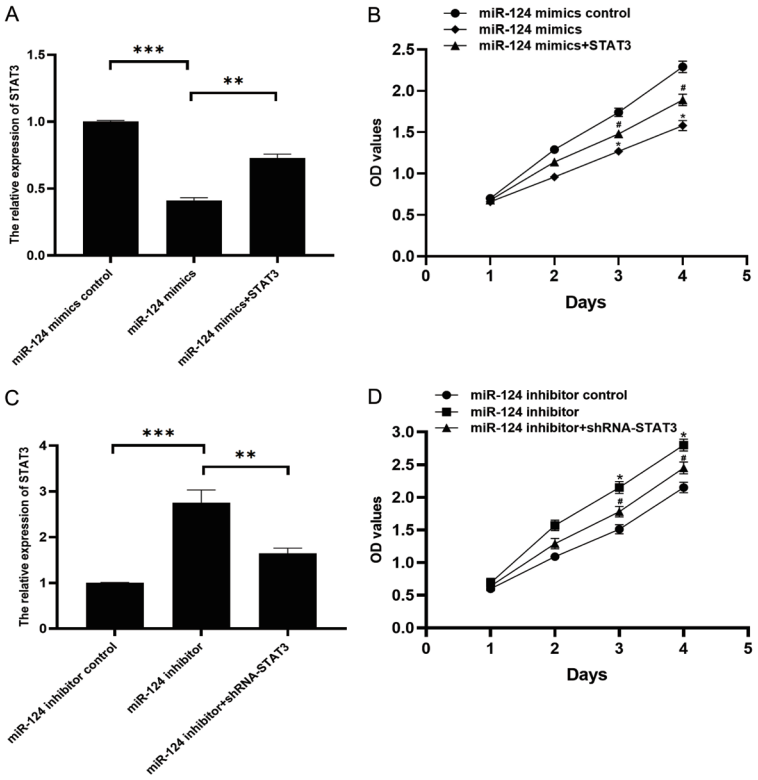

Figure 6. Effects of miR-124 Mimics + STAT3 or miR-124 Inhibitor + shRNA-STAT3 on Cell Proliferation. A. The expression of STAT3 in miR-124 mimics control group, miR-124 mimics group, and miR-124 mimics + STAT3 group. B. The cell viability at different time points in miR-124 mimics control group, miR-124 mimics group and miR-124 mimics + STAT3 group. C. The expression of STAT3 in miR-124 inhibitor control group, miR-124 inhibitor group and miR-124 inhibitor + shRNA-STAT3 group. D. The cell viability at different time points in miR-124 inhibitor control group, miR-124 inhibitor group and miR-124 inhibitor + shRNA-STAT3 group. *P<0.05 vs miR-124 mimics control group or miR-124 inhibitor control group; #P<0.05 vs miR-124 mimics group or miR-124 inhibitor group; **P<0.01 vs miR-124 mimics group or miR-124 inhibitor group; ***P<0.001 vs miR-124 mimics control group or miR-124 inhibitor control.

Cell proliferation is a critical biological process, and uncontrolled proliferation of malignant cells contributes to cancer development and progression. miRNAs regulate cell proliferation and cell death by modulating key molecules, including cyclin-dependent kinase (CDK) and other cell cycle proteins. Some studies have shown that the high expression of certain miRNAs promotes the G1/S phase transition, accelerating cell cycle progression, while others can block the G0/G1 phase transition, thereby slowing down cell cycle progression [18]. To clarify the effect of miR-124 on the proliferative ability of esophageal cancer cells, the CCK-8 assay in this study revealed that transfection with miR-124 mimics significantly reduced cell proliferation of esophageal cancer cells compared to the control group. Conversely, transfection with miR-124 inhibitor significantly enhanced the proliferative activity of esophageal cancer cells. These results suggest that miR-124 can inhibit the proliferative ability of esophageal cancer cells. Invasion and metastasis are key characteristics of malignant tumors. miR-21 is highly expressed in esophageal cancer, and its down-regulation has been shown to reduce cell migration and invasion [19]. He et al. found that overexpression of miR-143 reduced both the proliferative activity and invasive abilities of esophageal cancer cells [20]. To clarify the effect of miR-124 on the migration and invasion of esophageal cancer cells, Transwell assays were conducted. The results indicated that the average number of migrated cells in the miR-124 mimics group was

