MENA promotes esophageal squamous cell carcinoma cell invasion and migration via MMP-2, MMP-9 and AKT activation

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is a fatal disease with poor prognosis. The predominant reason for ESCC-related death is metastasis caused by tumor cell invasion. Human MENA protein is a member of Ena/Vasp family, which plays a critical role during tumor cell invasion. However, the biological effect of MENA in ESCC cell lines remains unclear

Methods: In this study, fluorescent quantitative real-time PCR (qRT-PCR) were conducted to detect the mRNA expression of MENA in tumor and para-cancer tissue, CCK-8 assay and clone formation assay were conducted to evaluate cell proliferation activity, Transwell assay and wound-healing assay were conducted to detect the changes of cell invasion and migration capacity, siRNA and MENA expression vector were constructed to explore biological function of MENA in ESCC cell lines. Western blot analysis were conducted to detect the expressions of MENA , molecular markers of epithelial-mesenchymal transition (EMT), Akt, p-Akt, MMP-2 and MMP-9 respectively in ESCC cell line.

Results: The qRT-PCR experiment results showed that MENA expression in ESCC tissue of 35 patients was relatively higher than that in tissue adjacent to cancer. CCK-8 assay suggested that tumor cell proliferation capacity was suppressed followed by the knockdown of MENA expression in Mena high ESCC cell TE13 and was potentiated by the overexpression of MENA in Mena low ESCC cell TE1. Transwell assay and wound healing assay demonstrated that interfering in MENA could inhibit TE13 cells invasion and migration capacity by affecting the expressions of Matrix metalloproteinase-2(MMP-2) and Matrix metalloproteinase-9 (MMP-9), in contrast, overexpression of MENA in Mena low ESCC cell TE1 could promote invasion and migration by up-regulated expression of MMP-2 and MMP-9. Western blot analysis indicated that interfering of MENA expression could affect EMT-related molecular markers (E-cadherin, N-cadherin, Snail, Slug), Akt and p-Akt

Conclusions: Our study reveal that MENA could promote the ESCC cell invasion and migration by upregulate MMP-2, MMP-9 expression and Akt activation. Meanwhile, interfering of MENA expression could affect EMT in ESCC cells. This indicated that MENA may be a potential molecular therapeutic target for ESCC metastasis

Background

Esophageal cancer is one of the most common malignant tumor in the digestive system [1], East Asia is the region with the highest incidence and mortality of esophageal cancer [2]. Esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) is the two main histological type of EC, EAC is more common in the developed countries compared with ESCC, which is mainly occurring in Asia, especially in China. [3–6]. At present, ESCC is the main histological subtype which account for more than 90% of cases of all esophageal cancers, and the 5-year survival rate of ESCC patient is still quite poor[7, 8]. Most critically, nearly 90% of ESCC patients are in the advanced stage at the time of diagnosis and more than half of them exhibit the local invasion and metastasis, which is the

leading cause of death of ESCC[9, 10]. Although there is new progress in therapeutic strategies, a large number of patients with ESCC remain poor clinical outcome. Therefore, it is particularly necessary to seek the novel prognostic biomarker for metastasis and molecular therapeutic target.

Human MENA encoded by the ENAH gene is a member of the ENA/VASP family of actin filament elongation factors [11,12] Recent studies found that MENA was highly expressed in a variety of human cancers, such as breast cancer, non-small cell lung cancer, malignant melanoma, and pancreatic cancer. [13, 14]. In addition, it shows that high expression of MENA is highly correlated with tumor metastasis and malignancy[15, 16]. However, no report about MENA expression and its biological function in ESCC cell have not been explored. In this study, we analyzed MENA gene expression levels in ESCC samples and examined MENA expression in a panel of ESCC cell lines, furthermore we investigated the effects of MENA knockdown on tumor cell migration and invasion as well as the capability of cell proliferation, and further preliminarily elucidated the possible molecular mechanisms.

Methods

Tissue samples

This study was carried out under the approval from the Ethics committee of Zhengzhou University, and all patient signed the written informed consent. ESCC tissue and paired adjacent normal tissue samples were obtained from 27 ESCC patients undergoing esophagectomy at Anyang tumor hospital from 2014–2016. All Fresh tissues were immediately frozen in liquid nitrogen waiting for RNA extraction.

Cell lines and culture

The ESCC cell lines TE1, TE13, Kyse-70, Kyse-450, Kyse-510 and normal human esophagus epithelial cell Het-1A were purchased from the Cell Bank of Chinese Academy of Sciences. All cells were maintained in RPMI 1640 medium(Biological Industries, Cromwell, CT,) containing 10% heat-inactive fetal bovine serum (FBS, Biological Industries, Cromwell, CT,) and were cultured at 37 °C under 5% humidified CO2 enriched atmosphere.

Real-time qRT-PCR

Quantitative real-time RT-PCR (real-time qPCR) assay was performed as described previously [17]. Briefly, total RNA was extracted from ESCC tumor tissue and and paired adjacent normal tissue by using TRIzol Reagent (Tiangen, Beijing, China). Target genes were amplified by QuantStudio™ 3 Real-Time PCR System(Thermo Fisher Scientific, Carlsbad, CA) with the following specific primers: MENA forward 5'-TCAAGGGTAAGGGAAACTGG-3', and reverse 5'-TGGCTCACAAGTGGTCCTCC-3'; GAPDH forward 5'-CTCCTCCTGTTCGACAGTCAGC-3', and reverse 5’-CCCAATACGACCAAATCCGTT-3'. The data analysis was conducted using the comparative threshold cycle method (ΔΔCt) in which all MENA Ct values in the ESCC tumor tissue were first normalized to GAPDH

Protein extraction and Western blotting

Western blotting assay was performed as our previous report [17]. Briefly, Cells were washed twice with ice-cold PBS, and collected cells were lysed in buffer (Solarbio, Beijing, China). Protein concentration was determined using BCA Protein Assay Kit (Solarbio, Beijing,, China,)according to according to manufacturer's instruction. Total protein (0.4 mg) was added to 4 × protein sample buffer(Solarbio, Beijing, China), heated at 100 °C for 5 min and separated by SDSPAGE with 3 μl of pre-stained protein molecular weight marker (Thermo Fisher Scientific, Carlsbad, CA) as a standard, which was transferred to a polyvinylidene fluoride (PVDF) membrane and incubated with the following respective primary antibodies including anti-MENA (1:5000), anti-MMP-2 (1: 1000), anti-MMP-9 (1:10000), anti-Akt (1:1000), anti-p-Akt (1:1000), anti-E-Cadherin (1:5000), anti-N-Cadherin (1:5000), anti-Snail (1:2000), anti-Slug (1:2000) and control anti-GAPDH (1:500) overnight at 4 °C, followed by secondary antibodies (goat anti mouse, 1:5000, goat anti rabbit, 1:2000) conjugated with horseradish peroxidase. Membranes were developed using the ECL detection system (Sloarbio, Beijing, China). Quantification of Western blotting was performed using Image J to determine the relative protein level, and the results were from at least three independently repeated experiments.

