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ORIGINAL ARTICLE

Micro ribonucleic acid-93 promotes proliferation and migration of esophageal squamous cell carcinoma by targeting disabled 2

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Abstract

Background: Accumulated evidence has revealed that the dysregulation of micro ribonucleic acids (miRNAs) may contribute to esophageal squamous cell carcinoma (ESCC). MiR-93, which is a member of the miRNA cluster miR-106b~25, has been widely studied for its tumor promoting effect on different types of cancers. However, our knowledge of miR-93 function in ESCC remains unclear.
Methods: The expression levels of miR-93 in ESCC and the adjacent non-tumor tissues were measured by real-time polymerase chain reaction. Cell counting kit-8, flow cytometry, and 5-ethynyl-2′-deoxyuridine incorporation and transwell migration assays were employed to explore the effects of miR-93 on proliferation and migration capabilities in EC109 cells. To determine the possible target gene of miR-93, cell transfection, Western blot analysis and luciferase reporter gene assays were performed.
Results: A significant upregulation of miR-93 expression in ESCC tissues was determined, combined with a downregulation of the predicted target gene, disabled 2 (DAB2). The introduction of miR-93 significantly promotes cell proliferation, cell cycle progression, and the metastatic capability of EC109 cells. By cell transfection and luciferase reporter assay, DAB2 was confirmed as a direct target of miR-93. In addition, the knockdown of DAB2 by small interfering RNA displayed a consentaneous phenocopy with miR-93 overexpression in EC109 cells.
Conclusion: Our results indicate that miR-93 acts as a tumor promoter in ESCC, and its promotion effects on ESCC cell proliferation and migration depend largely upon DAB2 suppression.

Introduction

Esophageal cancer is one of the most common malignancies and was expected to be the seventh cause of cancer deaths in the United States in 2015.[1] Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of esophageal cancer in Eastern Asia and shows a relatively high morbidity compared to western countries.[2] However, the prognosis for patients with ESCC remains unfavorable as a result of delayed diagnosis and the poor efficacy of conventional treatment;[3] further elucidating the pathogenesis of ESCC is critical.

Human disabled 2 (DAB2) is a putative tumor suppressor gene, which was initially identified as DOC-2.[4] It was first reported downregulated in both ovarian cancer cell lines and tumor tissues,[5] and the loss or decrease of DAB2 expression was later observed in a multitude of cancer types, such as lung, nasopharyngeal, bladder, and esophageal cancers.[6–9]

Previous studies have shown that DAB2 plays pivotal roles in several important signal pathways, and the downregulation of DAB2 expression could increase the propensity for tumor metastasis by facilitating transforming growth factor (TGF)-β stimulated epithelial-to-mesenchymal transition.[10–12] In the past few years, several possible mechanisms have been proposed to explain the downregulation of DAB2 expression in different cancers, most of them concerning epigenetic regulation. Aberrant gene methylation was reported to contribute to the loss of DAB2 protein in lung and nasopharyngeal cancers, while micro ribonucleic acid (miRNA) regulation played an important role at post-transcription level in ovarian cancers.[6,7,13]

MicroRNAs are a class of small non-coding RNAs, which negatively regulate gene expression at the post-transcriptional level by leading to degeneration or translational suppression of the target messenger (m)RNAs.[14] Recent evidence suggests that miRNAs may act as oncogene or tumor suppressor genes and play important roles in regulating cancer development.[15] In ESCC, aberrantly expressed miRNAs have previously been observed, indicating that dysregulation of miRNAs may contribute to ESCC progression.[16] Among those miRNAs, miR-93, which is a member of the miRNA cluster miR-106b~25, has been widely studied for its tumor promoting effect on different types of cancers.[17–19] Interestingly, a recent study in lung cancer shows that DAB2 is a direct target of miR-93, and the miR-93/DAB2 pathway plays an important part in lung cancer progression.[20] However, our knowledge of miR-93 function in ESCC remains unclear. In the present study, we analyzed the expression of miR-93 in esophageal cancer and corresponding normal tissues, and found that miR-93 expression was obviously upregulated. Subsequent experiments *in vitro* showed that miR-93 could promote the proliferation and migration of ESCC cells by targeting DAB2.

Materials and methods

Patients and tissue specimens

A total of 26 pairs of ESCC samples and corresponding non-cancerous tissues were obtained from patients who underwent esophagectomies at the First Affliated Hospital of Soochow University from 2011 to 2013. Each patient signed a consent form prior to enrolment and the ethics committee of Soochow University approved the study. The patients had not received any neo-therapies. All tissue samples were frozen in liquid nitrogen directly after collection and stored at −80°C until use.