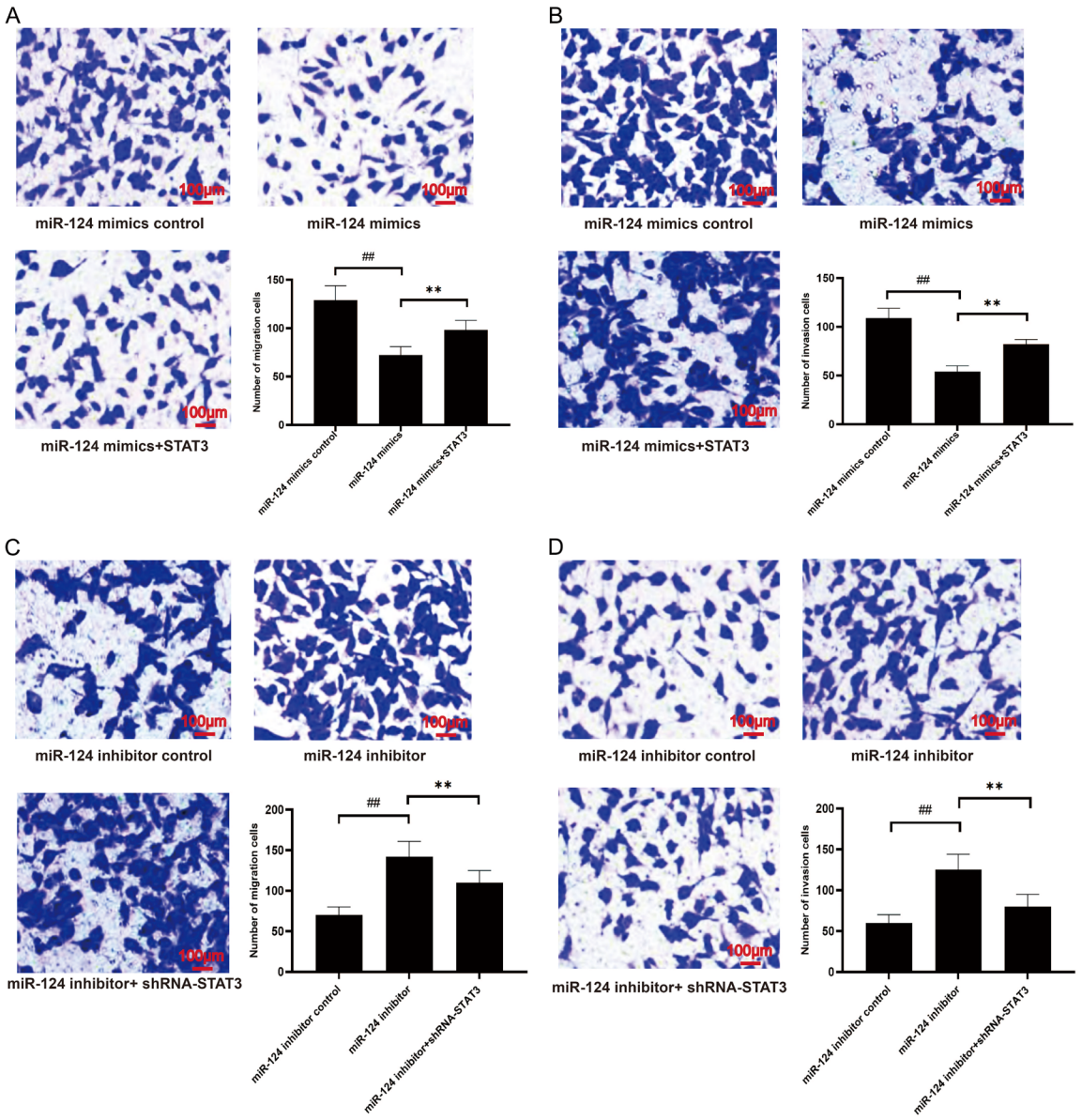

Figure 7. Effects of miR-124 Mimics + STAT3 or miR-124 Inhibitor + shRNA-STAT3 on Cell Migration and Invasion (×200). A. Number of migrated cells in miR-124 mimics control group, miR-124 mimics group, and miR-124 mimics + STAT3 group; B. Number of invaded cells in miR-124 mimics control group, miR-124 mimics group, and miR-124 mimics + STAT3 group; C. Number of migrated cells in miR-124 inhibitor control group, miR-124 inhibitor group, and miR-124 inhibitor + shRNA-STAT3 group; D. Numbers of invaded cells in miR-124 inhibitor control group, miR-124 inhibitor group, and miR-124 inhibitor + shRNA-STAT3 group; ##P<0.01 vs miR-124 mimics control or miR-124 inhibitor control; **P<0.01 vs miR-124 mimics or miR-124 inhibitor.

significantly lower than that in the control group, while that in the miR-124 inhibitor group was significantly higher. These findings suggest that miR-124 could inhibit the migration of esophageal cancer cells. Similarly, the Transwell invasion assay results showed that the average number of invaded cells was significantly lower in the miR-124 mimics group, while the average number of invasive cells was significantly higher in the miR-124 inhibitor group, further supporting the conclusion that miR-124 inhibits the invasive ability of esophageal cancer cells. These results are consistent with previous studies [21, 22].

Signal transducer and activator of transcription-3 (STAT3) is a member of the signal transduction and transcription activator family, wide-

ly expressed in the cytoplasm, where it is activated and translocated to the nucleus to bind to DNA, playing a critical role in signal transduction and transcription. The activation of STA3 is strictly controlled, and its abnormal over-activation can lead to tumor development. Several studies have reported that STAT3 is abnormally expressed in a variety of human tumors, and its activation is closely related to tumor proliferation, differentiation, apoptosis, angiogenesis, invasion, metastasis, and immune escape [23]. Another study reported that STAT3 expression was up-regulated in gastric cancer tissues, and its expression level was closely related to the degree of tumor differentiation and tumor stage in gastric cancer patients [24]. STAT3 is closely associated with the occurrence and development of esophageal cancer. Study has shown significant differences in STAT3 expression between esophageal cancer tissues, atypical hyperplastic tissues, and normal esophageal tissues [25]. Our study confirmed