MUC13 promotes the development of esophageal cancer by upregulating the expression of O-glycan process-related molecules

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Abstract

Background

Esophageal cancer is one of the most common malignant tumor in the world, which is characterized by poor prognosis, aggressiveness and poor survival. Mucin 13 (MUC13) is a member of the membrane-bound mucin and located on chromosome 3q21.2 and consisted of α and β subunits. It has been found that MUC13 is overexpressed in a variety of tumor cells and acts a vital role in the invasiveness and malignant progression of several types of tumors. However, the role and regulatory mechanism of MUC13 in the progression of esophageal cancer remain unclear.

Methods

The expression level of MUC13 was detected in 15 esophageal cancer tissues and 15 pairs of adjacent non-tumor tissues by immunohistochemistry (IHC). In addition, the expression of MUC13 mRNA level in human esophageal cancer cell lines (EC9706 and ECA109 and TE-1) was measured by qRT-PCR. *In vitro*, after silencing MUC13 with lentiviral interference technology, CCK8 assay, clone formation assay and flow cytometry were applied to investigate the proliferation activity, clone formation ability and anti-apoptosis ability of EC9706 and ECA109 cells. The tumor xenograft growth assay was used to confirm the influence of MUC13 knockdown on the growth of esophageal tumors *in vivo*. The qRT-PCR assay and western blot experiments were taken to study the mechanism of MUC13 regulating the pro-proliferation and anti-apoptotic of esophageal cancer.

Results

The results showed that MUC13 was over-expressed in esophageal cancer tissues and cell lines (EC9706 and ECA109 and TE-1), especially in EC9706 and ECA109 cells, but low-expressed in the human esophageal epithelial cell line (HEEC). Next, silencing MUC13 inhibits the proliferation, blocks cell cycle progression and promotes cell apoptosis *in vitro*, and also restrains the growth of esophageal cancer tissues *in vivo*. Finally, MUC13 affects the pro-proliferation and anti-apoptotic by regulating the expression of GLANT14, MUC3A, MUC1, MUC12 and MUC4 that closely related to O-glycan process.

Conclusions

This study proved that MUC13 is an important molecule that regulates the O-glycan process and then affects the progress of esophageal cancer. MUC13 may be a novel therapeutic target for patients with esophageal cancer.

Background

Esophageal cancer is one of the most malignant cancers, mainly due to its extremely aggressiveness and poor survival rate [1–3]. In recent decades, the application of new technologies, new equipment and neoadjuvant treatments has made great progress in the prevention and control of esophageal cancer [4, 5]. However, the prognosis of patients suffering from esophageal cancer is poor, and the number of people whose survival time exceeds five years is far from expectations. Therefore, it is urgent to rummage novel therapeutic targets for patients with esophageal cancer.

Mucins are glycoproteins secreted on the surface of cells which can lubricate the epithelial surface of mucosal tissues and provide chemical barrier. Studies have shown that cell surface mucin 13 (MUC13) protects the degradation of β-catenin by interacting with GSK-3β, thereby increasing the nuclear translocation of β-catenin and promoting the occurrence, development, invasion and immunosuppression of cancer by signal transduction [6]. The expression of MUC13 also leads to the activation of NF-κB p65 nuclear translocation and phosphorylation of IκB, which in turn upregulates the expression of important proteins involved in glucose metabolism to promote the invasion of pancreatic cancer[7, 8]. In addition, MUC13 can promote the progression of intrahepatic cholangiocarcinoma through the EGFR/PI3K/AKT pathway [9], distinguish intraductal papillary mucinous tumors from non-mucinous cysts, and is associated with high-risk lesions [10]. Studies have also shown that IL-6 increases the expression of MUC13 by activating the JAK2/STAT5 signaling pathway and promotes the progression of colon cancer [11]. Reducing miR-132-3p may promote the spread of gastric cancer by targeting MUC13 [12]. Transcription of MUC13 can enhance the proliferation of glioblastoma stem cells [13]. MUC13 is also abnormally expressed in ovarian cancer and changes the cellular characteristics of SKOV-3 cells [14]. MUC13 is often elevated not only in various malignant tumors but also in certain benign pathologies, so it seems to be a non-specific disease biomarkers. Nevertheless, MUC13 is significantly elevated in some cancer patients. In this case, its relationship with tumor progression deserves further study [15]. In addition, MUC family proteins are closely related to the process of O-glycans which exists in a variety of tumors and is of great significance in stimulating tumor cell proliferation and invasion as well as mediating cell adhesion [16–18]. However, the potential function, prognosis and therapeutic significance of MUC13 in esophageal cancer have not been determined. In this study, we researched the biological and clinical significance of MUC13 in esophageal cancer and its carcinogenic molecular mechanism, providing a new perspective for esophageal cancer treatment.

Methods

Differently Expression Genes (DEGs) analysis

The cancer public database (Oncomine) was applied to download the original data of sequencing or expression profiling chip and analyzed the DEGs in the cancer tissue and the adjacent non-tumor tissue group. After that, the survival prognostic significance of DEGs in cancer was determined by the Kaplan-Meier plotter, and then Oncomine database was used to confirm the differential expression of DEGs between tumor tissues and normal tissues for the next step in research. In addition, a molecular signaling

pathway network regulation map was constructed through using the genes co-expressed with differentially expressed genes in big data, and the related function enrichment map was drawn.

Cell Lines and Cell Culture

The human esophageal cancer cell lines (EC9706, TE-1 and ECA109) and the human esophageal epithelial cell line (HEEC) were purchased from the Shanghai Cell Bank Collection (Shanghai, China), and cultured in DMEM supplemented with 10% fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials, China) and maintained in a incubator at 37°C, 5% CO_2.

Immunohistochemistry (IHC)

The paraffin sections of esophageal cancer and adjacent non-tumor tissues (HEsoS030PG03, Shanghai Outdo Biotech Co., Ltd.) were deparaffinized with xylene and hydrated with absolute ethanol, 95% ethanol, 80% ethanol, 70% ethanol and distilled water, respectly. After 30min microwave heat recovery by citric acid antigen retrieval method, the sections were cooled to room temperature and washed with PBS (0.01M, pH 7.4), and then blocked with 2% BSA blocking solution at room temperature for 2 hours. After blocking, the sections were incubated with the primary MUC13 (1: 50, Santa Cruz, USA) antibody for 2 h at 37°C. Then rinsed three times in PBS, followed by incubation of secondary antibody (D110087,Sangon Biotech Co., Ltd) at 37°C for 2 h. DAB substrate kit was used to perform the chromogenic reaction. During which slices were observed under the microscope to confirm the color end point. Then, the nucleus is counter-stained with hematoxylin for 3 minutes. The slides were dehydrated in a series of graded ethanol solutions and transparented with the xylene, and then mounted with a neutral gum. Finally, semi-quantitative analysis of immunohistochemical results was conducted by Image ProPlus software, and positive cell percentages were calculated using IHC plug-in after color deconvulutionfor H&E DAB.