Cell line, cell culture, and cell transfection

Human esophageal squamous cell line EC109 was purchased from the Cell Bank of the Chinese Academy of Science and was cultivated in RPMI Medium 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (Gibco) and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C. All transfections were carried out using Lipofectamin 2000 (Invitrogen, Carlsbad, CA, USA) in serum-free conditions according to manufacturer's instructions. MiR-93 mimics, miR-93 inhibitor, DAB2-small interfering (si)RNA and negative control-siRNA were synthesized directly (GenePharma, Shanghai, China) and transfected into cells at a final concentration of 50 nM. The sequence of DAB2-siRNA was acquired from a previous study.[21]

Ribonucleic acid (RNA) extraction and quantitative real time-polymerase chain reaction analysis

The total RNA of EC109 cells or tissue specimens was extracted using the TRIzol reagent (Invitrogen) according to the user's manual. Reverse transcription was performed with a reverse transcriptase kit (Invitrogen) using Oligo dT Primer (Takara, Shiga, Japan). The mRNA and miRNA levels were verified by quantitative real time-polymerase chain reaction (qRT-PCR) using SYBR Green qPCR Mix (Invitrogen) and were performed on an ABI 7500HT system (Applied Biosystems, Foster City, CA, USA). U6 small nuclear RNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as an internal control for normalization, and the primer sequence used for qRT-PCR analysis is listed in Table 1. All samples were run in triplicate and the relative miR-93 or DAB2 mRNA level of each sample normalized to the internal control was calculated using the $2^{-\Delta\Delta CT}$ method.[22]

Western blotting analysis

Cells or tissues were lysed in RIPA buffer (Cell Signaling Technology, Boston, MA, USA) with protease inhibitors (Sangon Biotech, Shanghai, China) and centrifuged. The protein concentrations were then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). After blocking with 1.0% fetal bovine serum for one hour, the membranes were incubated overnight at 4°C with diluted (1:2000) primary antibodies (polyclonal rabbit anti-*DAB2*; BD Biosciences, Franklin Lakes, NJ, USA) and then subjected to incubation with horseradish peroxidase-conjugated secondary antibodies (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for two hours at room temperature. The protein bands were finally detected by electrochemiluminescence kit (Pierce, Rockford, AL, USA). An antibody against GAPDH (Santa Cruz Biotechnology) confirmed equal loading of samples. The band density was quantified by

Table 1 Primers for RT or amplification of the mature miR-93 and U6, DAB2 and GAPDH mRNA

Name	Sequence, 5′-3′	
RT primers		
U6	CGAGCACAGAATCGCTTCACGAATTTGCGTGTCAT	
miR-93	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACCTGCA	
qRT-PCR primers		
U6	F: CGAGCACAGAATCGCTTCA;	R: CTCGCTTCGGCAGCACATAT
GAPDH	F: GAAGGTGAAGGTCGGAGTC;	R: GAAGATGGTGATGGGATTTC
DAB2	F: CCAACAGAAAGCAAAGATATCC;	R: GTTGGTCGAGGAAGAGAAC

DAB2, disabled 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger ribonucleic acid; miR-93, micro RNA-93; qRT-PCR, quantitative real time-polymerase chain reaction.

Quantity One 4.6 software (BioRad, Hercules, CA, USA) and the DAB2 protein level was normalized to GAPDH protein.

Luciferase reporter gene assay

A 281bp gene fragment containing the predicted miR-93 binding site in 3′UTR of DAB2 gene and its mutant counterpart were separately synthesized (Genewiz, Suzhou, China) and subcloned into the psiCHECK-2 dual luciferase vector (Promega, Madison, WI, USA) to generate the DAB2-3′-UTR-wild and DAB2-3′-UTR-mutant vectors. For the luciferase assay, EC109 cells cultured in 24 well plates were transiently co-transfected with the reporter vectors (DAB2-3′-UTR-wild or DAB2-3′-UTR-mutant) and miR-93 mimics or the miR-control (miR-negative control [NC]) using Lipofectamine 2000 according to the manufacturer's protocol. Cells were collected after 48 hours and lysed for measuring the luciferase activity using a Dual Luciferase Reporter Assay Kit (Promega). Each experiment was performed at least three times.

Cell proliferation assay

Cells were seeded in 96-well plates at a density of 5×10^3 cells/well at 12 hours post-transfection. Cell proliferation was evaluated by cell counting kit (CCK)-8 assay and 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay. For the CCK-8 assay, 10 μl of CCK-8 solution (Beyotime, Shanghai, China) was added to the culture medium per well and incubated for an additional two hours in an incubator. The absorbance at a wavelength of 450 nm (OD450) was determined daily using a microplate reader (SpectraMax 250, Molecular Devices, Sunnyvale, CA, USA) over four consecutive days. The EdU incorporation assay was performed using a Cell Light EdU DNA imaging Kit (RiboBio, Guangzhou, China) according to the manufacturer's instructions. After 48 hours of transfection, cells were incubated with EdU (50 mM) for two hours, followed by fixation with 4% formaldehyde for 30 minutes and permeabilization with 0.5% Triton X-100 for 10 minutes. Apollo dyeing reaction buffer was then added to each well and the cells were incubated in the dark for 30 minutes. After staining with Hoechst 33342 for 30 minutes, the proportion of nucleated cells incorporating EdU was determined by fluorescence microscopy. All experiments were independently performed in triplicate.