Cell transfection assay

A MENA expression plasmid Pex-3(pGCMV/MCS/Neo) containing the full-length human Mena cDNA and Small interfering RNA was obtained from the (Gene Pharma, Shanghai, China). Empty vector was used as a negative control for MENA expression plasmid. Transient transfections were performed when ESCC cells reached 60%-80% confluence in 6 well plates using Lipo8000 (Beyotime, Shanghai, China) for expression plasmid transfection and LipoRNAi (Beyotime, Shanghai, China) for siRNA transfection, according to the manufacturer's instructions. At 48 hours after transfection, MENA expression was examined by western blotting. The siRNA sequences were: siMENA#1, sense 5'-GGUCCUAUGAUUCAUUACATT-3', and antisense 5'-UGUAAUGAAUCAUAGGACCTT-3'; siMENA#2, sense 5'-GCGAGAAAGAAUGGAAAGATT-3', and antisense 5'- UCUUUCCAUUCUUUCUCGCTT-3'; negative control (NC), sense 5'-UUCUCCGAACGUGUCACGUTT-3', and antisense 5'-ACGUGACACGUUCGGAGAATT-3'.

Proliferation assay

Cells were plated in a volume of 200 μl at a density of 3000 cells per well in 96-well plates, which was allowed for siRNA and MENA expression plasmid transfection after 24 hours of growing. The proliferation rate of cells was evaluated using the CCK-8 Cell Proliferation kit (APExBIO Technology LLC, Huston, USA) according to manufacturer's instructions, and each experiment was done in triplicate.

Colony formation assay

Cells were trypsinized after finishing with siRNA or MENA expression plasmid transfection, resuspended cells with PBS and calculate. 500 cells were seeded in a signal well of a six-well-plate and cultured at 37ºC in an atmosphere of 5% CO2 for 10 days. Colonies were washed twice with PBS, and fixed with 4% paraformaldehyde solution for 20 min. After fixation, 0.1% crystal violet was added for staining for 30 min. After being left to dry at room temperature, photographs were recorded, and three replicate experiments were set for each group. Colony numbers were analyzed using Image J.

Migration and invasion assays

To determine whether the invasion and migration ability of ESCC cells was mediated by siRNA and MENA expression plasmid. Transwell assay was performed in Transwell chambers (8-μm pores, Corning, Shanghai, China). For invasion assay, 0.5 mg/ml Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA, USA) was coated in the bottom of the transwell chamber,

and for migration assay, there was no Matrigel Basement Membrane Matrix coated in he upper chamber, the rest of the following steps are all the same.1 × 105 cells in a volume of 200 μl were added into the upper transwell chamber, 600 μl RPMI 1640 medium with 20% FBS was added into the lower chamber. The cells were incubated in 37℃ 5% CO2 for 48 hours. After incubation, the cells were washed with PBS for 3 times and fixed with 75% methanol for 20 min. 0.5% crystal violet was used for cell staining. The stained cells were counted in 5 random fields under an inverted microscope. Each experiment was carried out in three separate wells, and independent experiments were repeated three times.

Wound healing assay

Wound healing assay was used for investigating whether MENA could affect ESCC cell migration. The cells were transfected with siRNA or MENA expression plasmid in a 6 well plate, after transfection, a scratch wounds were created by scraping the cell monolayers with a 200 μl sterile pipette tip. Suspended cells were washed using PBS, photomicrograph was taken immediately, and after 24 hours of incubation, photomicrographs were taken at the same position, respectively. Migrations at least three independently repeated experiments were quantified by measuring distances from the wound edges.

Statistical analysis

Statistical analyses were carried out with the Statistical Package for the Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± standard error (SEM) The comparisons of two samples were carried out using t test and paired-sample t test. $p < .05$ was considered as statistical significance.

Results

mRNA of MENA is overexpressed in human GC

To confirm whether MENA mRNA expression was higher in ESCC tumor tissue than in normal tissue, MENA mRNA expression level was examined by quantitative Real-Time PCR (qRT-PCR) in 27 human ESCC tumor tissue and paired normal adjacent tissue. The qRT-PCR results shows that 22(81.5%) ESCC tumor tissue expressed higher MENA mRNA than paired normal adjacent tissue(Fig. 1C), and it can be seen that the data distribution of the adjacent tissues is relatively concentrated, while the data distribution of the tumor tissues is relatively discrete, which reflects the high heterogeneity of the tumor tissues to some extent.

Expression of MENA in a panel of ESCC cell lines

We detected MENA protein expression in a series of ESCC cell lines as well as normal esophageal epithelial cell by Western blotting. We found that MENA protein expression was significantly higher than normal esophageal epithelial cell Het-1A which is consistent with results of qPCR. However, MENA expression was different among these ESCC cell lines, TE13 cells exhibited the highest MENA expression than other ESCC cell lines, especially in TE1 which is even harbored equal expression of MENA compared with Het-1A (Fig. 1A). Thus, we compared TE1 and TE13 in cell proliferation by CCK-8 assay, colony formation by colony formation assay and cell invasion and migration by transwell assay and wound-healing assay, TE13 showed much stronger capabilities of cell proliferation (Fig. 2C), colony formation(Fig. 2A and B), cell invasion and migration (Fig. 2D, E, and F). Therefore, to further explore the functional roles of MENA in ESCC cells, TE13 with the highest MENA level and TE1 with the lowest MENA level were employed for further investigation.

