

# ESM1 May Be Used as a New Indicator for the Diagnosis and Prognosis of Early and Advanced Stage Digestive Tract Cancers

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**Background:** The biological function and prognostic significance of endothelial cell specific molecule 1 (ESM1) in various cancers have been validated. This study aimed to explore the expression and clinical diagnosis values in patients with stomach adenocarcinoma (STAD) and esophageal carcinoma (ESCA).

**Methods:** Online database Gene Expression Omnibus was used to screen for abnormally expressed genes in STAD and ESCA. Besides, 36 STAD and 36 ESCA patients were enrolled, and their corresponding control groups were also 36 people each. Reverse transcription-quantitative polymerase chain reaction and Western blot were performed to analyze the expression of ESM1. Overall survival (OS) curve and receiver operating characteristics curve (ROC) analysis were used to assess the prognosis, and the sensitivity and specificity of ESM1 for the diagnosis of STAD and ESCA, respectively. Additionally, the effects of ESM1 on cell viability, migration, and invasion were analyzed by cell counting kit-8, transwell migration and invasion assays.

**Results:** The results showed that the poor OS of STAD and ESCA patients was correlated with high ESM1. Besides, ESM1 was increased in ESCA and STAD in in vivo and in vitro studies. ESM1 has a high accuracy [area under the curve (AUC) > 0.79] at stage I and IV of STAD and ESCA. Knockdown of ESM1 suppressed the cell viability, migration, and invasion and increased the apoptosis rate of AGS and TE1 cells.

**Conclusion:** Our study suggested that ESM1 might be used as a new indicator for the diagnosis and prognosis of early and advanced stage digestive tract cancers.

**Keywords:** STAD, ESCA, ESM1, ROC, diagnosis, prognosis

## Introduction

Digestive malignancies refer to a group of different types of cancer that occur in the gastrointestinal tract and related organs.<sup>1</sup> According to a previous study, half of the top ten most common cancers and cancer-related causes of death are digestive tract cancers.<sup>2</sup> Stomach adenocarcinoma (STAD) and esophageal carcinoma (ESCA) are two cancers of the digestive tract with a high incidence. STAD, which is the third leading cause of cancer-related death, is a multifactorial disease that is closely related to dietary habit, genetics, and multiple pathogenic infections.<sup>3</sup> ESCA is the eighth most common cancer in the world with a lower 5-year survival, which is around 15–25%.<sup>4</sup> Regrettably, both STAD and ESCA suffer from a low rate of early-stage diagnosis, which poses a great threat to the prognosis and survival rate of patients. In addition, chemoradiotherapy, molecular targeted therapy and immunotherapy also bring great physical and mental pain and economic burden to STAD and ESCA patients with advanced stage. Therefore, the search for potential biomarkers is of great value for the diagnosis and prognosis of early and advanced stage digestive tract cancers.

Endothelial cell specific molecule 1 (ESM1), which is located on chromosome 5q11.2,<sup>5</sup> is a gene that encodes soluble secreted proteoglycans, also known as endorphins.<sup>6,7</sup> ESM1 is expressed in a variety of cells, including human vascular endothelial cells, liver cells, and bronchi and pulmonary submucosal gland cells.<sup>5</sup> ESM1 is involved in regulating

endothelial cell function, angiogenesis, inflammation, and other physiological and pathological processes.<sup>8,9</sup> In addition, increasing studies have confirmed the role of ESM1 in different cancers, including ovarian cancer,<sup>10</sup> bladder cancer,<sup>11</sup> and head and neck squamous cell carcinoma.<sup>12</sup> Besides, a recent study indicates that ESM1 promotes the occurrence and development of ESCA, and promotes the malignant behavior of esophageal cancer cells.<sup>13</sup> However, the effects of ESM1 on STAD have not been investigated.

In this study, we screened the abnormal gene, ESM1, in STAD and ESCA by microarray dataset analysis, and further explored the effects of ESM1 on overall survival (OS), cell viability, migration, and invasion through clinical and in vitro studies, which might provide new ideas for the diagnosis and prognosis of early and advanced stage STAD and ESCA.

## Methods and Materials

### Bioinformatics Analysis

We searched for STAD and ESCA datasets from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database and downloaded. R language was used to analyze the data, and the differentially expressed genes were defined as  $p < 0.05$  and  $|\log_2(\text{fold change})| > 1$ . Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment was conducted using DAVID database (<https://david.ncifcrf.gov/>) and plotted using the clusterProfiler package of the R software. In addition, the effects of ESM1 on the survival rate in STAD and ESCA were analyzed using the Kaplan–Meier plotter database.

### Human Study Design and Sample Collection

This study was approved by Beijing Aerospace General Hospital. The study included 36 patients diagnosed with STAD and 36 patients with acute simple gastritis (healthy group). In addition, 36 ESCA patients and 36 patients with esophagus inflammation (healthy group). Pregnant and lactating women were excluded in this study. All STAD and ESCA patients had complete clinical data and had not received radiotherapy or chemotherapy. Informed consent was obtained from all patients. The tissue specimens and corresponding adjacent ones from STAD and ESCA patients were collected and stored in liquid nitrogen for use. All tissue samples were verified by pathological examination. Besides, fasting blood samples were obtained from all study participants by venous cannula and divided into two parts, one of which was loaded into vials supplemented with anticoagulant and then mixed upside down. Then, the blood samples were centrifuged at 1000 rpm for 15 min to obtain plasma samples. The other part was loaded into vials supplemented without anticoagulant and placed at room temperature for 20 min for solidification. After that, the blood samples were centrifuged at 3000 rpm for 5 min to get serum samples. All the samples were stored at  $-80^{\circ}\text{C}$  for subsequent analysis. Basic information of all patients including age, sex, drinking status, smoking status, tumor grade, and American Joint Committee on Cancer (AJCC) stage was collected and shown in [Table 1](#).

### Cell Culture

Human gastric adenocarcinoma cells (AGS) and esophageal cancer cells (TE1) were obtained from Procell Life Science&Technology Co., Ltd. (Wuhan, China). TE1 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Corning, NY, USA) containing 10% heat-inactivated fetal bovine serum (FBS, Rebiosci Biotechnology Co., Ltd. Shanghai, China), and 1% penicillin/streptomycin (Rebiosci). Besides, AGS cells were maintained in the Ham's F-12 medium (Absin Biotechnology Co., Ltd. Shanghai, China) containing 10% FBS and 1% penicillin/streptomycin. All cells were incubated in a humidified incubator at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ .

### Cell Transfection

ESM1 short hairpin RNA (shESM1) and shRNA negative control (shNC) used in this study were synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China). For cell transfection, AGS and TE1 cells ( $2 \times 10^5$  cells/well) were inoculated in a 6-well plate (Corning). After the cell confluence reached 80%, transfection was performed using Lipofectamine 3000 (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The cells were transfected for 48 h, and then reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed to detect the expression of ESM1.

**Table 1** Clinicopathologic Characteristics of Study Subjects

Clinicopathologic Characteristics	Healthy (%) (n = 36)	STAD (%) (n = 36)	Healthy (%) (n = 36)	ESCA (%) (n = 36)
Age (years)				
< 65	21 (58.3)	13 (36.1)	20 (55.6)	14 (38.9)
≥ 65	15 (41.7)	23 (63.9)	16 (44.4)	22 (61.1)
Sex				
Male	19 (52.8)	22 (61.1)	20 (55.6)	23 (63.9)
Female	17 (47.2)	14 (38.9)	16 (44.4)	13 (36.1)
Smoking status				
Never	23 (63.9)	16 (44.4)	25 (69.4)	14 (38.9)
Ever	8 (22.2)	6 (16.7)	6 (16.7)	7 (19.4)
Current	5 (13.9)	14 (38.9)	5 (13.9)	15 (41.7)
Drinking status				
Never	13 (36.1)	4 (11.1)	10 (27.8)	3 (8.3)
Ever	18 (50.0)	21 (58.3)	19 (52.8)	20 (55.6)
Current	5 (13.9)	11 (30.6)	7 (19.4)	13 (36.1)
Tumor grade				
Well	–	13 (36.1)	–	16 (44.4)
Moderate	–	15 (41.7)	–	14 (38.9)
Poor	–	8 (22.2)	–	6 (16.7)
AJCC stage				
I	–	9 (25.0)	–	13 (36.1)
II	–	15 (41.7)	–	11 (30.6)
III	–	7 (19.4)	–	8 (22.2)
IV	–	5 (13.9)	–	4 (11.1)

**Abbreviations:** STAD, stomach adenocarcinoma; ESCA, esophageal carcinoma; AJCC, American Joint Committee on Cancer.

## RNA Extraction and RT-qPCR

TRIzol reagent (Senbeijia Biotechnology Co., Ltd, Nanjing, China) was applied to extract total RNA from tissues and cells. Then, RNA was reverse transcribed into cDNA using the Hifair<sup>®</sup> III 1st Strand cDNA Synthesis SuperMix for qPCR kit (Yeason Biotechnology, Shanghai, China), and the qPCR amplification experiment was performed using the Hieff<sup>®</sup> qPCR SYBR Green Master Mix kit (Yeason) with the reaction conditions: 95°C for 5 min, 40 cycles of 95°C for 10 sec, and 60°C for 30 sec, and a melt curve stage. Primers used in this study were synthesized by Genewiz Biotechnology Co., Ltd (Suzhou, China) and listed as follows: endothelial cell specific molecule 1 (ESM1), forward, 5'-AGCTGGAATTCCATGAAGAG-3' and reverse, 5'-TCTCTCAGAAGCTTAGCCG-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-GACTCATG ACCACAGTCCATGC-3' and reverse, 5'-AGAGGCAGGGATGATGTTCTG-3'. The gene expression was calculated by the  $2^{-\Delta\Delta CT}$  method. The expression levels of ESM1 were normalized against the levels of GAPDH.

## Western Blot

The tissue and cell samples were lysed using RIPA lysis buffer (Yeason) for 30 min. After that, the homogenate was homogenized at 4°C for 30 min and centrifuged at 12,000 rpm for 20 min. Then, the supernatant was taken and stored at –80°C for further analysis. The commercial Bicinchoninic Acid (BCA) kit (Yeason) was obtained to analyze the protein concentration. Next, proteins were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Beyotime Biotechnology, Shanghai, China). Then, 5% bovine serum albumin (BSA; Yeason) was used to reduce the non-specific binding of antibodies in membranes. After being blocked, the membranes were incubated with the primary antibodies [ESM1 (ab103590; Abcam, Cambridge, MA, USA; 1/1000) and GAPDH (ab9485; Abcam; 1/2500)] at 4°C overnight. Next, the goat-anti-rabbit secondary antibody (ab6721; Abcam; 1/2000) was incubated with the membrane at 37°C for 2 h after washing the membrane for

three times (10 min each) with Tris-buffered saline Tween (Beyotime). Finally, an enhanced chemiluminescence solution (Beyotime) was used to detect the protein signal.

### Cell Counting Kit-8 (CCK-8) Assay

Cell viability was detected by a commercial CCK-8 kit (Vazyme, Nanjing, China). Firstly, the cells were seeded into a 96-well plate at the density of  $1 \times 10^3$  cells/well and maintained in the incubator for 24 h. At the same time, the blank group and the control group were both set. Three replicate wells were set up. Then, 10  $\mu$ L of CCK-8 solution was added to each well to incubate with cells for 2 h. Finally, the absorbance was assessed at 450 nm using a microplate reader (Synergy HT, Bio-Tek, USA).

### Transwell Migration and Invasion Assays

The AGS and TE1 cells were resuspended in serum-free medium. For the migration assays, the cells ( $5 \times 10^4/100 \mu$ L) were seeded onto the chamber without Matrigel. For the invasion assays, the cells ( $5 \times 10^4/100 \mu$ L) were seeded onto the chamber with 100  $\mu$ L of Matrigel. The bottom chamber was prepared using 800  $\mu$ L of DMEM supplemented with 10% FBS as a chemoattractant. After 24 h of incubation, the cells on the outer surface were washed with PBS, fixed with 4% paraformaldehyde (Biosharp, Hefei, China) for 20 min, and stained in a dye solution containing 0.1% crystal violet (Yeason) for visualization.