Flow cytometry assay

At 48 hours post-transfection, cells were harvested for cell cycle analysis. The collected cells were washed with phosphate buffered saline three times and fixed in 70% ethanol. The fixed cells were then washed again and finally stained with propidium iodide (PI) staining buffer (Beyotime), which contained 200 μg/ml RNase and 50 μg/ml PI, for 30 minutes at 37°C shielded from light. Analyses of the cell cycle were performed by flow cytometer (LSR, BD Biosciences).

Transwell migration assay

The migration ability of EC109 cells was measured by transwell migration assay. At 24 hours post-transfection, cells were seeded into the inserts (5×10^4 cells/well) of the Transwell chamber (Corning Costar, Cambridge, MA, USA). The inserts were then put into the bottom chambers loaded with 20% fetal bovine serum-containing medium, and the cells were cultured in a humidified incubator at 37°C for 24 hours. The inserts were then taken out and the cells remaining on the upper surface (non-migrant) were gently scraped off. Cells on the lower surface of the inserts (migrant cells) were fixed with 75% ethanol for 30 minutes, and stained with 0.1% crystal violet for one hour at room temperature. Images of the migrant cells were captured using a photomicroscope (Axiovert 200 M, Carl Zeiss, Oberkochen, Germany). Three randomly selected images per well were used for cell counting and an average was finally calculated. Each assay was performed in triplicate.

Statistical analysis

All data were presented as mean ± standard error. A two-tailed paired *t*-test was used for comparison of miR-93 or

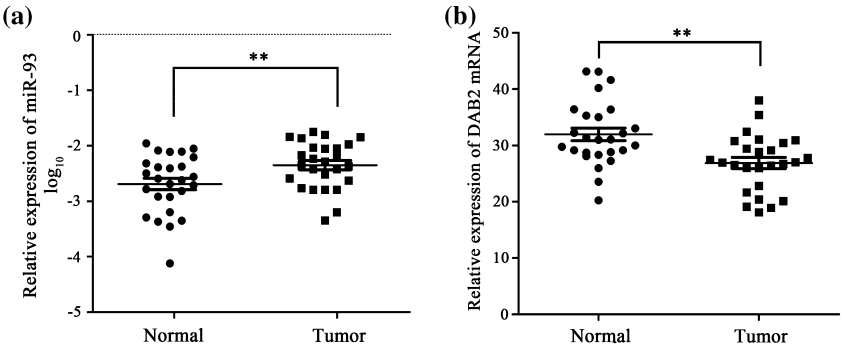

Figure 1 The expression levels of micro ribonucleic acid (miR)-93 and disabled 2 (DAB2) messenger (m)RNA in esophageal squamous cell carcinoma (ESCC) tissue specimens. (**a**) Quantitative real-time-polymerase chain reaction (qRT-PCR) analysis of miR-93 expression in 26 pairs of ESCC tissues and corresponding noncancerous tissues. The relative expression levels of miR-93 were normalized to U6. (**b**) The relative DAB2 mRNA levels detected by qRT-PCR in the same 26 paired ESCC tissues. DAB2 mRNA expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase. $^{**}P < 0.01$.

DAB2 mRNA expression between ESCC and the adjacent non-tumor tissues. For the in vitro assays, one-way analysis of variance was used for multiple group comparisons and an unpaired *t*-test was used for comparisons between two groups. All statistical analyses were performed using SPSS statistics software (version 16.0, SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Upregulation of micro ribonucleic acid (miR)-93 and downregulation of disabled 2 (DAB2) in esophageal squamous cell carcinoma (ESCC)

To detect the expression level of miR-93 and DAB2 mRNA, qRT-PCR was performed on 26 pairs of ESCC tissues and adjacent noncancerous tissues. As shown in Figure 1, we identified a significant upregulation of miR-93 expression in ESCC tissues when compared with normal tissues ($P < 0.05$), while the DAB2 mRNA expression level in ESCC tissues showed a clear reduction ($P < 0.01$).

MiR-93 promotes cells proliferation of ESCC

In order to make clear the biological effects of miR-93 on ESCC proliferation, CCK-8 and EdU incorporation assays were performed. Results of CCK-8 assay showed that ectopic miR-93 expression significantly stimulated EC109 cell growth compared with the control ($P < 0.01$), while transfection of the miR-93 inhibitor showed significant growth retardation in EC109 cells ($P < 0.05$) (Fig. 2a). EdU incorporation assay also indicated that miR-93 could promote ESCC cell proliferation. Ectopic miR-93 expression significantly increased the proportion of cells with EdU-positive nuclei in EC109 cells ($P < 0.05$), while transfection of the miR-93 inhibitor had the opposite effect ($P < 0.05$) (Fig. 2b).