MENA mediates ESCC cells proliferation and colony formation

We silenced MENA expression in TE13 cells with MENA specific siRNA in order to evaluate whether MENA could mediate ESCC cell proliferation and colony formation. Western blotting results shows that MENA expression was significantly suppressed after MENA-siRNA transfection in TE13 cells (Fig. 3A). Cell proliferation and colony formation were suppressed in siMENA group, compared with siNC group (Fig. 3B, C, D). Since MENA protein expression was much lower in TE1 cells compared with TE13 cells (Fig. 1A and B), therefore, we constructed an MENA expression vector (pGCMV) and transfected TE1 cells with it to further confirm the effect of MENA in ESCC cells proliferation and colony formation (Fig. 4A). The cell growth rate and colony formation were enhanced in TE1 cells transfected with MENA expression plasmid compared with TE1 cells transfected with empty vector. (Fig. 4B, C, D). These results indicate that MENA could mediate cell proliferation and colony formation.

MENA promotes cell migration and invasion in ESCC cells

Wound healing assay revealed that migration area of TE13 cells in MENA-siRNA group was all lower after 24 hours than those in siNC group (Fig. 3G). However, migration area of TE1 cells transfected with MENA expression plasmid is higher after 24 hours than those in control group (Fig. 4G). Meanwhile, We also conducted transwell assay to evaluate the effects of MENA expression on ESCC cell migration and invasion. Silencing MENA expression in TE13 cells significantly decreased the number of cells that migrated or invaded through the membrane in the transwell chamber (Fig. 3E, F). Conversely, overexpression of MENA enhanced the migration and invasion of TE1 cells compared with control group(Fig. 4E, F). These results suggest that the expression level of MENA strongly correlated with the ESCC cell migration and invasion, therefore increased MENA expression may promote ESCC invasion and tumor metastasis.

Over expression of MENA promotes ESCC cell EMT

Epithelial-mesenchymal transition plays important role during tumor cell invasion and metastasis [18, 19], this biological process is mainly manifested as down-regulated expression of E-cadherin, substrate degradation, and up-regulated expressions of N-cadherin and two EMT inducer Snail and Slug [20]. In order to study whether MENA could affect ESCC cell EMT, we explored the EMT specific markers via western blotting. We found that after TE1 cells was transfected with MENA expression vector EMT-related molecular markers significantly changed, E-Cadherin was down-regulated, N-Cadherin and two EMT transcription factor Snail and Slug were up-regulated (Fig. 5A and B). In TE13 cells, Snail, Slug and mesenchymal marker N-Cadherin were down-regulated in MENA-siRNA group compared with siNC group, however E-Cadherin shows no difference between hMENA-siRNA group and siNC group. Based on the EMT specific markers expression of above, we suggest that MENA plays a role during ESCC cell perform EMT.

MENA expression correlates with MMP-2 and MMP-9 expression in ESCC cells

Strong correlation between MENA and cell invasion was fully demonstrated with MENA knock-down in TE13 cells and MENA over expression in TE1 cells, and given that Matrix metalloproteinases(MMP) is highly required during tumor cell invasion[21]. We conducted Western blotting and Immunofluorescence to further confirm whether knock-down and over expression of MENA could affect the expressions of MMP-2 and MMP-9 proteins, which are considered as critical player in the process of tumor invasion and metastasis[22, 23]. We found that MENA-siRNA significantly downregulated MMP-2, MMP-9 expressions, compared with siNC group (Fig. 6A and B)., the Immunofluorescence showed that MENA-siRNA group gave weaker fluorescence signal of MMP-9 than siNC group(Fig. 6C), which was consistent with the outcomes from Western blotting. Conversely, Western blotting experiment shows that TE1 cells transfected with MENA expression vector showed higher expression of MMP-2 and MMP-9 compared with TE1 cells transfected with control plasmid(Fig. 6A, B). These findings suggest that MENA mediated cell invasion may be achieved by degrading and up-regulating of MMP-2 and MMP-9 expression.

MENA expression affects AKT signaling pathway

Akt signaling pathway is critical in tumor genesis and plays important role during cell proliferation [24]. Due to knock-down MENA strongly inhibited cell proliferation and cell colony formation in TE13 cells and over expression of MENA in TE1 cells gave the opposite outcomes. In line with these results, we observed decreased Akt and phosphorylated Akt expression in MENA-siRNA group compared with siNC group via western blotting(Fig. 6A, B), and over expression of MENA gave increased Akt and phosphorylated Akt expression(Fig. 6A, B). In summary, the results implied MENA affect Akt signaling pathway in ESCC cells

Discussion

Tumor metastasis is the most dangerous process in the development of tumors. According to statistics, only about 10% of cancer related mortality are caused by primary tumors, whereas metastatic tumor account for 90% of cancer related mortality [25]. The high metastasis rate of esophageal squamous cell carcinoma has always been the main reason for the high mortality rate of this cancer. How to predict and suppress the metastasis of the tumor as soon as possible is a major problem in the treatment of esophageal squamous cell carcinoma.

In the current study, we found mRNA expression of MENA were significantly higher in primary ESCC tissues compared with adjacent non-tumor tissues, and interestingly, qPCR analysis shows that data distribution of the adjacent tissues is relatively concentrated, while the data distribution of the tumor tissues is relatively discrete, this reflects the high heterogeneity of tumor tissues. The MENA expression was remarkably higher in ESCC cell lines than it in normal human esophagus epithelial cell Het-1A which is consistent with the qPCR results from ESCC patient tissue. We noticed that expression of MENA in TE13 is significantly higher than other ESCC cell lines while expression of MENA in TE1 is the lowest among ESCC cell lines. We then compared TE1 and TE13 in proliferation, colony formation, invasion and migration in vitro, and we found that TE13 is much stronger than TE1 in proliferation, colony formation, invasion and migration.

To better explore the role of MENA in ESCC cell lines, we knocked down MENA expression in TE13 cells and over-expressed MENA in TE1 cells, we found that silencing MENA expression in TE13 cells significantly suppressed cell proliferation and colony formation, whereas MENA overexpression in TE1 cells enhanced cell proliferation and colony formation. These results indicated that MENA may promote tumor cell growth.