RNA Extraction and Quantitative Real-time PCR

Total RNA was extracted from the cells by using TRIzol® Plus RNA Purification Kit (Invitrogen, USA) and RNase-Free DNase Set (Qiagen, China) in accordance with the manufacturer's protocol. GAPDH cDNA was used as an internal control for quantification. The following primers were used for qRT-PCR amplification: MUC13 (NM_033049.4) expression were

5'- TCAAGTGTCCTGATGCCTGC-3' (forward)

and 5'- GCTCCCTTCTGCTCCAAGAT-3' (reverse);

Human GAPDH (BC059110) expression were

5'- AGAAGGCTGGGGCTCATTTG-3' (forward)

and 5'- AGGGGCCATCCACAGTCTTC-3' (reverse);

MUC1 (NM_001018016.3) expression were

5’- TGCCGCCGAAAGAACTAC-3’ (forward)

and 5’- TGCCGCCGAAAGAACTAC-3’ (reverse);

MUC4 (NM_001322468.1) expression were

5’- CAGGGACGACGGGACTTA-3’ (forward)

and 5’- ACAGGGCACAGAGGTAGGG-3’ (reverse);

MUC12 (NM_001164462.2) expression were

5’- GAACGAAGTCGCAAATGA-3’ (forward)

and 5’- TGGGATAGGCTGAATAAGAT-3’ (reverse);

MUC3A (NM_005960.2) expression were

5’- TTTCGAGGACGACGGAACAG-3’ (forward)

and 5’- TCACACTGAGGACGAGGTCA-3’ (reverse);

GALNT14 (NM_001253826.2) expression were

5’- CCCCAGTGGTTCTTGTCC-3’ (forward)

and 5’- GGATGTCTGAGGTGGTTGC-3’ (reverse).

The conditions of 95°C for 60 s, 40 cycles of 95°C for 15 s, 63°C for 25 s were set for performing PCR.

Silencing of MUC13

Three small interfering RNA (siRNA) target sequence for MUC13 gene were designed as following:

5’- GGCAACUCAGCUGAUGCUGUATT-3’ (forward)

and 5’-UACAGCAUCAGCUGAGUUGCCTT-3’ (reverse);

5’- CCUGUGCAGAUAAUUCGUUAUTT-3’ (forward)

and 5’-AUAACGAAUUAUCUGCACAGGTT-3’ (reverse);

5’- CGACUGUAAGGACAAAUUUCATT-3’ (forward)

and 5’-UGAAAUUUGUCCUUACAGUCGTT-3’ (reverse).

The siRNA constructs were synthesized and cloned into GV115 plasmid vector. The MUC13-siRNA plasmid was then transfected into EC9706 and Eca109 cells with lentivirus following the manufacturer’s

protocol.

Western blot

The proteins were extracted from the cells harvested 72 hours after transfection using the total protein extraction kit (Thermo Pierce, USA) and Halt Protease and Phosphatase Inhibitor Cocktail kit (Thermo Pierce, USA), and then the BCA Protein Assay Kit (Biyuntian, Germany) was utilized to measure the consistency of the total proteins. After separating by SDS-PAGE gels, proteins were transferred onto a PVDF membrane (Millipore, USA) which was soaked in methanol for 20 sec in advance. The membrane was blocked with 5% BSA at room temperature for 1 h. The primary MUC13, MUC1, MUC4 and GALNT14 antibodies (1:1000, Abcam, UK), GAPDH antibody (1:10000, Abcam, UK), MUC12 antibody (1:500, Santa Cruz, USA) as well as the primary MUC3A antibody (1:200, Santa Cruz, USA) were incubated overnight at 4°C. After washingd with PBS for 5 min, the membrane was incubated at 37°C for 1 h with secondary antibody (1:5000, Thermo Pierce, USA). The ECL working solution was prepared following SuperSignal® West Dura Extended Duration Substrate kit instructions and used to develop the transfer film. Finally, the optical density values of the bands were analyzed and the GAPDH was adopted as control.

CCK8 assay

The amber dehydrogenase in living cells, especially in the mitochondria of proliferating cells, can reduce WST-8 which containing in Cell Counting Kit-8 (CCK8) reagent to yellow formazan. The cell proliferation could be evaluated by detecting the absorption value at 450nm wavelength. In brief, after siRNA transfection of EC9706 and ECA109 cells for 72 h, 100μL of cell suspension was added and incubated for 24 h at 37°C. Then, 10 μL CCK8 was supplemented and incubated for 2 h. At last, the absorbance was gauged at 450 nm on days 1, 2, 3, 4 and 5 respectively.

Clone formation assay

Clone formation is one of the effective methods for measuring cell proliferation ability. The cells in the logarithmic growth phasewere inoculated in a 6-well plate at a density of 500 cells/well after transfection and continued to culture until the number of cells in most single clones is greater than 50. After the culture is terminated, the cells were fixed with 1 mL of 4% paraformaldehyde for 30 min and stained with 1000 μL of crystal violet staining solution for 20 min. After cells were washed with deionized distilled water (ddH_2O) for 3 times and images were captured with a digital camera.

Cell cycle analysis

Cells were harvested with trypsin when they have grown to a coverage rate of about 80%. After centrifuging at 1300 rpm for 5 min, the cells were washed by D-Hanks (pH 7.4) pre-cooled at 4°C and fixed in 75% ethanol pre-cooled at 4°C for 2 h. Then, the cells were washed with D-Hanks once again after discarding ethanol. Finally, cells were stained by working solution (PI: RNase stock solution : D-Hanks = 25: 10: 1000) for 30 min in the dark. The cells solution was collected for cell-cycle analysis by flow cytometry (Millipore, USA).

Apoptosis analysis

Cells were harvested with trypsin after transfection and centrifuged at 300 rpm for 5 min, and resuspended in 200 μL 1×binding buffer containing 2 μL Annexin V-FITC and 7AAD (PI) for 30 min at room temperature. According to the amount of cells, 200-300 μL 1×PBS was added to resuspend the cells for apoptosis analysis by flow cytometry (Millipore, USA).