MiR-93 promotes cell cycle progression in ESCC

Promotion of cell growth in cancer is often associated with concomitant activation of cell cycle pathways. Therefore, we investigated the contribution of miR-93 on cell cycle progression in ESCC. The results of flow cytometry assay showed that compared with the control, the cell percentage in the G0-G1 stages significantly decreased in miR-93 transfected EC109 cells, while the percentage in S stage increased markedly ($P < 0.01$). The exact opposite result was obtained when the miR-93 inhibitor (anti-miR-93) was transfected into the EC109 cells ($P < 0.05$) (Fig. 3a).

MiR-93 promotes the metastatic capability of EC109 cells

To examine the influence of miR-93 on the migration capability of ESCC, we performed a transwell migration assay. The results showed that the number of migration cells in the miR-93 transfected group was significantly higher than the control ($P < 0.05$, Fig. 3b). On the contrary, inhibition of miR-93 expression resulted in a significant decrease in the number of migration cells compared with the control ($P < 0.05$, Fig. 3b). The results indicated that miR-93 could promote cell migration in the EC109 cell line.

DAB2 is a functional target of miR-93 in ESCC

To further elucidate the mechanism of promotion effect of miR-93 on proliferation and migration in ESCC, we carried out an *in silico* prediction by TargetScan (Whitehead Insti-