Previous studies have shown that MENA proteins localize to focal adhesions, the leading edge of lamellipodia, and the tips of filopodia, and it helps tumor cells to form lamellipodia and filopodia which facilities ECM degradation[26]. Frank B Gertler an etc identified MENA as regulator of carcinoma cell invasion and plays a critical role during breast cancer metastasis[27]. Therefore, In our study, we found silencing MENA expression in TE13 remarkably inhibited cell invasion and migration, in siMENA group almost no cell invaded into the lower chamber whereas the siNC group exhibited much stronger capability of invasion and migration. TE1 cells gained higher capabilities of invasion and migration after transfected with MENA expression vector.

The initial step in carcinoma cell invasion is form invadopodia, which protrude into the matrix and secrete proteases focally that degrade the matrix [28]. The acquired invasion ability of carcinoma cells is often supported by their secretion of matrix metalloproteases (MMPs) [29]. In current study, We found that knock down MENA expression inhibited MMP-2 and MMP-9 secretion while over expressed MENA promote MMP-2 and MMP-9 secretion. Furthermore, Immunofluorescence showed that weaker fluorescent signal in siMENA group compared to siNC group. We also found that interference MENA expression in ESCC cell lines could affect Akt signaling pathway which plays critical roles in tumor cell proliferation, migration and invasion.

Epithelial-mesenchymal transition in cells often enhances the cell's ability to invade and migrate. EMT occurs as a decrease in the expression of E-cadherin and an increase in the expression of N-cadherin and EMT inducer such as snail and slug [30, 31]. High expression of E-cadherin will increase the adhesion between cells, while high expression of N-cadherin and Vimentin will make the cells lose adhesion and promote cell movement [32]. Our study showed that knocked down MEAN expression could suppress EMT in TE13 which expressed down-regulated expression of N-Cadherin, snail and slug whereas up-regulated expression of E-Cadherin. In contrast, over-expression of MENA could promote EMT in TE1 which exhibited down-regulated expression of E-Cadherin but up-regulated expression of N-Cadherin, snail and slug.

The abnormal expression of Akt signaling pathway plays an important role in tumorigenesis and development. The phosphorylation of Akt can inhibit some apoptosis-promoting proteins, and inhibiting Akt in tumor cells will significantly reduce cell proliferation [33, 34]. Given that knocking down Mena and overexpressing Mena respectively inhibited and promoted the proliferation and colony formation of ESCC cells, and that the abnormal expression of the Akt signaling pathway particularly affected tumor cell proliferation, we suspect that intervention in the expression of Mena in ESCC cells would affect Akt signaling pathway. So we used Western blot to detect changes in Akt and phosphorylated Akt expression in ESCC cells after knocking down and over-expressing MENA, the results were completely in line with our expectations. Knockdown of MENA inhibited Akt and phosphorylated Akt expression, while overexpression MENA promoted Akt and phosphorylated Akt expression.

MENA has different isoform due to alternative splicing, including the $MENA^{INV}$ and $MENA^{11a}$ [35, 36]. $MENA^{INV}$ is highly expressed specifically in aggressive tumors and has a very strong pro-invasion effect. Recent studies show that $MENA^{INV}$ can promote formation of cell membrane protrusion which is induced by epidermal growth factor both in vivo and in vitro, and it can enhance the ability of tumor cells to degrade extracellular matrix, thereby promoting the migration and invasion of tumor cells [37, 38]. However, $MENA^{11a}$ is specifically expressed in tumor tissues with low invasion and high adhesion. The expression of $MENA^{11a}$ in orthotopic tumors tissue is significantly higher than $MENA^{INV}$, and the epithelial-like tumor cells also show higher expression of $MENA^{11a}$ than mesenchymal-like tumor cells in breast cancer [39, 40]. The distinct functional properties of MENA isoform have exacerbated the complexity of tumor-related research about MENA. Given that $MENA^{INV}$ and $MENA^{11a}$ have distinct functions in a variety of tumors, our follow-up research will focus on splice variant of MENA in ESCC.

In conclusion, we have demonstrated amplified expression of MENA mRNA in esophageal squamous cell carcinoma. and we confirmed that MENA enhance ESCC cell growth, colony formation, cell migration, and invasion in vitro via mediate MMP-2, MMP-9 secretion, Akt activation and EMT status. Taken together, our research suggests that MENA might serve as a candidate biomarker for ESCC metastasis and a potential therapeutic target in the treatment of ESCC patients.

Conclusion

Our study reveal that MENA could promote the ESCC cell invasion and migration by upregulate MMP-2 ,MMP-9 expression and Akt activation. Meanwhile, interfering of MENA expression could affect EMT in ESCC cells. This indicated that MENA may be a potential molecular therapeutic target for ESCC metastasis.

Abbreviations

ESCC	Esophageal squamous cell carcinoma
EAC	esophageal adenocarcinoma
qRT PCR	quantitative real time quantitative PCR
EMT	Epithelial mesenchymal transition
MMP-2	Matrix metalloproteinases-2
MMP-9	Matrix metalloproteinases-9
SEM	Standard error

Declarations

Ethics approval and consent to participate

The study was approved and supervised by the research ethics committee of Zhengzhou University, Zhengzhou, China. All procedures performed in studies were in accordance with the ethical standards. All patients were anonymous and provided written informed consent.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TF, HL and YL conceived of the study and participated in its design and coordination and drafted the manuscript. GH helped to correct experimental methods. YL carried out the Transwell assay, wound-healing assay and cell culture. NG, JL, ZG carried out part of the western blotting. Z Y and JD performed the Immunofluorescence. All authors read and approved the final manuscript.