### Flow Cytometry

The apoptosis rates of AGS and TE1 cell lines were assessed using Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) double staining solution using the Apoptosis Detection Kit (Vazyme). Transfected cells were digested with 0.25% trypsin (Absin) without ethylenediamine tetraacetic acid (EDTA) for 1 min and collected after centrifugation (1800 rpm, 4°C, 5 min). Then, the cells were washed twice with pre-cooled PBS (Procell) and re-suspended in 100  $\mu$ L of  $1 \times$  binding buffer. After that, 5  $\mu$ L of Annexin V-FITC and 5  $\mu$ L PI Staining Solution were added to incubate with cells for 10 min at room temperature in the dark. Subsequently, 400  $\mu$ L  $1 \times$  binding buffer was added to incubate with cells. Finally, the cells were immediately loaded onto the FACS CantoII flow cytometer (BD Biosciences, CA, USA), and the cell apoptosis rate was assessed by the BD FACSDiva software. Flow cytometry was conducted three times with three biological repetitions each time.

### Statistical Analysis

The SPSS 21.0 software was used to analyze the data. Data are expressed as mean  $\pm$  standard deviation (SD). Student's *t*-test was used for comparison between the two groups. One-way analysis of variance (ANOVA) was used for comparison among groups. Statistical analyses were performed using GraphPad Prism software (v8.0.1, GraphPad Software Inc., San Diego, CA, USA). Potential diagnostic value of ESM1 in STAD and ESCA were presented by receiver operating characteristics (ROC) curve analysis.  $p < 0.05$  indicates that the difference is statistically significant.

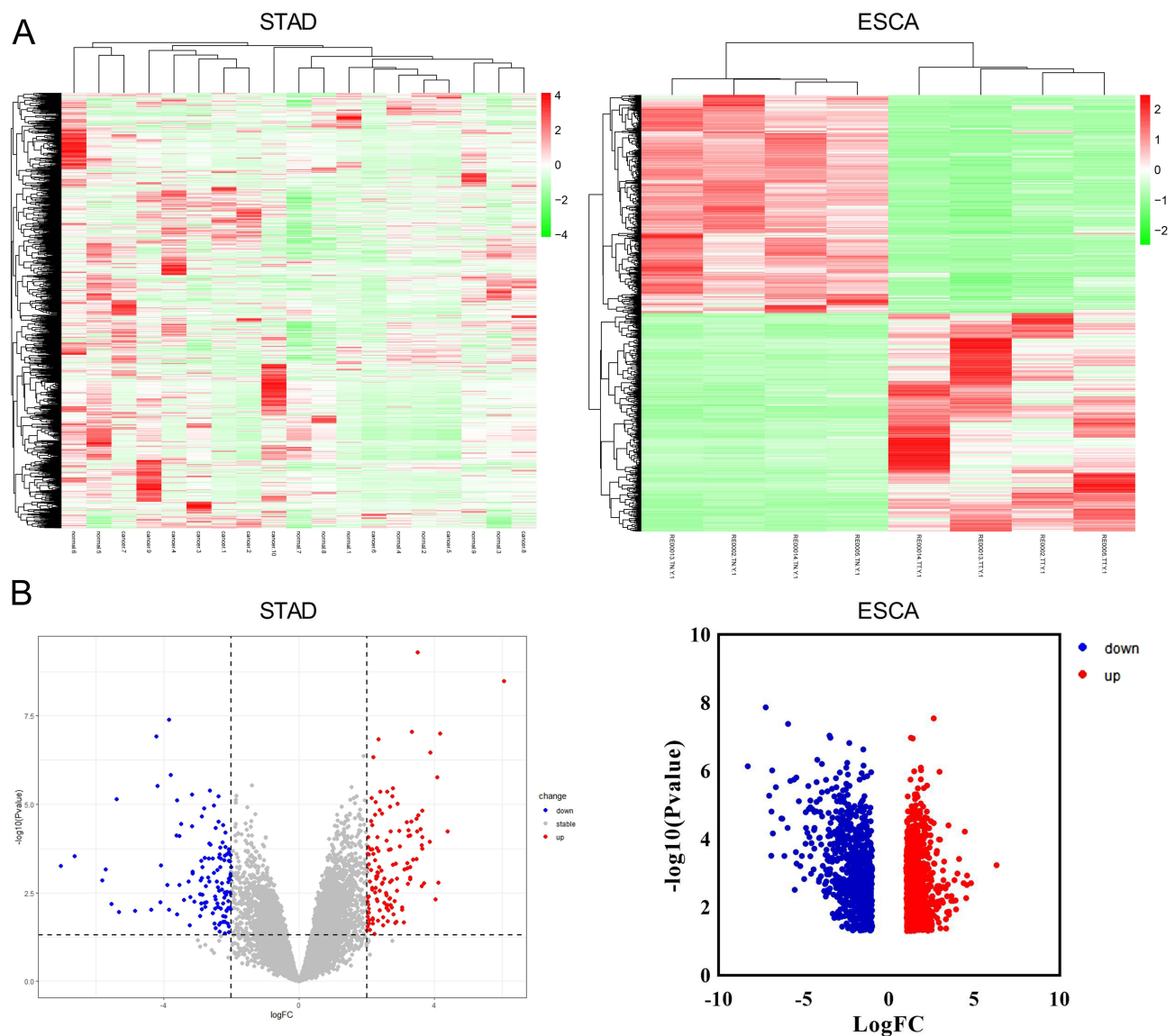
## Results

### Identification of Differentially Expression Genes (DEGs) in STAD and ESCA

The data were obtained from GEO database. The significant DEGs between the STAD or ESCA and normal samples were illustrated in the heatmap (Figure 1A) and volcanic map (Figure 1B).

### Functional Enrichment Analysis of Genes

From the microarray dataset, 1265 abnormally expressed genes in ESCA samples were screened and 93 abnormally expressed genes in STAD samples. Through the Venn diagram, we screened 40 genes that were abnormally expressed in both ESCA and STAD (Figure 2A). KEGG enrichment analysis was performed for the gene functional enrichment. The results indicated that the 40 abnormally expressed genes are mainly involved in rheumatoid arthritis, protein digestion and absorption, and cytokine–cytokine receptor interaction pathways (Figure 2B).



**Figure 1** Identification of DEGs in STAD and ESCA. (A) DEGs between the normal and STAD or ESCA tissues were shown using heatmap. Green and red represented upregulated and downregulated DEGs; (B) Upregulated (red), downregulated (blue), and non-significant genes (black) between the normal and STAD or ESCA tissues using volcano plot.