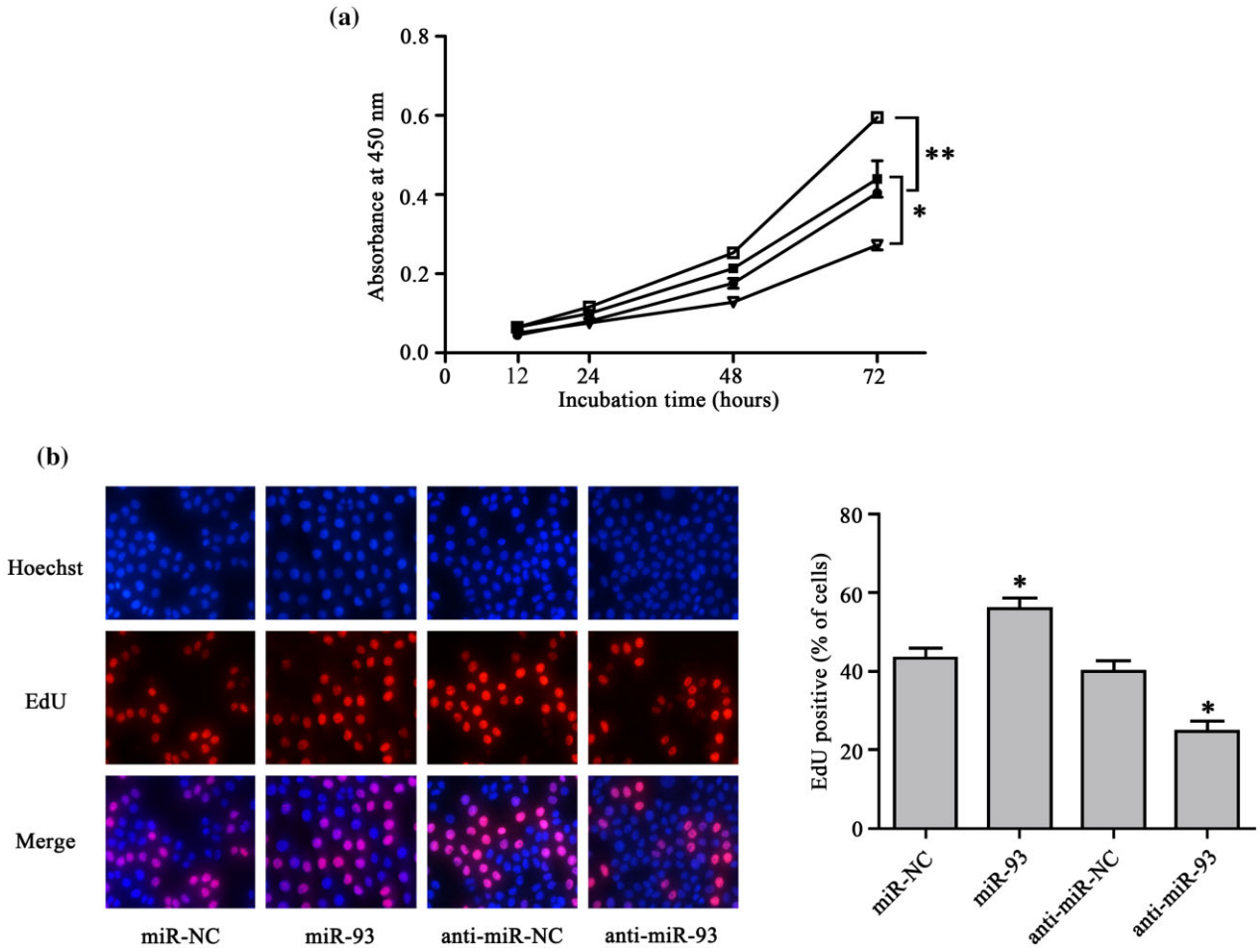

Figure 2 Effects of micro ribonucleic acid (miR)-93 overexpression or knockdown on proliferation in EC109 cells. (**a**) Cell counting kit-8 assay was performed on EC109 cells at 12, 24, 48, and 72 hours after transfection of miR-93 mimics, miR-93 inhibitor (anti-miR-93) or negative control. (**b**) After 48 hours of transfection, an 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay was performed on EC109 cells. All experiments were performed in triplicate and the percentage of nucleated cells incorporating EdU was calculated. $*P < 0.05$; $**P < 0.01$. MiR-NC: negative control for miRNA mimics, anti-miR-NC: negative control for miRNA inhibitor, miR-93: miR-93 mimics, anti-miR-93: inhibitor mimics of miR-93. —●—, miR-NC; —■—, anti-miR-NC; —⊟—, miR-93; —▽—, anti-miR-93.

tute, Cambridge, MA, USA) and found that the DAB2 gene, which was proved downregulated in ESCC tissues in our work, was a candidate target of miR-93. Therefore we hypothesized that miR-93 promoted ESCC progression by directly targeting DAB2. To test the prediction, we first performed cell transfection in EC109 cells. The results showed that after an effective transfection of miR-93 mimics (Fig. 4a), the protein level of DAB2 expression reduced significantly ($P < 0.01$) (Fig. 4c); however, the DAB2 mRNA did not decrease as significantly (Fig. 4b). Accordingly, the knockdown of miR-93 expression by transfection of the miR-93 inhibitor (Fig. 4a) led to a significant increase in DAB2 expression, shown by Western blot assay (Fig. 4c). These findings demonstrated that miR-93 could regulate DAB2 expression in ESCC cells.

To test whether miR-93 directly targets DAB2, a luciferase reporter gene assay was performed. Using TargetScan software, we found the predicted miR-93 target site (positions 1690–1696), which is highly conserved across species (Fig. 4d). The synthesized DAB2 3′-UTR segments containing the target site (wild type/mutant) were subcloned to psiCHECK-2 dual luciferase vector (Fig. 4e), and the reporter constructs were subsequently co-transfected with miR-93 mimics or inhibitor into EC109 cells. As expected, a significant decline of luciferase activity was observed in the cells transfected with miR-93 and DAB2-3′-UTR-wild vector compared with the control, while the invariable luciferase activities were detected in cells transfected with miR-93 and DAB2-3′-UTR-mutant vector (Fig. 4f). In sum, these results indicate that miR-93 can directly inhibit DAB2 expression by targeting its 3′UTR, and that there are more obvious changes to the DAB2 protein than the mRNA level when transfection with miR-93 is performed, suggesting