Xenograft studies

All the animal experiments were carried out in strict accordance with the principles and procedures approved by the Committee on the Ethics of Animal Experiments of First People's Hospital of Xuzhou. 4 weeks old female BALB/c nude mice (Shanghai Lingchang Biotechnology Co. Ltd) were selected to xenograft studies. First, 7×10^6/200μL EC9706 cells transfected with siMUC13 or siCtrl were injected subcutaneously into the right hindlimbs of mice. We used the formula: $\pi/6 \times (\text{length} \times \text{width}^2)$ to calculate tumor size every 2 days and weighed the body weight of the mice (g). At end of experiment, the mice were euthanized with 2% sodium pentobarbital.

Statistical analysis

All experiments were repeated three times independently and two-tailed unpaired Student t test was applied to analyze data. $P<0.05$ was taken as statistically significant.

Results

MUC13 is one hub gene of esophageal cancer

To explore new targets for esophageal cancer, the cancer public database (Oncomine) was applied to confirm the DEGs (Figure 1A). It is well known that MUC family proteins are closely related to the process of O-glycans and the occurrence and development of tumors [16–18]. So we selected MUC13 as one hub gene. At present, although there are evidences that MUC13 can be used as biomarkers of esophageal cancer, their functions and mechanisms in depth have not been studied yet [19, 20]. Next, the differential expression of MUC13 between tumor tissues and normal tissues (Figure 1B) and the survival prognostic significance of MUC13 (Figure 1C) was determined by Oncomine database and the Kaplan-Meier plotter, respectively. After that, it found that MUC13 and its interacting multiple proteins such as GLANT14, MUC3A, MUC1, MUC12, and MUC4 are closely related to the O-glycan process by PPI protein interaction network analysis (Figure 1D) and functional enrichment analysis (Figure 1E).

Overexpression of MUC13 in esophageal cancer tissues and cell lines

The expression level of MUC13 in esophageal cancer tissues and adjacent non-tumor specimens was measured by IHC. As is shown in Figure 2A and 2B, MUC13 is significantly overexpressed in esophageal tumor samples, but is low expressed in adjacent non-tumor tissues. There appears significant difference in MUC13 positive cell percentage between esophageal cancer tissues and adjacent non-tumor

specimens (Figure 2C, n = 30, $P = 0.007$). *In vitro* experiments, the expression levels of MUC13 in EC9706, TE-1 and Eca109 cell lines were analyzed by qRT-PCR assay. As is shown in Figure 2D, high expression of MUC13 mRNA level was detected in EC9706, ECA109 and TE-1 cell lines, especially in EC9706 and ECA109 cell lines, but low expression of MUC13 mRNA level was confirmed in TE-1 cell line. We selected EC9706 and ECA109 cell lines to perform subsequent experiments. In all, these findings indicate that MUC13 have an overly expression in esophageal cancer.

Silencing of MUC13 by infection of lentivirus-mediated siRNA in esophageal cancer cells

MUC13 silence was obtained by transfection with MUC13-specific siRNA plasmids. The qRT-PCR and Western blot were taken to examine the transfection efficiency, respectively. Compared with the negative control siRNA (siCtrl), the mRNA and protein expression levels of MUC13 were both significantly decreased in EC9706 ($P < 0.001$) and ECA109 ($P < 0.001$) cells transfected with MUC13-specific siRNA (siMUC13) (Figure 3). In brief, silencing of MUC13 was effectively achieved by infection of lentiviral-mediated siRNA in esophageal cancer cells.

Silencing of MUC13 inhibited proliferation and promoted apoptosis in esophageal cancer cells in vitro

CCK8 assay, clone formation assay and cell cycle analysis were used to observe the effects of MUC13 silencing on proliferation of EC9706 and ECA109 cells. As showed in Figure 4A and 4C, compared with siCtrl group, the ability of cell proliferation of siMUC13 group was distinctly declined. Moreover, the cellular clone formation rate of silencing of MUC13 cells was remarkably restrained ($P < 0.001$) in comparison with siCtrl group (Figure 4B and 4D). Next, as illustrated in Figures 4E and 4F, compared with siCtrl group, the more cells were present in the G1 phase in siMUC13 group, a result that implied that silencing of MUC13 blocked cell-cycle progression in the EC9706 ($P < 0.01$) and ECA109 ($P < 0.01$) cells. Finally, apoptosis rate in siCtrl group or siMUC13 group was detected with Annexin V-FITC/PI staining by flow cytometry. The results showed that the apoptotic proportion was significantly higher in siMUC13 group than in siCtrl group in the EC9706 ($P < 0.001$) and ECA109 ($P < 0.001$) cells (Figure 4F). Overall, it is indicated that silencing of MUC13 could decrease cell proliferation, clone formation, promote cell cycle arrest and induce cell apoptosis.

Silencing of MUC13 suppressed tumor growth in vivo

To observe whether silencing of MUC13 reduce tumorigenicity of esophageal cancer *in vivo*, EC9706 cells transfected with siMUC13 or siCtrl were subcutaneously inoculated into nude mice. As showed in Figure 5A, 5B and 5C, silencing of MUC13 distinctly decreased the tumor volume and the tumor weight, respectly. Therefore, the results proved that MUC13 knockdown restrains tumorigenicity of esophageal cancer.

Molecular mechanism analysis

Western blot and qRT-PCR were employed to verify the expression level of 5 proteins (GLANT14, MUC3A, MUC1, MUC12 and MUC4) that interact with MUC13 in EC9706 cell. As showed in Figure 6A and 6B, compare to siCtrl group, GLANT14, MUC3A, MUC1, MUC12 and MUC4 were significantly up-regulated in both protein and mRNA level in siMUC13 group. In summary, these data indicate correlation between the pro-proliferation and anti-apoptotic effects of MUC13 with the expression of GLANT14, MUC3A, MUC1, MUC12 and MUC4 in esophageal cancer.

Discussion

The prognosis of esophageal cancer is poor and the incidence has increased in recent years, so it is urgent to find new therapeutic targets for patients with esophageal cancer. Recent research on esophageal cancer has focused on seeking to improve the detection and prediction methods necessary before treatment and emerging multimodality treatment methods [1, 2, 21–23]. Although many studies have demonstrated that MUC13 is abnormally presented in a variety of malignant tumors[6–15], its function and mechanism in the invasiveness and malignant progression of esophageal cancer have not been studied in depth.