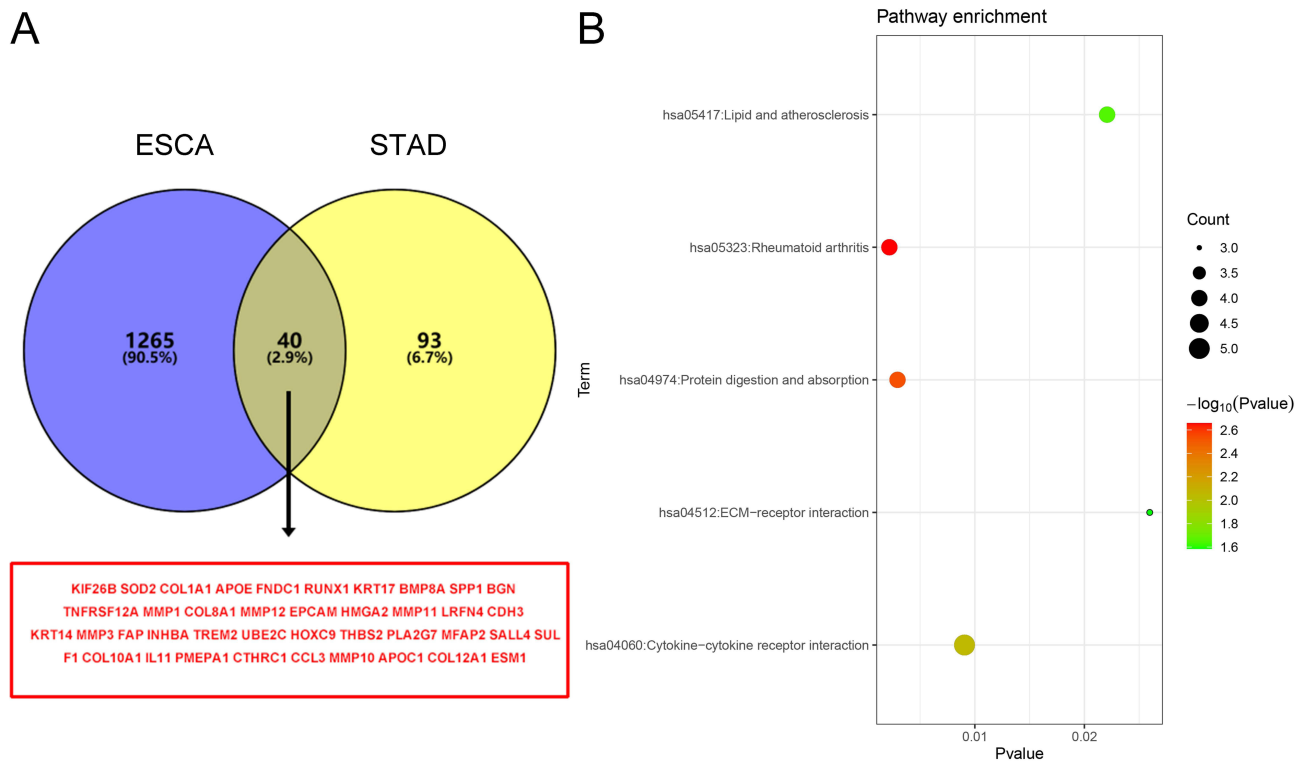
**Abbreviations:** STAD, stomach adenocarcinoma; ESCA, esophageal carcinoma; DEG, differential expression gene.

## High ESM1 Expression Predicted Poor OS in STAD and ESCA

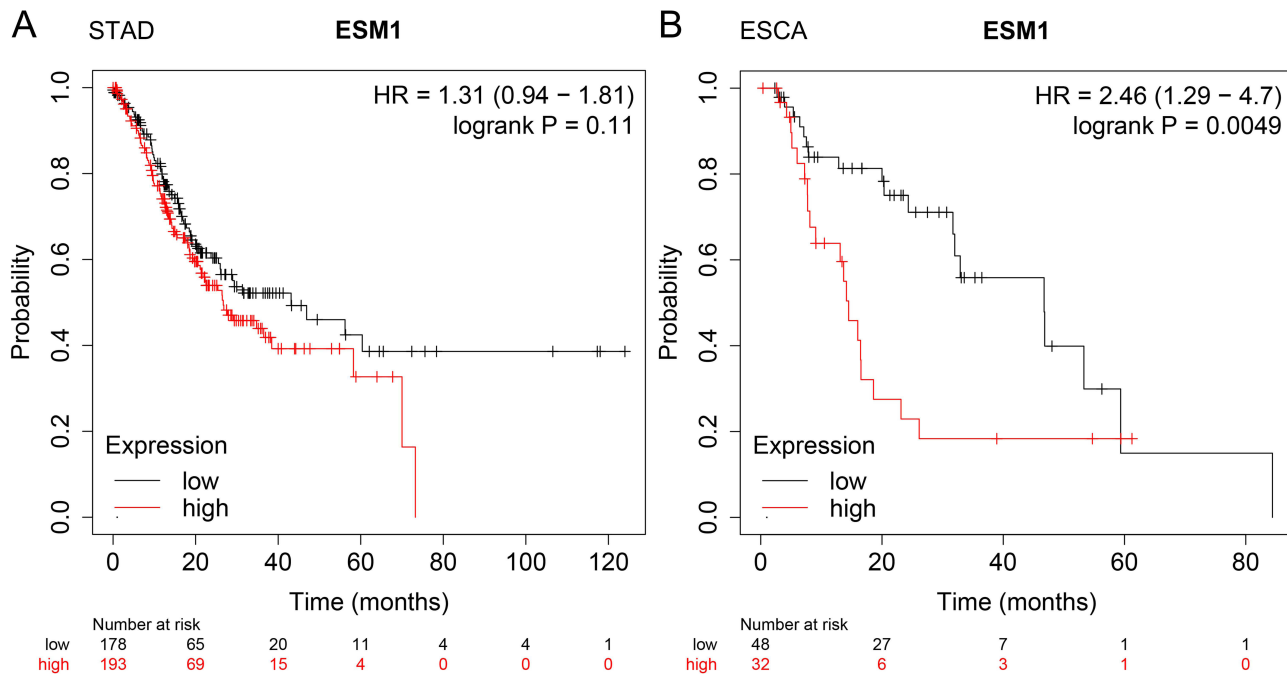
The survival rate of ESCA patients with high or low ESM1 expression was analyzed using Kaplan–Meier plotter database. The results showed that high expression of ESM1 showed low OS in STAD (Figure 3A) and ESCA (Figure 3B).