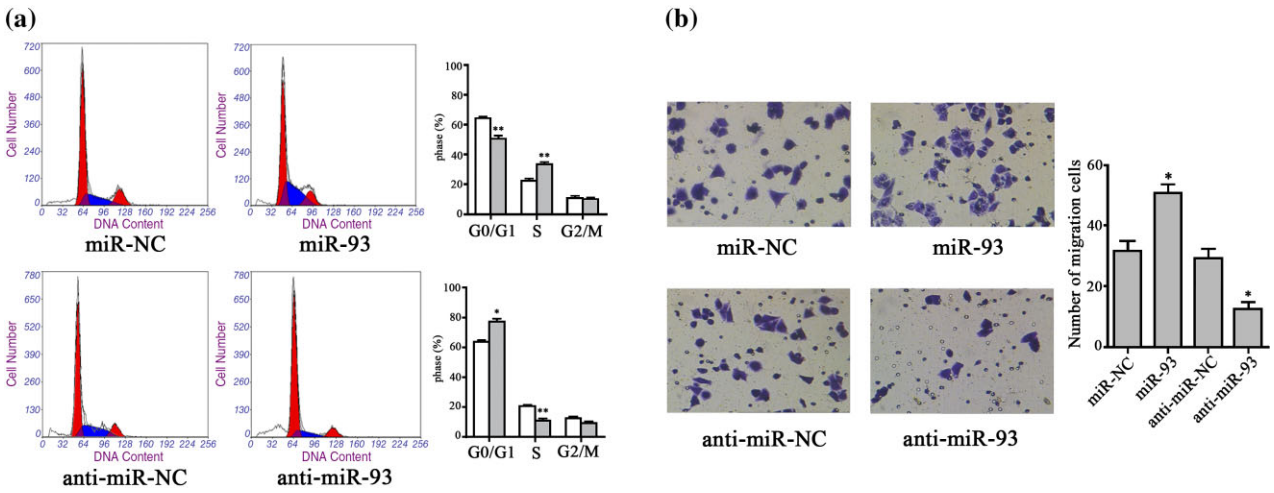

Figure 3 Effects of micro ribonucleic acid (miR)-93 overexpression or knockdown on EC109 cell cycles and migration capability. (**a**) Flow cytometry assay was performed on EC109 cells at 48 hours after transfection of miR-93 mimics, miR-93 inhibitor (anti-miR-93) or negative control. (**b**) Transwell migration assay was employed to examine the migration ability of EC109 cells after transfection of miR-93 mimics, miR-93 inhibitor or negative control. The number of migration cells were counted and compared between groups. All the results were from three independent experiments. $*P < 0.05$; $**P < 0.01$. MiR-NC: negative control for miRNA mimics, anti-miR-NC: negative control for miRNA inhibitor, miR-93: miR-93 mimics, anti-miR-93: inhibitor mimics of miR-93. ▭, miR-NC; ▬, miR-93; ▭, anti-miR-NC; ▬, anti-miR-93.

that miR-93 regulates DAB2 expression mainly by translational suppression.

Depletion of DAB2 contributes to the proliferation and migration capabilities of EC109 cells

To further explore whether the depletion of DAB2 could induce similar phenotypes as miR-93 overexpression in ESCC, the EC109 cells were treated with siRNA, which could knockdown DAB2 expression. The knockdown effect of the synthesized si-DAB2 was confirmed by Western blot (Fig. 5a). CCK-8 and EdU incorporation assays were performed to examine the effects of DAB2 knockdown on cell proliferation. As expected, the CCK-8 results showed a significantly higher proliferation rate at 72 hours in cells transfected with si-DAB2 compared with the control (Fig. 5b), and consistent results were also observed using EdU incorporation assay (Fig. 5c).

The effect of DAB2 knockdown on ESCC cell cycles was evaluated by flow cytometry assay, and the results showed a significant decline in percentage of G0/G1 cells ($P < 0.05$) when DAB2 was knocked down by RNA interference, and an elevation in the percentage of S stage cells was also determined ($P < 0.05$) (Fig. 5d). These results indicate that DAB2 protein can induce growth retardation in ESCC cells by arresting the G1/S transition.

Additionally, we evaluated the effect of DAB2 knockdown on cell migration in EC109 cells. The results of transwell migration assay showed an enhanced capability in si-DAB2 transfected EC109 cells compared with the control ($P < 0.05$) (Fig. 5e).

Discussion

Cancer development is a complex process characterized by the dysregulation of a host of oncogenes and tumor suppressor genes, and in recent years much attention has focused on miRNA regulation as an important post-transcriptional regulation style.[23] MiRNAs have been estimated to control the expression of more than 30% of all protein coding genes and have been proven to play pivotal roles in tumorigenesis.[24,15] As to esophageal cancer, miRNA dysregulation has also been reported to act as a tumor promoter and is closely associated to patient prognosis.[16]

MiR-93 is a member of the miRNA cluster, miR-106b~25, which has been extensively studied in recent years and observed upregulated in several common malignancies, such as lung, gastric, and human breast cancers.[25-27] Our results also showed a significant upregulation of miR-93 expression in ESCC tissues. However, conflicting results have also been found in colon cancer, revealing a significant decrease of miR-93 expression in tumoral colon tissues.[28] This result was later confirmed by another study, which showed that miR-93 could suppress colorectal cancer development by downregulating the Wnt/β-catenin pathway.[29] The distinct biological effects of miR-93 in different cancer types may attribute to the dissimilar target genes. For example,