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Figures

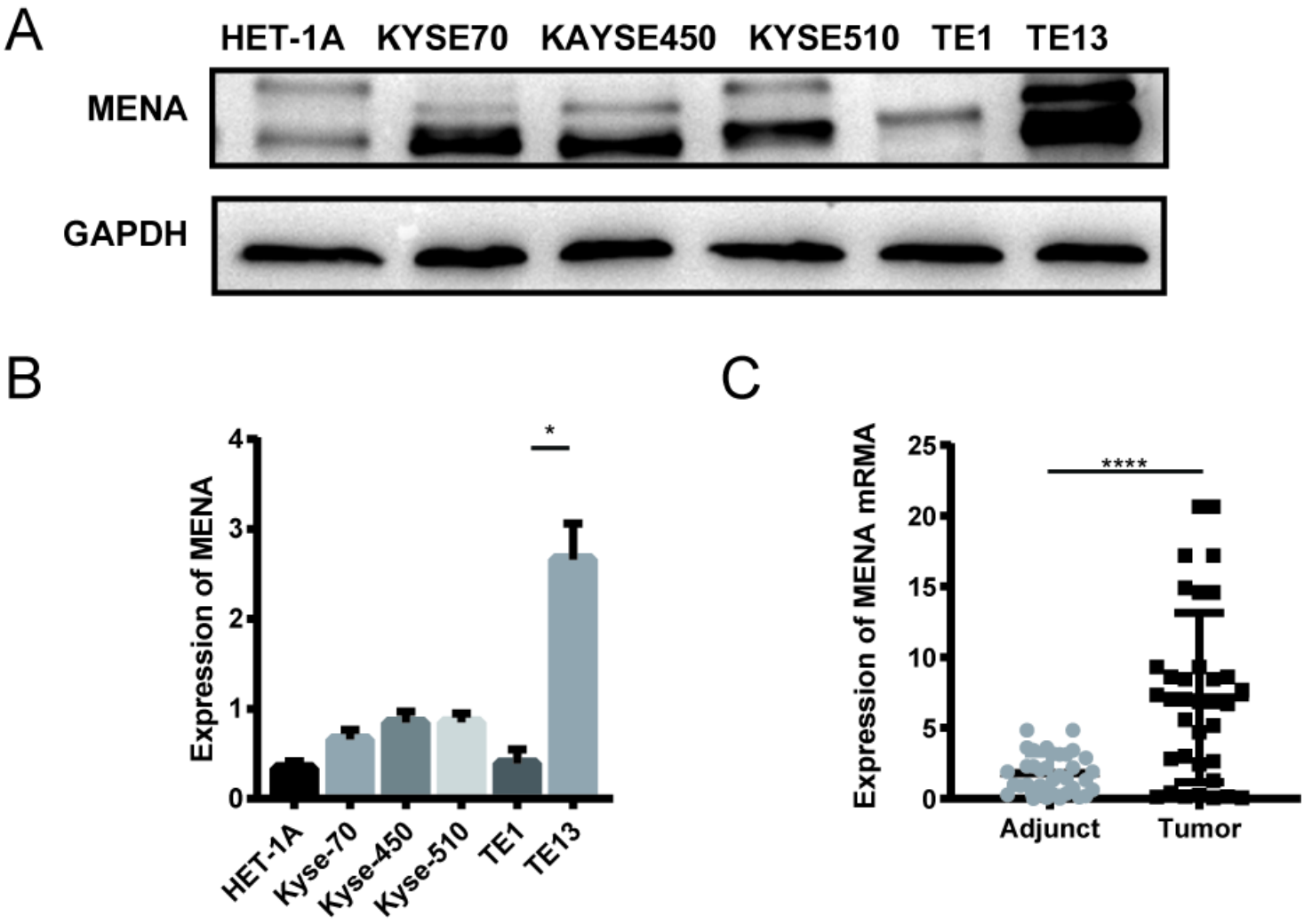

Figure 1

The mRNA of MENA is higher in ESCC surgical specimens and MENA protein expression in ESCC cell lines. (a) Western blotting analysis of MENA expression in different ESCC cell lines. (b) Relative expression of MENA in different ESCC cell lines compared to esophageal epithelial cell Het-1A. (c) The relative mRNA expression of MENA was significantly higher in ESCC tissues compared with the matched adjacent noncancerous tissues (*** P<0.001).