## ESM1 Showed an Upregulated mRNA and Protein Levels in STAD and ESCA

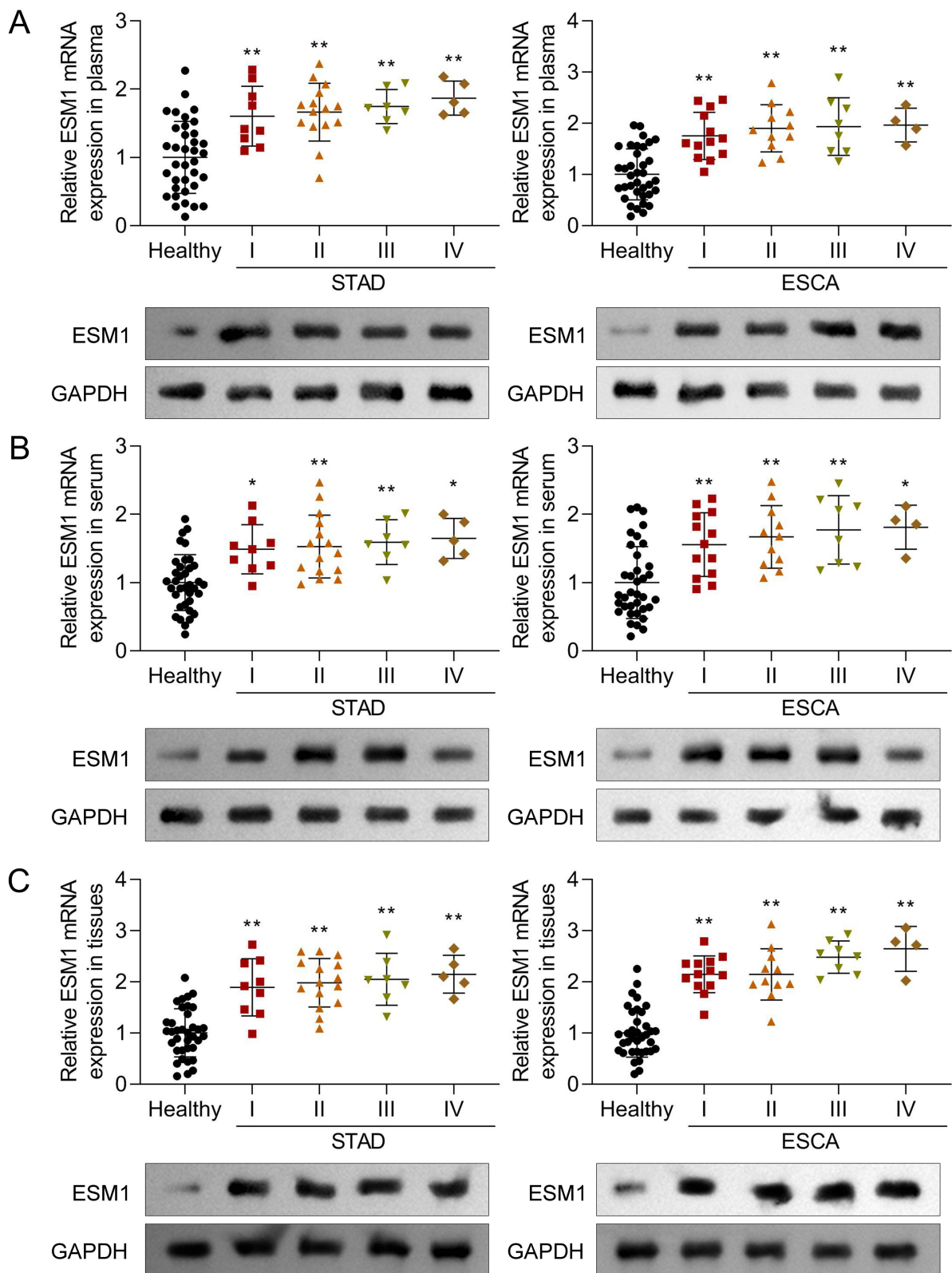
To further explore the biological functions of ESM1 in STAD and ESCA progression, we detected its mRNA and protein expression in plasma, serum, and tissues in clinical experiments. The results demonstrated that the mRNA and protein levels of ESM1 were upregulated at the stage of I, II, III, and IV in plasma, serum, and tissues of STAD or ESCA patients compared with the healthy groups (Figure 4A–C).



**Figure 2** Functional enrichment analysis of genes. **(A)** The Venn diagram was used to screen genes that were upregulated in both ESCA and STAD; **(B)** The diagram of genes for KEGG pathway enrichment analysis. The color indicates the p-value (from the lowest in green to the highest in red), and the bubble size indicates the number of genes. **Abbreviations:** STAD, stomach adenocarcinoma; ESCA, esophageal carcinoma; KEGG, Kyoto Encyclopedia of Genes and Genomes.



**Figure 3** High ESM1 expression predicted poor OS in STAD and ESCA. **(A)** Kaplan-Meier plotter database was used to analyze the survival rate of STAD patients with high or low ESM1 expression; **(B)** The survival rate of ESCA patients with high or low ESM1 expression was analyzed by Kaplan-Meier plotter database. **Abbreviations:** STAD, stomach adenocarcinoma; ESCA, esophageal carcinoma; OS, overall survival.