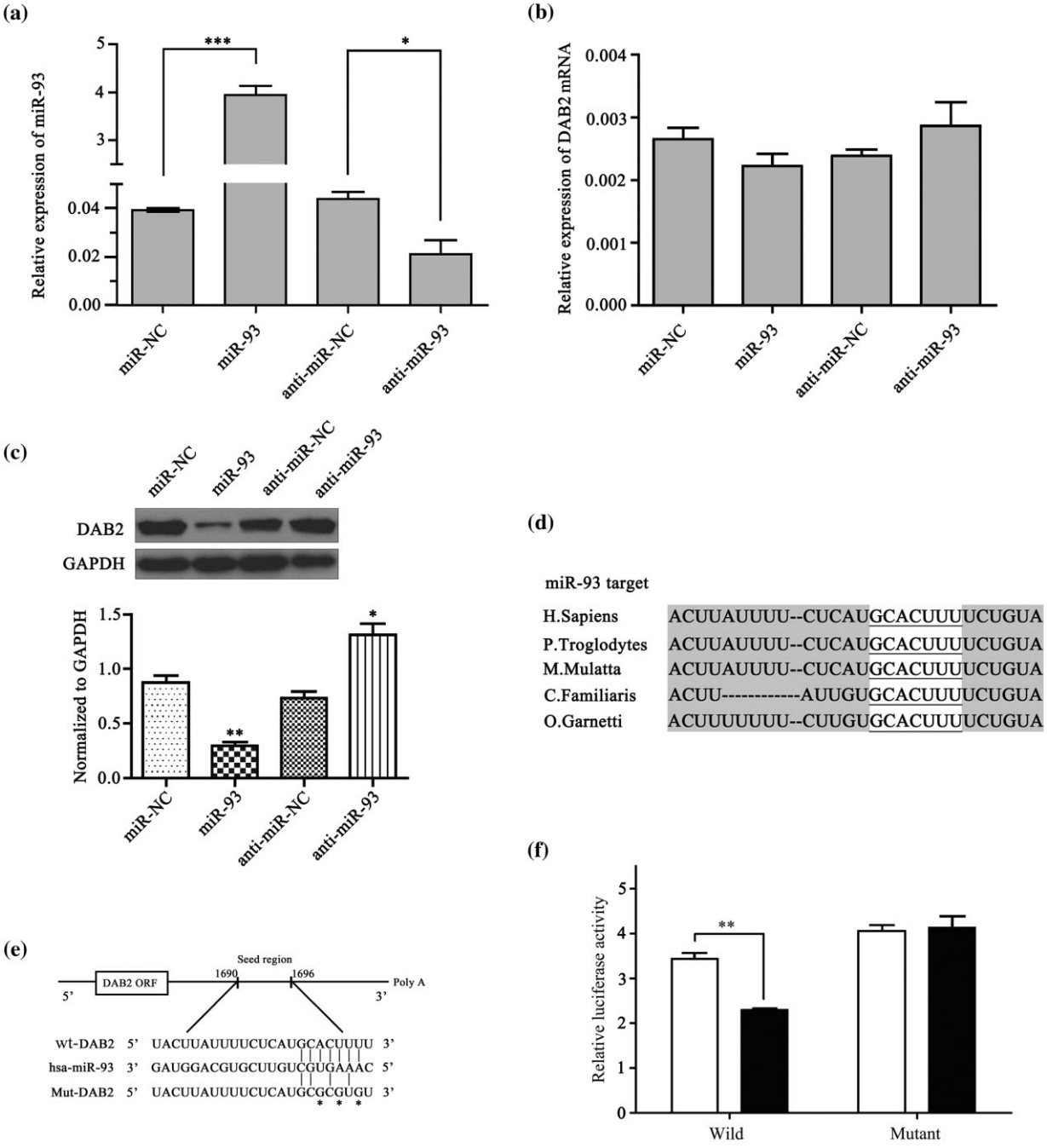

Figure 4 Micro ribonucleic acid (miR)-93 represses disabled 2 (DAB2) expression through directly binding to 3′-UTR of DAB2. (**a, b**) Real-time-polymerase chain reaction analysis of miR-93 and DAB2 messenger (m)RNA expression levels in EC109 cells transfected with miR-93 mimics, miR-93 inhibitor or negative control. The relative expression levels of miR-93 and DAB2 mRNA were normalized to U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively. (**c**) Western blot was used to analyze the expression of DAB2 in EC109 cells at 72 hours after transfection with miR-93 mimics, miR-93 inhibitor or negative control. GAPDH was used as a loading control. (**d**) Sequence alignment of predicted miR-93 target sites on DAB2 3′-UTR shows a high symmetry between humans and other species. The predicted miR-93 target sequence (GCACUUU) is shown underlined, and conserved nucleotides are shaded. (**e**) A 3′UTR fragment of DAB2 mRNA containing wild type or mutant miR-93 binding sequence was synthesized and subcloned into the psiCHECK-2 dual luciferase vector. Mutant was generated at the seed region of DAB2 3′UTR, as indicated by the asterisk. (**f**) EC109 cells were co-transfected with the reporter vectors (DAB2-3′-UTR-wild or DAB2-3′-UTR-mutant) and miR-93 mimics or control (miR-NC). Luciferase activity was detected at 48 hours post-transfection. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. MiR-NC: negative control for miRNA mimics, anti-miR-NC: negative control for miRNA inhibitor, miR-93: miR-93 mimics, anti-miR-93: inhibitor mimics of miR-93. ▭, miR-NC; ■, miR-93.