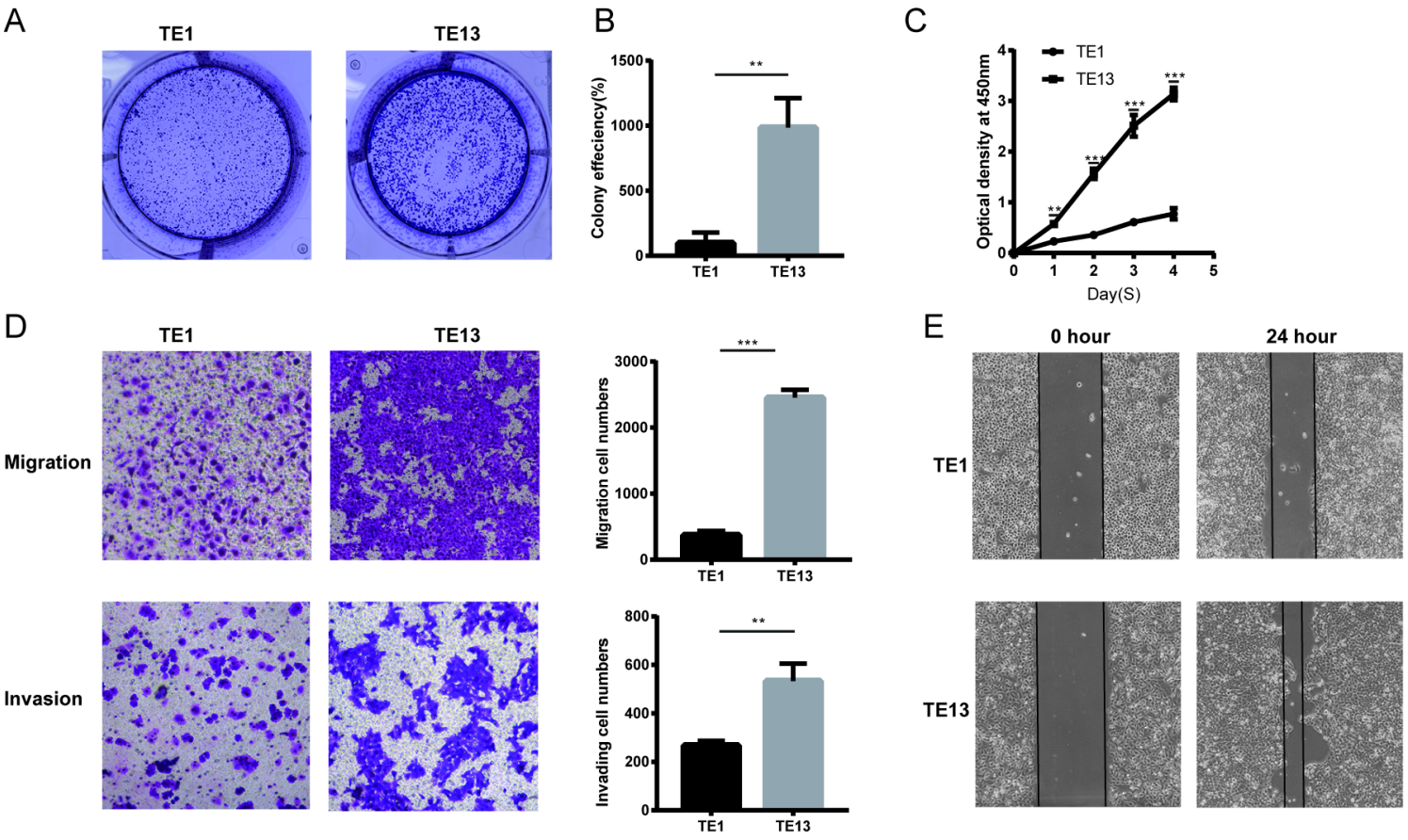

Figure 2

Cell proliferation, colony formation, invasion and migration of MENAhigh cell TE13 is significantly stronger than MENAlow cell TE1. (a) (b) Colony formation of TE13 cells is stronger than TE1 (** P<0.01). (c) Cell proliferation assay showed that TE13 proliferation is stronger than TE1. (c) TE13 showed higher invasion and migration abilities than TE1 (*** P<0.001 ** P<0.01). (c) Wound-healing assay detected that migration ability of TE13 is higher than TE1.

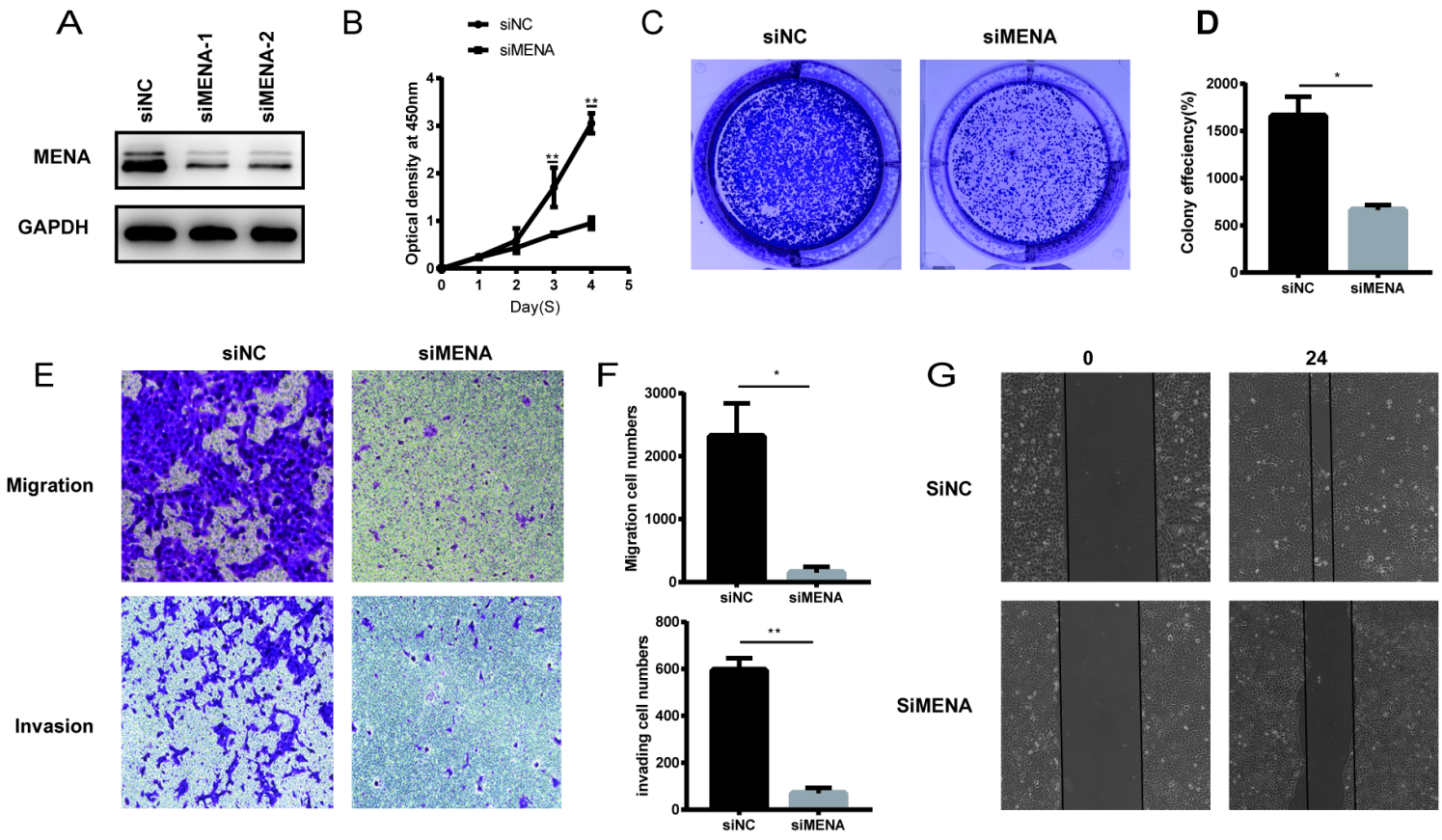

Figure 3

Knocking down MENA attenuates the cell proliferation, colony formation migration and invasion of esophageal squamous cell carcinoma (ESCC) cells. (a) (b) Knocking down MENA expression in the TE13 cells by siRNA transfection(** P<0.01). (c) Cell-proliferation assay shows that silencing MENA expression inhibited proliferation of TE13 cells. (d) (e) Representative results showing silencing MENA expression inhibited colony formation of TE13 cells(* P<0.05). (f) Knocking down MENA strongly attenuates the migration and invasion of TE13 cells(* P<0.05 ** P<0.01). (g) Wound-healing assay indicated that knock down MENA expression could inhibit cell migration.