**Figure 4** ESM1 showed an upregulated mRNA and protein levels in STAD and ESCA. The mRNA and protein levels of ESM1 in each group in (A) plasma, (B) serum, and (C) tissues were detected by RT-qPCR and Western blot. (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

**Abbreviations:** ESM1, endothelial cell specific molecule I; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

## ESM1 Might Be a Sensitive Biomarker for the Diagnosis of STAD

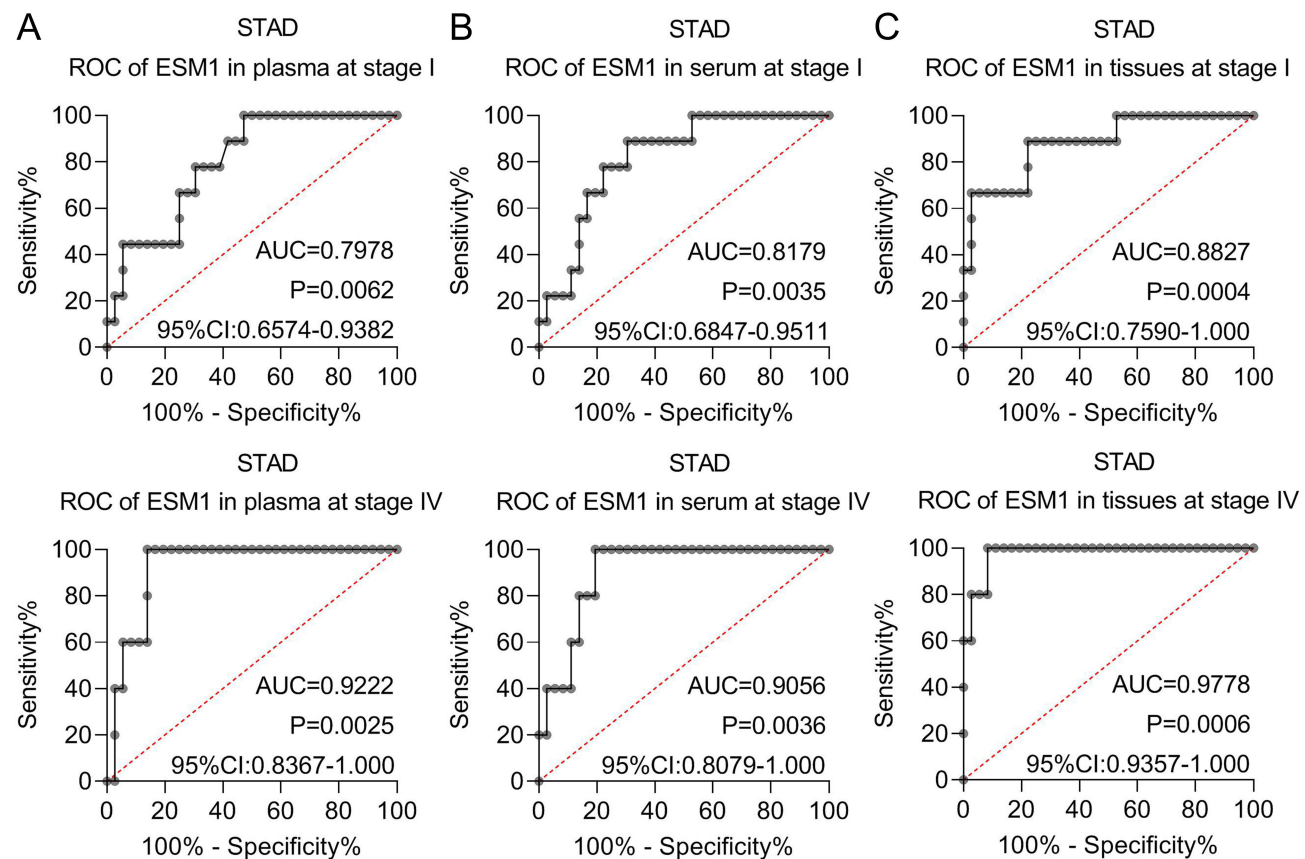
Next, ROC curve analysis was conducted to evaluate the sensitivity and specificity of ESM1 at the stage of I and IV in plasma, serum, and tissues of STAD. The area under the curve (AUC) is characterized by sensitivity and specificity and is often used to indicate the intrinsic validity of a diagnostic test.<sup>14</sup> The results implied that the AUC of ESM1 in plasma, serum, and tissues at stage I were 0.7978 (95% CI, 0.6574 to 0.9382), 0.8179 (95% CI, 0.6847 to 0.9511), and 0.8827 (95% CI, 0.7590 to 1.000), respectively. In addition, the AUC of ESM1 in plasma, serum, and tissues at stage IV were 0.9222 (95% CI, 0.8367 to 1.000), 0.9056 (95% CI, 0.8079 to 1.000), and 0.9778 (95% CI, 0.9357 to 1.000), respectively (Figure 5A–C). These results indicated that ESM1 might be a sensitive biomarker for the diagnosis of STAD.

## ESM1 Might Be a Sensitive Biomarker for the Diagnosis of ESCA

In ESCA, the AUC of ESM1 in plasma, serum, and tissues at stage I were 0.8611 (95% CI, 0.7582 to 0.9640), 0.7906 (95% CI, 0.6648 to 0.9164), and 0.9573 (95% CI, 0.9041 to 1.000), respectively. In addition, the AUC of ESM1 in plasma, serum, and tissues at stage IV were 0.9444 (95% CI, 0.8599 to 1.000), 0.8958 (95% CI, 0.7779 to 1.000), and 0.9931 (95% CI, 0.9717 to 1.000), respectively (Figure 6A–C). These results illustrated that ESM1 might be regarded as a sensitive biomarker for the diagnosis of ESCA.