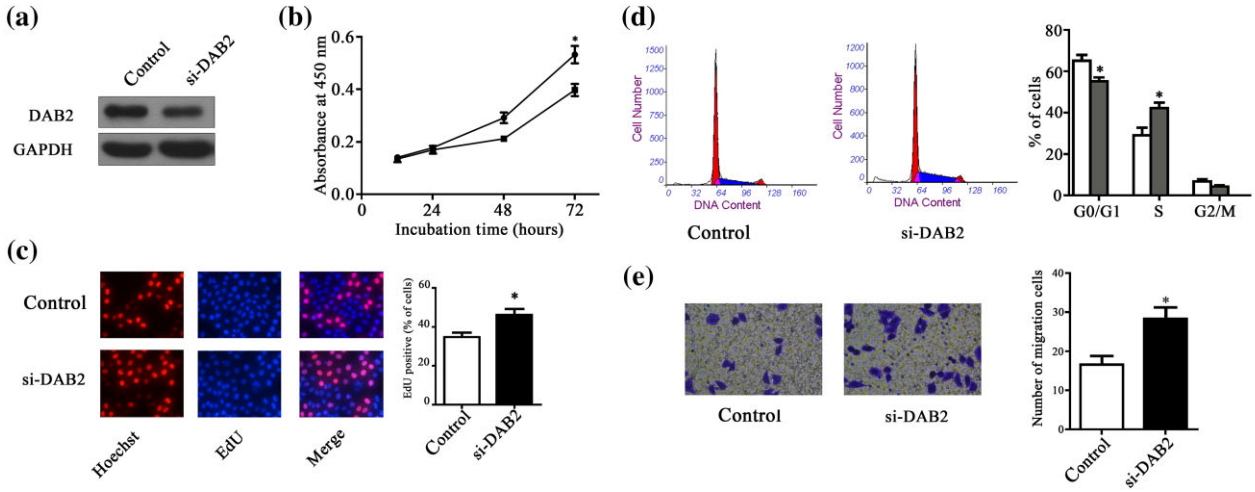

Figure 5 Effect of disabled 2 (DAB2) depletion on proliferation and migration capabilities of EC109 cells. (**a**) EC109 cells were transfected with DAB2 small interfering ribonucleic acid or the control. At 48 hours post-transfection, the DAB2 expression level was determined by Western blot. Glyceraldehyde 3-phosphate dehydrogenase was used as a loading control. (**b**) Cell counting kit-8 assay was performed on EC109 cells at 12, 24, 48, and 72 hours after transfection with si-DAB2 or the control. —■—, control; —●—, si-DAB2. (**c**) 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay was performed at 48 hours after transfection with si-DAB2 or control. Hoechst stained cells and EdU add-in cells were counted, respectively, under fluorescence microscopy, and the percentage of nucleated cells incorporating EdU was calculated. (**d**) Cell cycle progression of EC109 cells was measured by flow cytometry at 48 hours after transfection with si-DAB2 or control. ▭, control; ▬, si-DAB2. (**e**) Transwell migration assay was performed to evaluate the migration capability of EC109 cells after transfection of si-DAB2 or control. Each assay was performed in triplicate and repeated three times. *$P < 0.05$.

TGFβR2 was reported as a major target of miR-93 in nasopharyngeal carcinoma, and by suppressing TGFβR2, overexpression of miR-93 resulted in both attenuation of Smad-dependent TGF-β signaling and the activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B pathway.[30] Other studies have revealed that miR-93 could promote tumor growth and angiogenesis by targeting integrin-β8 in glioblastoma, and contribute to drug resistance by directly targeting phosphatase and tensin homolog.[31,32] In the present study, by cell transfection and luciferase reporter gene assay, we identified that in ESCC, DAB2 is a direct target of miR-93.

In recent years, the DAB2 gene, which encodes an epithelial phosphoprotein, has been determined to be a tumor suppressor gene.[4,6–8] Studies have shown that DAB2 could inhibit the growth of several cancer cells and the inhibitory effects could partly attribute to Wnt signaling restraint.[33,34] On the other hand, a loss of DAB2 has been reported to increase the propensity for metastasis by facilitating TGF-β-stimulated epithelial-to-mesenchymal transition.[12] In ESCC, DAB2 downregulation has been previously reported as an early event, and the study also identified an infrequent DAB2 promoter methylation in ESCC patients.[9] These results support the hypothesis that post-transcriptional regulation, such as miRNA control, may play a role in DAB2 gene silencing. In our study, a significant promotion of proliferation and corresponding change in EC109 cell cycles was observed either by miR-93 transfection or DAB2 knockdown, thus leading to the conclusion that miR-93 could inhibit ESCC cell proliferation by targeting DAB2. Aside from the promotion of cell proliferation, we also identified an increase in the migration ability of miR-93 transfected EC109 cells, while a decreased number of migration cells were observed with miR-93 depletion. Interestingly, the knockdown of DAB2 in EC109 cells by RNA interference brought nearly identical results to miR-93 overexpression in transwell migration assay. These results also strengthen the point that DAB2 plays an important role in the improvement of miR-93 mediated migration capability.

Conclusion

In summary, in the present study we demonstrated that the miR-93 expression level was markedly elevated in ESCC, and that miR-93 can promote proliferation and migration of ESCC cells by targeting the tumor suppressor gene DAB2. Our results also provide new insights for ESCC diagnostic and therapeutic strategy.

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Disclosure

No authors report any conflict of interest.

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