As we all know, O-glycan process is closely related to tumor development. Abnormal glycan structures are widely present in a variety of tumors, and are of great significance in stimulating tumor cell proliferation and invasion and mediating cell adhesion. It is worth noting that MUC family proteins are closely related to the O-glycan process [16–18]. In this study, it was found that the MUC family protein MUC13 gene is the most fourth gene based on differential expression level in the public cancer database (Oncomine), but there are few reports on the research of MUC13 in esophageal cancer. It also found that MUC13 and its interacting multiple proteins such as GLANT14, MUC3A, MUC1, MUC12 and MUC4 are closely related to the O-glycan process by PPI protein interaction network analysis and functional enrichment analysis. So we guess that MUC13 can affect the development of esophageal cancer by regulating the O-glycan process, and it is hoped to become a potential therapeutic target of esophageal cancer with certain clinical value.

In this study, we found that MUC13 is highly expressed in some human esophageal cancer tissues compared with adjacent non-tumor tissues (Figure 2A and 2B). This may be due to the high expression of MUC13 is related to specific types of esophageal cancer, and requires to be further identified. We also found that MUC13 is highly expressed in EC9706 and ECA109 and TE-1 cells and the basal expression level of MUC13 was relatively high, especially in EC9706 and ECA109 cells, while the expression in HEEC cell line was low (Figure 2C). According to recent studies, MUC13 is overexpressed in gastric cancer cells and liver cancer cells, making it a promising target for cancer therapeutic intervention [12, 24]. Therefore, it is speculated that the characteristics of MUC13 related to cancer can be used for the diagnosis and prognosis prediction of esophageal cancer. However, the relevance between the utterance level of MUC13 and the clinical classification of patients with esophageal cancer needs further study.

Subsequently, we showed that in vitro MUC13 knockdown resulted in a significant of anti-proliferation and pro-apoptosis in EC907 and ECA109 cells. *In vivo* assays showed that the tumor volume and weight of the siMUC13 group were significantly reduced. These results proved the tumorigenic effect of MUC13 in esophageal cancer. Another study reported that MUC13 knockdown significantly weakened the potential for cell migration and invasion [6, 12], indicating that MUC13 acts as a governing role in maintaining the aggressiveness of cancer. Therefore, the invasion and metastasis of MUC13 in esophageal cancer remain to be further studied.

In previous studies, it was found that MUC13 is abnormally expressed in a variety of malignant tumors [6–15], but its function and mechanism in the invasiveness and malignant progression of esophageal cancer have not been studied in depth. Therefore, it may provide extremely important clues for finding new targets for the treatment of esophageal cancer by studying the underlying mechanism of MUC13 in development of esophageal cancer. O-glycans are oligosaccharides attached to serine/threonine residues in protein peptide chains and the side chain hydroxyl groups of other amino acid residues. Most of these oligosaccharides are short and present different structures. In this study, we confirmed that GLANT14, MUC3A, MUC1, MUC12 and MUC4 regulatory factors which related to the O-glycan process were overexpressed and down-regulated with MUC13 knockdown in esophageal cancer cells, resulting in insufficient tumor growth and proliferation. A large number of studies have also shown that these molecules are involved in the regulation of a variety of malignant tumors, such as, MUC4 can be used as a new tumor antigen in pancreatic cancer immunotherapy [28], and MUC12 promotes renal cell carcinoma through the c-Jun/TGF-βsignaling pathway [29], MUC1 confers radioresistance in head and neck squamous cell carcinoma cells [30].But the regulatory network between MUC13 and these molecules needs to be further explored. Abnormal glycan structures are widely present in a variety of tumors and be of great significance in stimulating tumor cell proliferation and invasion and mediating cell adhesion [25–27]. Therefore, it proves that MUC13 as an important molecule regulates the O-glycan process could affect the process and development of esophageal cancer in this study. In all, this study lays a foundation for elucidating that MUC13 affects the proliferation and apoptosis of esophageal cancer and indicates MUC13 may be as a latent therapeutic target for esophageal cancer and has certain clinical value.

Although our study investigated the effect and the potential mechanism of MUC13 on the proliferation and apoptosis of them, the role of MUC13 on the migration and invasion of esophageal cancer cells has not been further studied. Secondly, the expression level of MUC13 has not been detected in a large number of clinical specimens. Therefore, it is necessary to conduct large-scale clinical studies to determine its value in esophageal cancer. Finally, although we have made a preliminary discussion on the mechanism of MUC13 regulation on the proliferation and apoptosis of esophageal cancer cells, further regulation mechanisms have not been studied in depth, and we only selected two strain of esophageal cancer cells for research, which does not have extensive applicability, we need to expand our investigation in the follow-up work.

Conclusion

This study proved that MUC13 is an important molecule that regulates the O-glycan process and then affects the progress of esophageal cancer. MUC13 may be a novel therapeutic target for patients with esophageal cancer.

Abbreviations

MUC13	Mucin 13
IHC	Immunohistochemistry
HEEC	Human esophageal epithelial cell line
siRNA	Small interfering RNA
CCK8	Cell Counting Kit-8

Declarations

Authors' contributions

CH1 raised the topic and designed experiments; CH2 controlled the direction of the study; HY conducted the experiment and data analysis; LS drafted the manuscript; ZG, XX, ZH, LZ, and WC participated in the experiment; LY and FK collected data.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Protocols for experiments involving animals were approved by Committee on the Ethics of Animal Experiments of First People's Hospital of Xuzhou.

Consent for publication

Not Applicable.

Competing interests

All authors report no conflicts of interest.