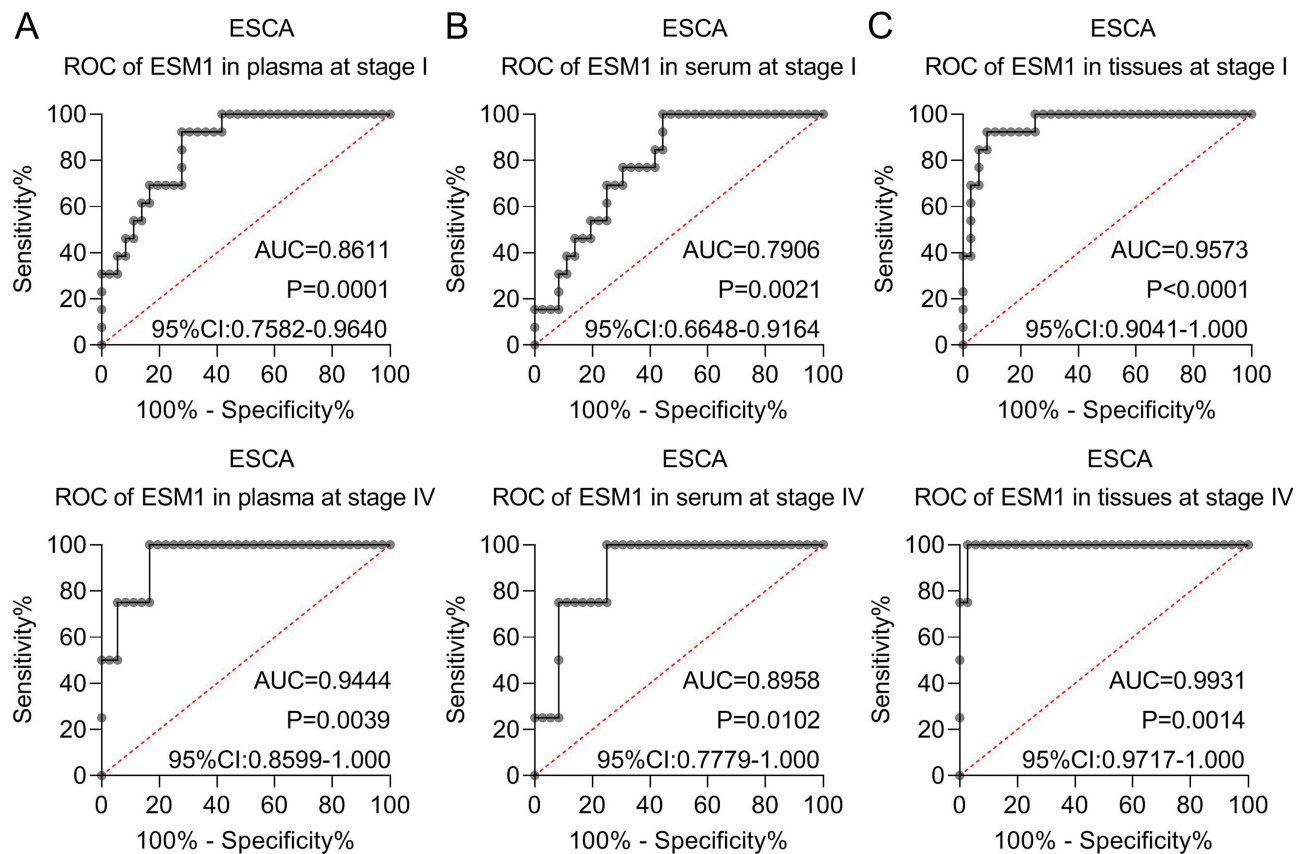
## Silencing ESM1 Decreased the Cell Viability, Migration, and Invasion and Increased the Apoptosis Rate in AGS and TE1 Cells

Subsequently, we conducted an in vitro study to further explore the effects of ESM1 on STAD and ESCA. The mRNA and protein levels of ESM1 were downregulated when ESM1 was inhibited in AGS and TE1 cells (Figure 7A).



**Figure 5** ESM1 might be a sensitive biomarker for the diagnosis of STAD. The ROC curves of ESM1 at stage I and II in (A) plasma, (B) serum, and (C) tissues in STAD. **Abbreviations:** STAD, stomach adenocarcinoma; ESM1, excess microsporocytes I; ROC, receiver operating characteristic.





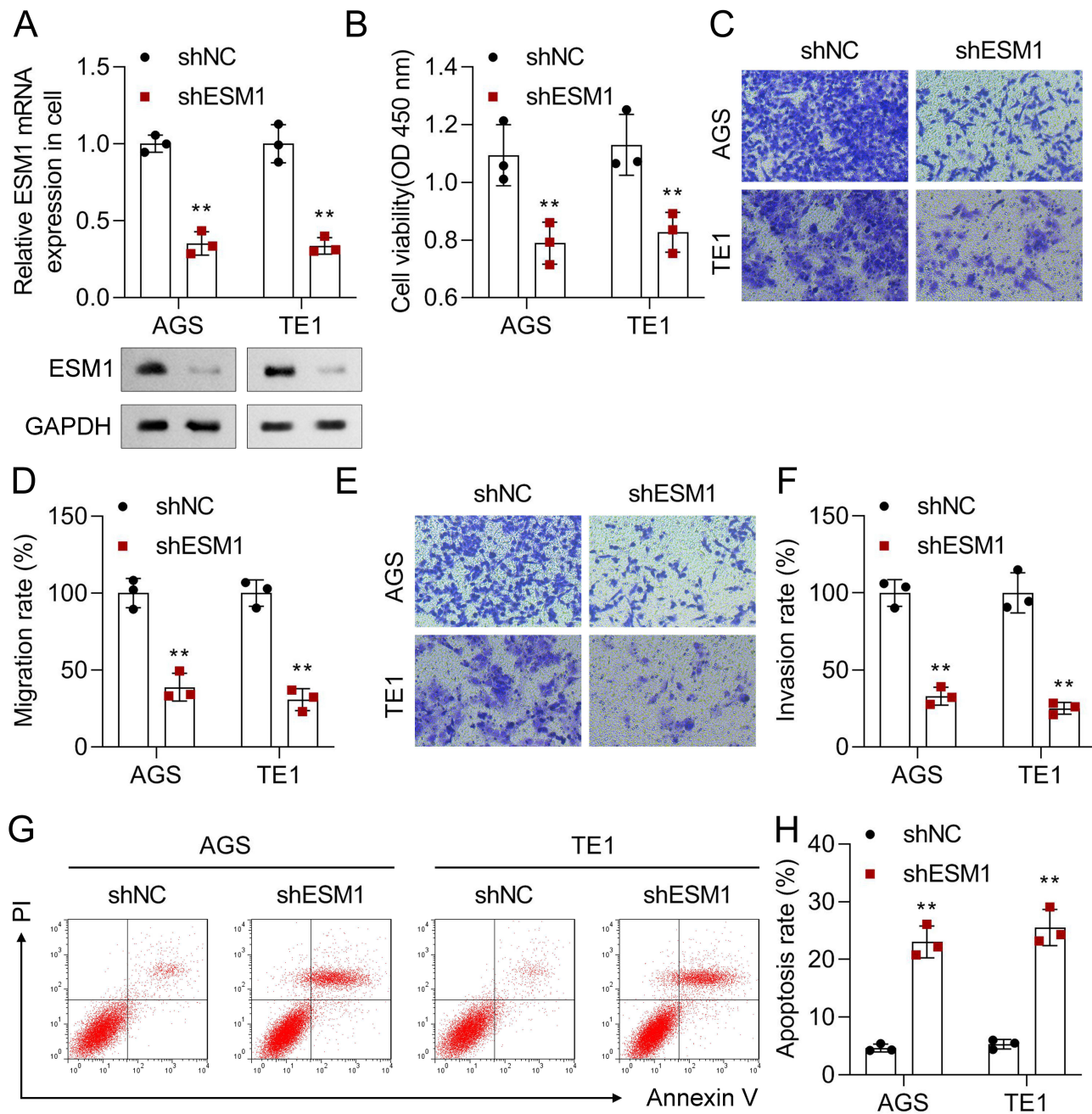
**Figure 6** ESM1 might be a sensitive biomarker for the diagnosis of ESCA. The ROC curves of ESM1 at stage I and II in (A) plasma, (B) serum, and (C) tissues in ESCA. **Abbreviations:** ROC, receiver operating characteristic; ESM1, endothelial cell specific molecule 1; ESCA, esophageal carcinoma.