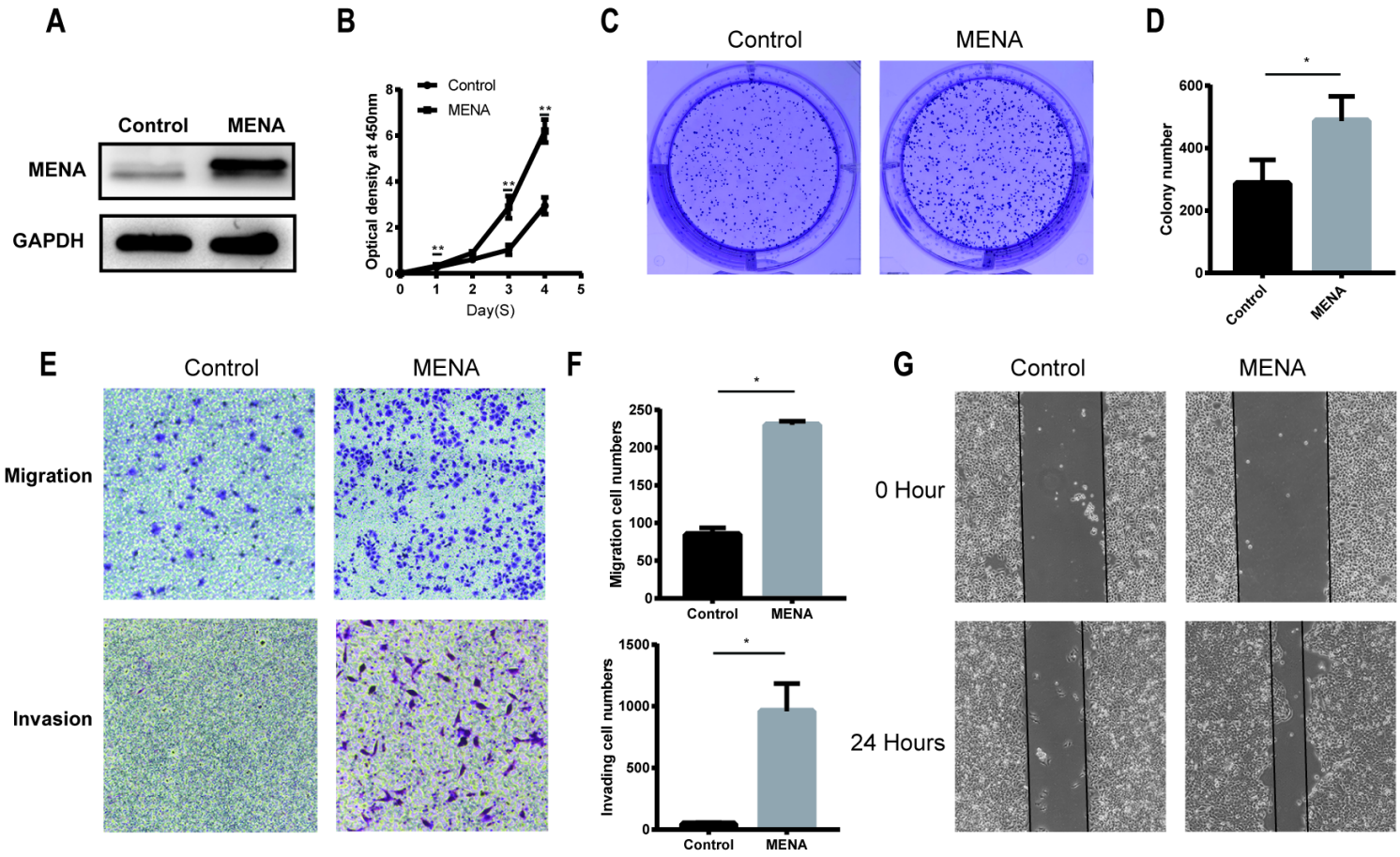

Figure 4

MENA promotes cell proliferation, colony formation, migration and invasion in TE1 cells. (a) (b) Upregulating MENA expression in the TE1 cells by MENA expression vector transfection(* P<0.05). (c) MENA promotes cell proliferation in TE1 cells. (d)(e) Colony formation assay shows that TE1 cells gained stronger colony formation ability after transfected with MENA expression vector(* P<0.05). (f)(g) Transwell assay shows that MENA over-expression promotes cell migration and invasion of TE1 cells(* P<0.05 * P<0.05). (h) Wound-healing assay detected stronger cell migration in TE1 cells after transfected with MENA expression vector.