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Figures

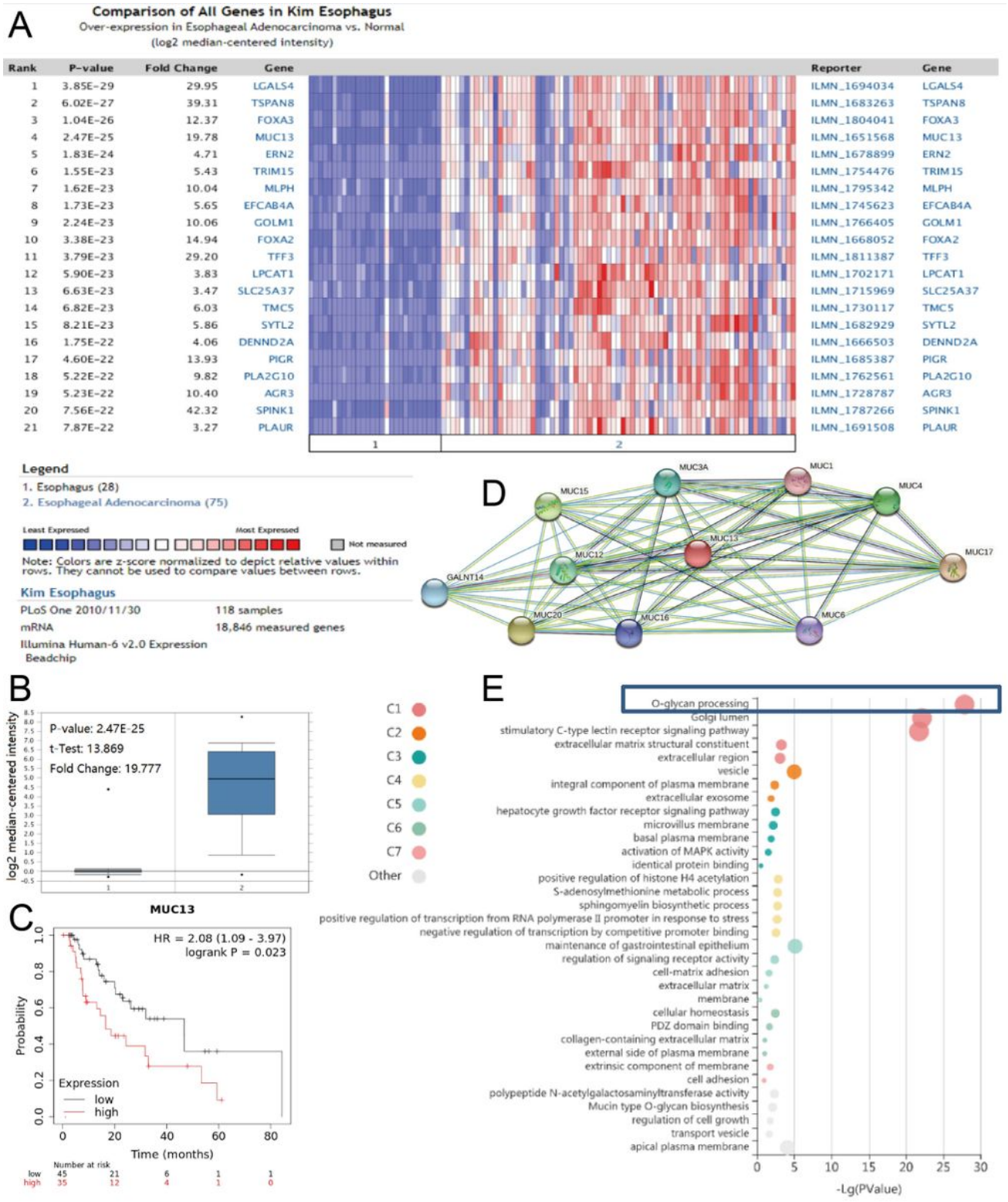

Figure 1

MUC13 is one hub gene of esophageal cancer. (A) The differentially expressed genes (DEGs) in the cancer tissue and the adjacent non-tumor tissue group were analyzed by the Oncomine database. (B) The differential expression of MUC13 between tumor tissues and normal tissues was determined by Oncomine database. (C) The survival prognostic significance of MUC13 was determined by the Kaplan-Meier plotter. (D) PPI protein interaction network analysis. (E) Functional enrichment analysis.