Moreover, silencing ESM1 suppressed the cell viability, migration, and invasion of AGS and TE1 cells (Figure 7B–F). Additionally, ESM1 inhibition increased the apoptosis rate in AGS and TE1 cells (Figure 7G and H).

## Discussion

The research for potential biomarkers in early and advanced stage cancers has become an effective strategy to improve the prognosis and survival rate of patients. A growing body of studies indicate that ESM1, as an oncogene, might be a clinical biomarker in multiple cancers.<sup>15,16</sup> In this study, we found that high expression of ESM1 showed low OS in STAD and ESCA. Similarly, a pan-cancer analysis demonstrates that the expression of ESM1 was apparently correlated with the OS of patients in ESCA and other cancers.<sup>15</sup> In addition, another clinical study indicates that ESCA patients with high ESM1 expression show a lower clinical survival rate.<sup>13</sup> One of the key findings supporting the role of ESM1 as an oncogene comes from studies investigating its expression level in tumor tissues compared to normal tissues. Our results found that the mRNA expression of ESM1 was upregulated in STAD and ESCA tissues in the present in vivo and in vitro studies, which was consistent with previous studies.<sup>13,17</sup> Various studies indicate that ESM1 is upregulated in various types of cancer, including breast, lung, colorectal, ovarian, and pancreatic cancers.<sup>5,10,18–20</sup> This aberrant overexpression of ESM1 is often associated with tumor progression, metastasis, and poor prognosis, highlighting its potential as a prognostic biomarker.

Furthermore, ROC analysis, an effective method of evaluating the performance of diagnostic tests,<sup>21</sup> was performed to detect the sensitivity and specificity of ESM1 in STAD and ESCA. The AUC value in ROC curve can be used to evaluate the accuracy of diagnosis.<sup>22</sup> Several studies have demonstrated that ESM1 levels can be detected in various body fluids, such as blood, serum, and urine, making it easily accessible for clinical testing.<sup>23,24</sup> Our results found that ESM1 has a high accuracy (AUC > 0.79) in plasma, serum, and tissues at stage I and IV, suggesting that ESM1 could be considered as an effective



**Figure 7** Silencing ESM1 decreased the cell viability, migration, and invasion and increased the apoptosis rate in AGS and TE1 cells. **(A)** The ESM1 mRNA and protein levels in AGS and TE1 cells after ESM1 inhibition were assessed by RT-qPCR and Western blot; **(B)** The cell viability in AGS and TE1 cells when ESM1 was inhibited was detected by CCK-8 assay; **(C)** Transwell migration assay was used to detect AGS and TE1 cell migration after silencing of ESM1; **(D)** The migration rate in shNC and shESM1 groups in AGS and TE1 cells; **(E)** The invasion of AGS and TE1 cells was analyzed by transwell invasion assay; **(F)** The invasion rate in shNC and shESM1 groups in AGS and TE1 cells; **(G)** Flow cytometry was performed to detect the apoptosis of AGS and TE1 cells; **(H)** The apoptosis rate in shNC and shESM1 groups in AGS and TE1 cells. (\*\* $p < 0.01$ ). **Abbreviations:** ESM1, endothelial cell specific molecule 1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; shRNA, short hairpin RNA; CCK-8, cell counting kit-8.

diagnostic biomarker in STAD and ESCA. Similarly, plasma ESM1 is a novel biomarker for predicting the tumor status in oral squamous cell carcinoma patients.<sup>25</sup> Moreover, high ESM1 expression has been associated with resistance to certain cancer treatments, indicating its potential as a predictive biomarker for treatment response.<sup>26</sup> In other cancers, ESM1 has also been found to have potential as a potent biomarker.<sup>12,13</sup> In in vitro study, we found that ESM1 inhibition suppressed the cell viability, migration, and invasion and upregulated the apoptosis rate in AGS and TE1 cells, implying that silencing of ESM1 could inhibit the progression of STAD and ESCA. Similar to our results, ESM1 inhibition suppresses the cell proliferation,

apoptosis escape, migration and invasion in ovarian cancer,<sup>10</sup> ESCA,<sup>13</sup> and hepatocellular carcinoma,<sup>27</sup> which indicates that ESM1 might be considered as a promising therapeutic target and prognostic indicator for different cancers.

In summary, clinical studies have shown that ESM1 was upregulated in STAD and ESCA, and may function as a potential biomarker for diagnosis and prognosis of early and advanced stage digestive tract cancers. Besides, the consistent findings of reduced cell viability, migration, and invasion, along with increased apoptosis upon ESM1 inhibition, suggested that ESM1 could be used to predict the aggressiveness and prognosis of digestive tract cancers. Thus, it may be possible to develop novel therapeutic strategies for treating digestive tract cancers by targeting ESM1.

However, there are several limitations in this study. The number of patients included in the study is small due to the difficulty of sample collection. Additionally, our research is deficient in substantiation from in vivo data. We will further explore these deficiencies in our future studies.

## Data Sharing Statement

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

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Beijing Aerospace General Hospital.

## Consent for Publication

Informed consent was obtained from all individual participants included in the study.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests in this work.

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