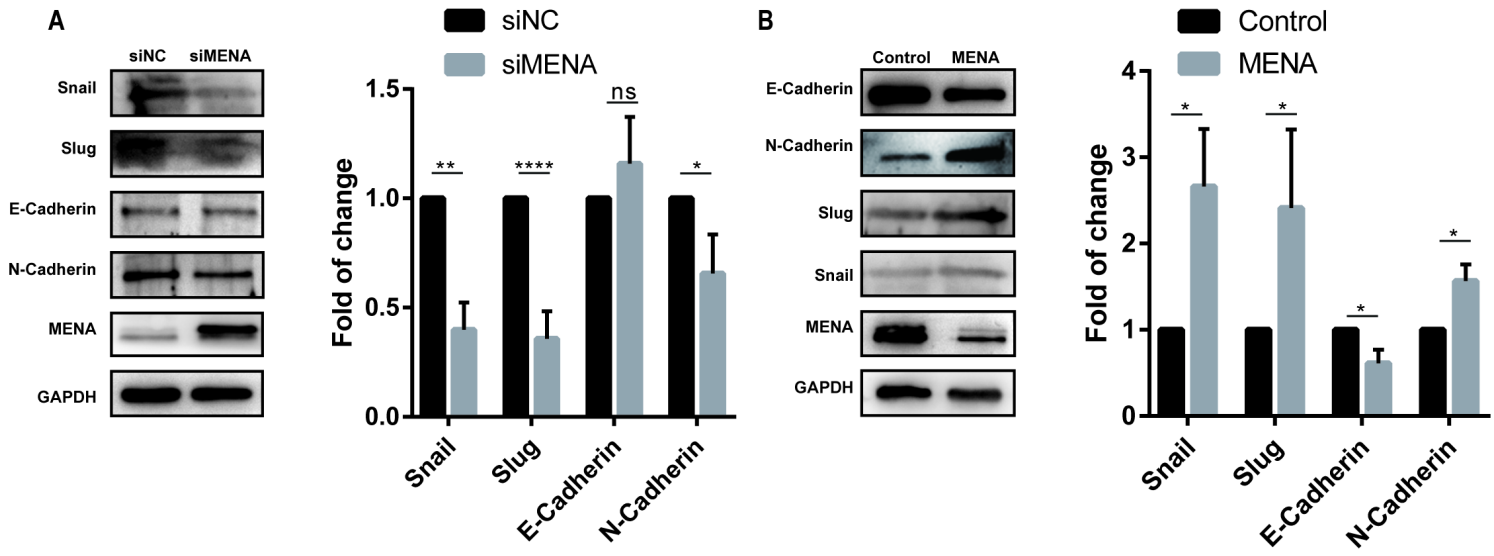

Figure 5

MENA regulates EMT status of ESCC cells. (a) EMT specific marker N-Cadherin(* P<0.05), Snail(** P<0.01) and Slug(*** P<0.001) was down regulated after knock down MENA expression. (b) In contrast, over expression of MENA up regulates N-Cadherin(* P<0.05), Snail(* P<0.05), Slug(* P<0.05) and down regulates E-Cadherin(* P<0.05).

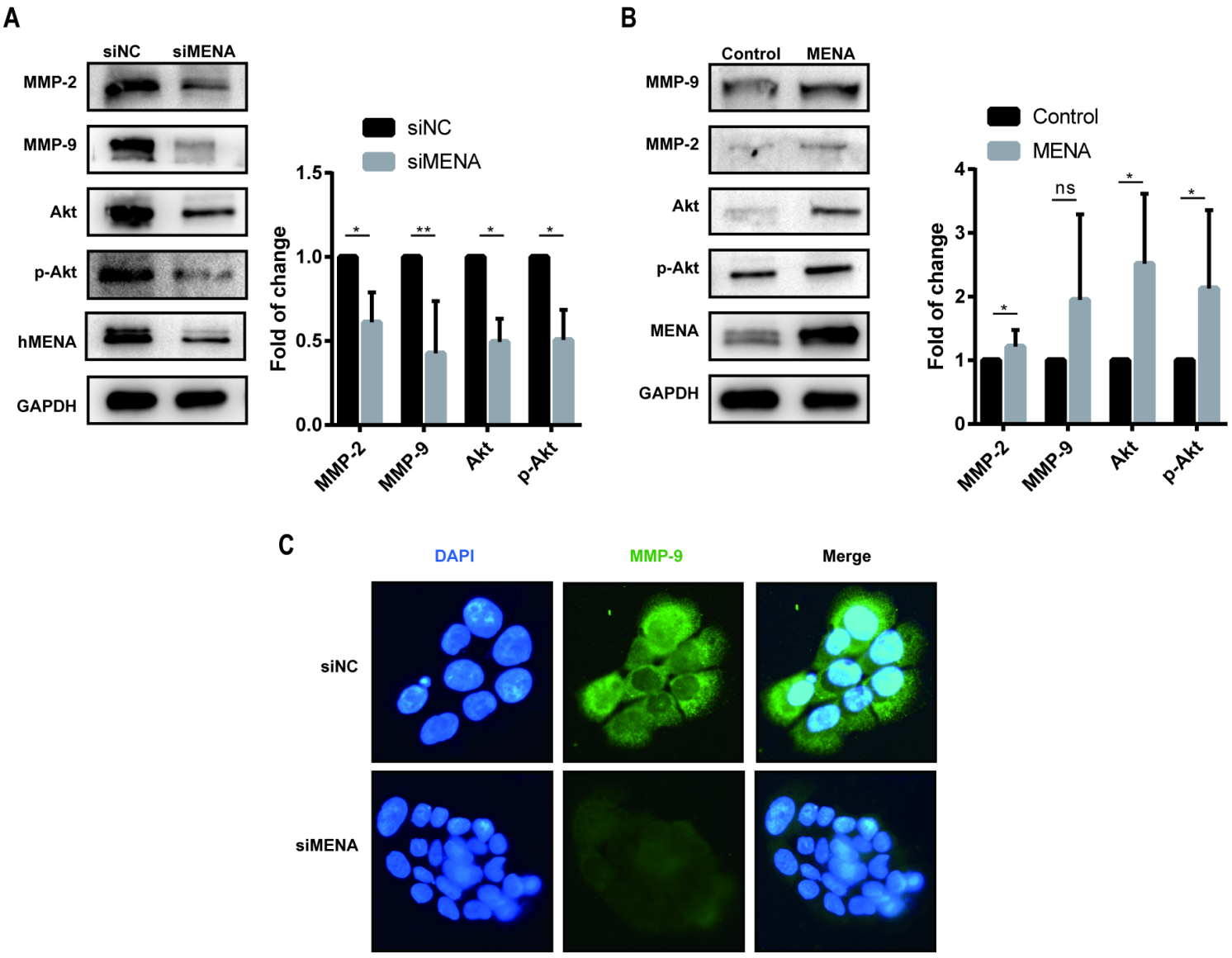

Figure 6

Interfering MENA expression affects Akt signaling pathway and MMP-2, MMP-9 expression. a Akt(* P<0.05), p-Akt(* P<0.05), MMP-2(* P<0.05) and MMP-9(** P<0.01) expression are inhibited in TE13 cells after knock down MENA expression. (b) However, Over expression of MENA promotes Akt(* P<0.05), p-Akt(* P<0.05) and MMP-2(* P<0.05) expression. (b) Immunofluorescence assay detects weaker fluorescence signal in siMENA group compared to siNC group.

Supplementary Files

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