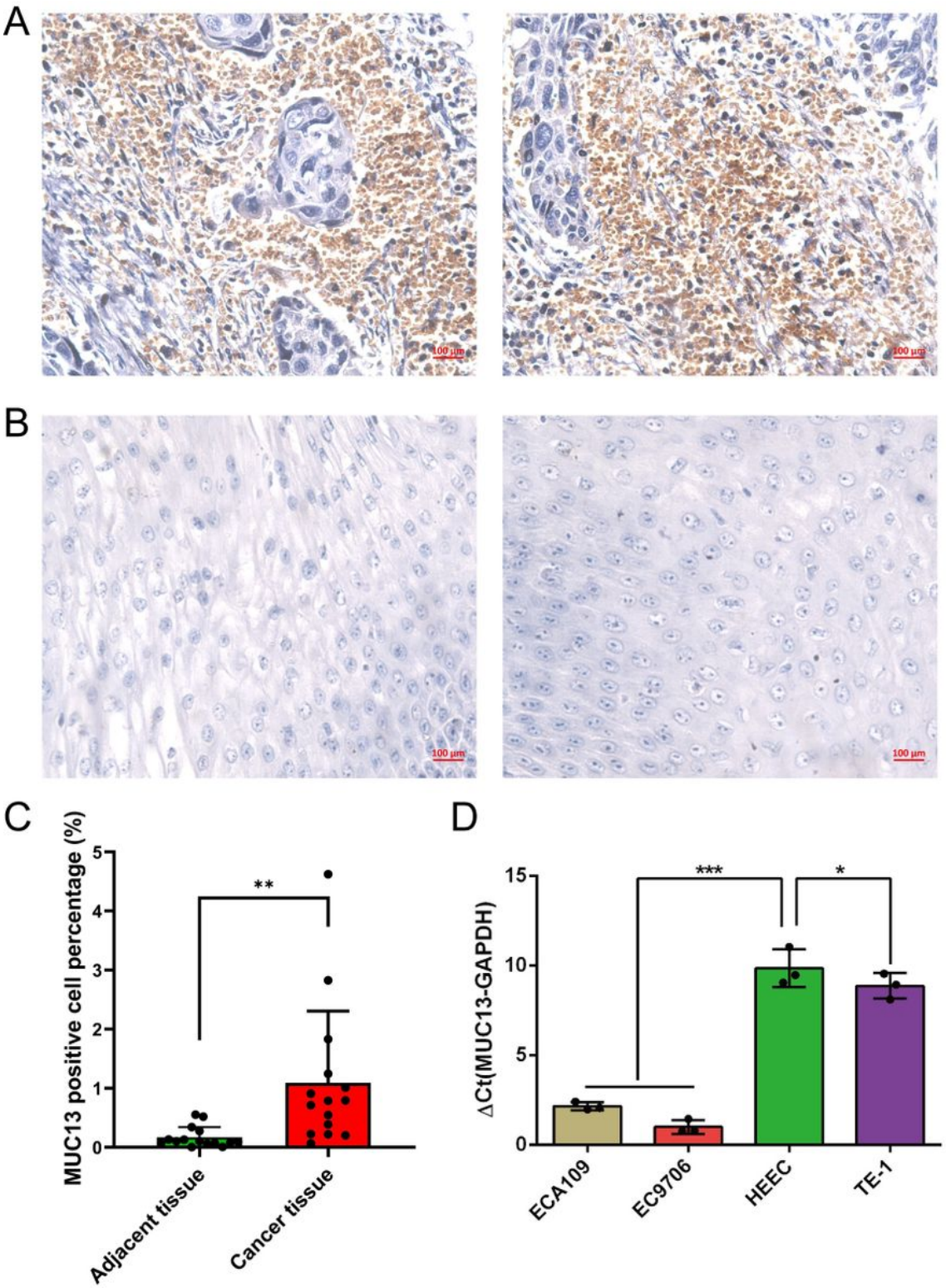

Figure 2

MUC13 expressions in esophageal cancer cell lines. (A) Representative images of immunohistochemical staining for MUC13 in esophageal tumor tissues: strong expression. (B) Representative images of immunohistochemical staining for MUC13 in adjacent non-tumor tissues: absent expression. (C) MUC13 positive cell percentage of Esophageal tumor tissues vs Adjacent non-tumor tissues, n = 30, P = 0.007.

(D) High expression of MUC13 mRNA level in ECA109 and EC9706 cell lines. Data were expressed as the mean ± s.d., *P < 0.05, ***P < 0.001.

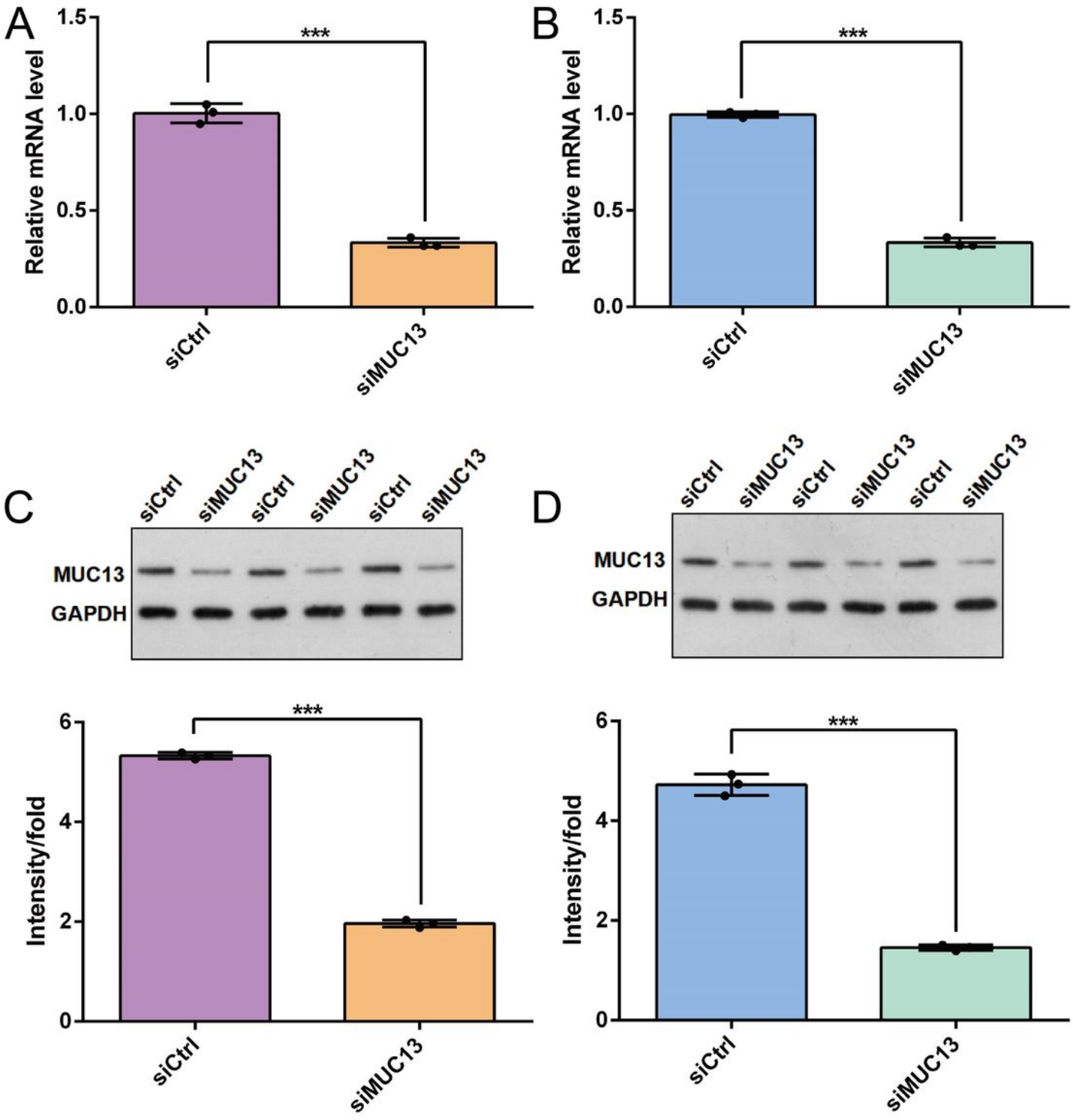

Figure 3

The knockdown efficiency of MUC13 by infection of lentivirus-mediated siRNA and the negative control siRNA in (A) EC9706 cells and (B) ECA109 cells were verified by qRT-PCR. Data were expressed as the mean ± s.d., ***P < 0.001. The knockdown efficiency of MUC13 by infection of lentivirus-mediated siRNA

and the negative control siRNA in (C) EC9706 cells and (D) ECA109 cells were verified by Western blot. Data were expressed as the mean ± s.d., ***P < 0.001.