that STAT3 expression was significantly up-regulated in esophageal cancer tissues compared with normal esophageal tissues, and the STAT3 expression was also significantly elevated in the esophageal cancer EC-1 cells compared with normal esophageal mucosal cells. In addition, Gu et al. found that STAT3 was significantly upregulated in esophageal cancer tissues and cells, and it enhance the migratory and invasive ability of esophageal cancer cells through the transcriptional activator ZEB1 [26]. Zhu et al. reported that silencing STAT3 expression using siRNA significantly reduced cell proliferation by inducing cell cycle arrest in the G0/G1 phase [27]. miRNAs mainly regulate the expression of target genes by binding to the 3'UTR of downstream target genes, either through complete or partial complementarity, thereby exerting their biological functions. TargetScan and miRanda are commonly used bioinformatics tools to predict miRNA target genes. To identify the downstream targets of miR-124, TargetScan and miRanda were used to predict the possible target genes of miR-124, and it was found that STAT3 had a complementary pairwise binding site for miR-124, which was further validated using dual luciferase reporter gene assay. To further validate the relationship between miR-124 and STAT3 expression, Spearman correlation analysis was performed. The results revealed a significant negative correlation between miR-124 and STAT3 expression, further supporting the notion that miR-124 inhibits STAT3 expression.

Despite the inspiring findings, there are still some shortcomings in this study, including its single-center design, limited sample size, and reliance on cell experiments. Notably, animal or human experiments were not conducted, which may provide more comprehensive insights compared to cell experiments. Additionally, the STAT3 signaling pathway was not intervened with to explain whether the antitumor effect of miR-124 are mediated through STAT3 signaling. Future studies involving multicenter collaborations and animal models or human experiments are necessary to further validate these findings.

In summary, miR-124 is significantly down-regulated in esophageal cancer cells and tissues. Overexpression of miR-124 effectively inhibits the proliferation, migration and invasive ability of esophageal cancer cells possibly through regulating STAT3, serving as a potential therapeutic target for the treatment of esophageal cancer.

Disclosure of conflict of interest

None.

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