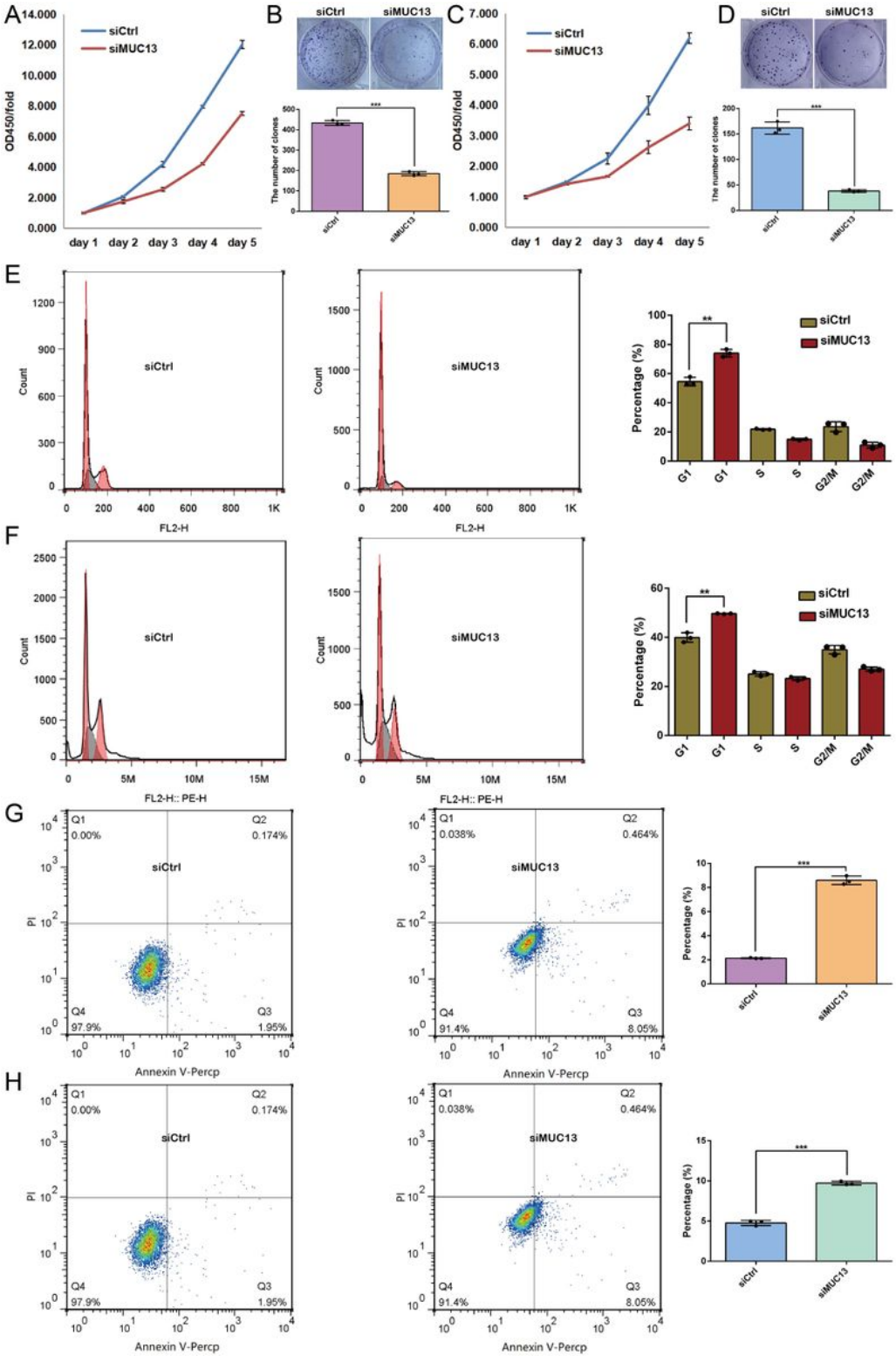

Figure 4

MUC13 knockdown suppressed esophageal cancer cells proliferation and promoted esophageal cancer cells apoptosis. Proliferation of EC907 (A) and ECA109 (C) cells was significantly inhibited in the absence of MUC13. Data were expressed as the mean ± s.d.. The ability of forming colonies of MUC13 knockdown

cells was significantly restrained compared with control cells in EC907 (B) and ECA109 (D) cell lines. Data were expressed as the mean ± s.d., ***P < 0.001. The cycle of EC907 (E) and ECA109 (F) cells was significantly arrested in G0/G1 phase in the absence of MUC13. Data were expressed as the mean ± s.d., **P < 0.01. The proportion of apoptotic cells was significantly increased in MUC13 knockdown group compared with control cells in EC907 (G) and ECA109 (H) cell lines assessed by flow cytometry. Data were expressed as the mean ± s.d., ***P < 0.001.

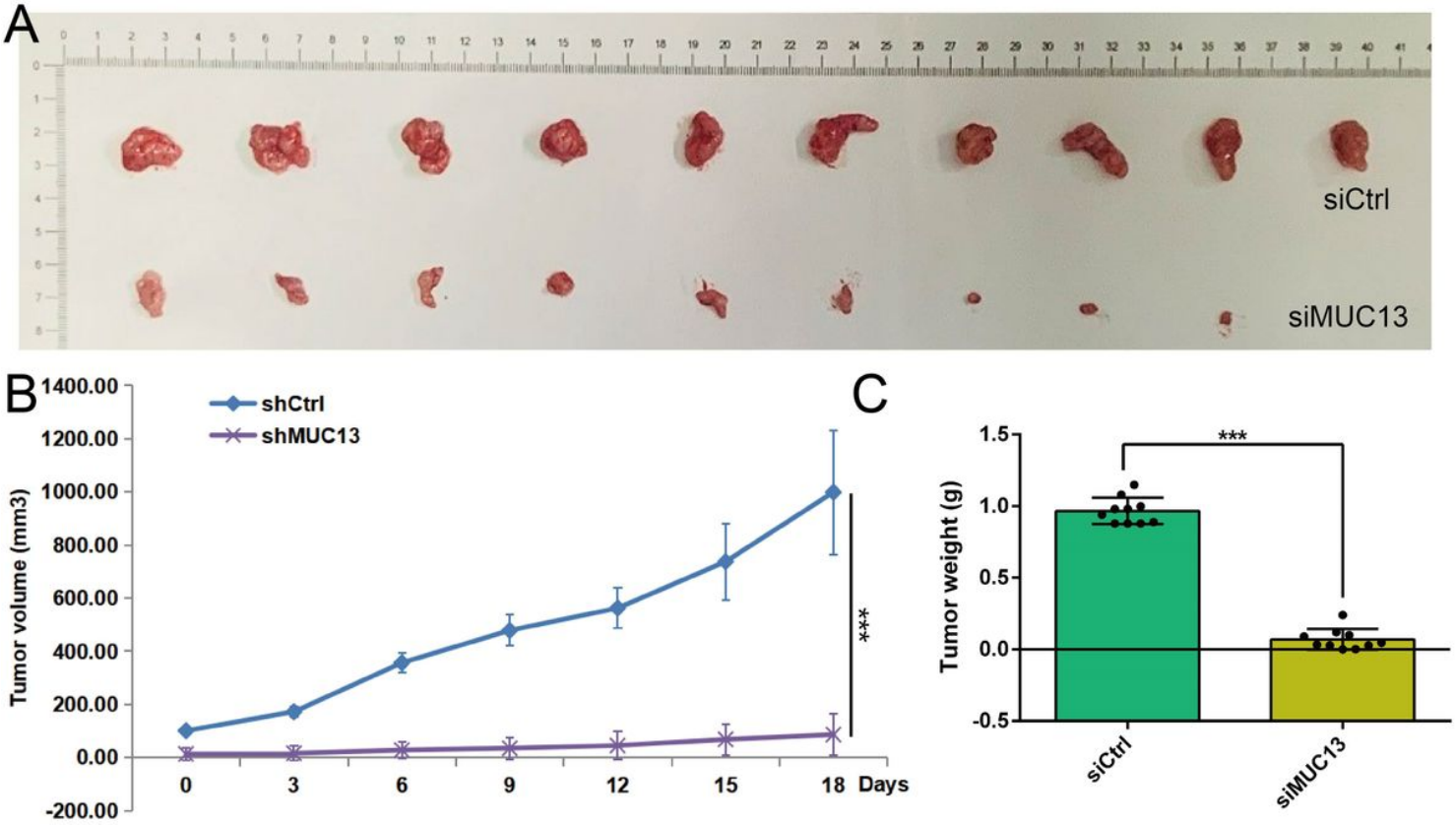

Figure 5

Silencing of MUC13 suppressed tumor growth in vivo. (A and B) The tumor volume was obviously decreased in MUC13 knockdown group. Data were expressed as the mean ± s.d., ***P < 0.001. (C) The tumor weight was significantly declined in MUC13 knockdown group. Data were expressed as the mean ± s.d., ***P < 0.001.

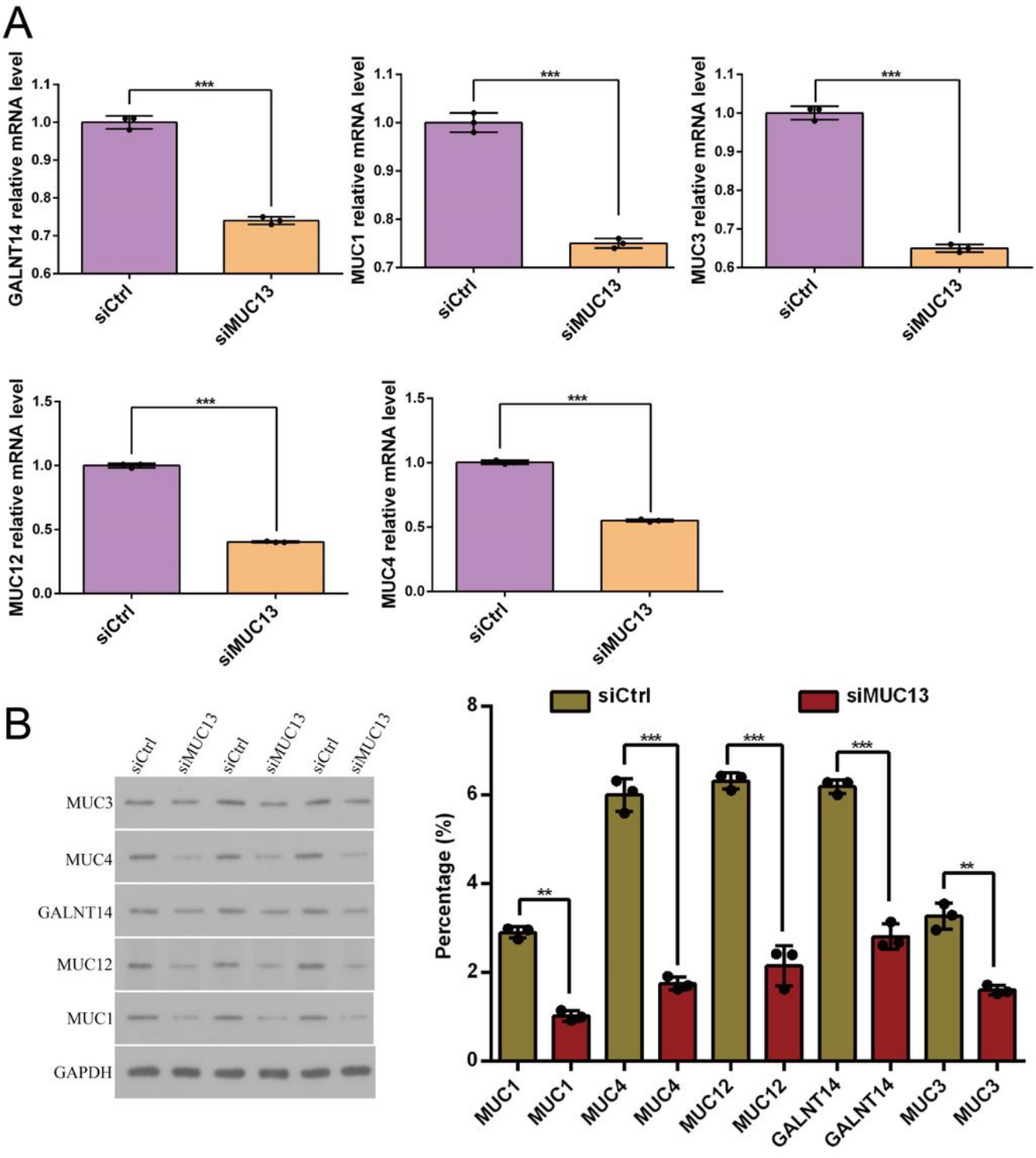

Figure 6

Verification the expression of 5 proteins that interact with MUC13 in EC9706 cell. (A) GLANT14, MUC3A, MUC1, MUC12 and MUC4 were significantly up-regulated in mRNA level. Data were expressed as the mean ± s.d., ***P < 0.001. (B) GLANT14, MUC3A, MUC1, MUC12 and MUC4 were significantly up-regulated in protein level. Data were expressed as the mean ± s.d., **P < 0.01 and ***P < 0.001.