# Serum exosomal miR-4787-3p as potential lymph node metastasis and prognostic marker in esophageal squamous cell carcinoma.

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**Primary research**

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# Abstract

**Background:** To explore the miR-4787-3p expression levels in the serum exosome and tissue and its role in lymph node metastasis and prognosis in ESCC.

**Methods:** The miRNA array was conducted to detect the ESCC serum exosomal miRNAs expression. A receiver operating characteristic (ROC) curve was constructed to determine the predictive ESCC with lymph node metastasis efficacy of serum exosomal miR-4784-3p. The Cox regression analysis was preformed to explore prognostic factors for ESCC.

Transwell assay and CCK-8 assays were utilized to evaluate cell migration, invasion, and proliferation, respectively.

**Results:** High serum exosomal miR-4787-3p expression was demonstrated in lymph node metastasis group (P =0.011). The serum exosomal miR-4787-3p expression was significantly associated with histologic grade (P = 0.010), and TNM stage (P = 0.033). However, there was no significant relationship between tissue miR-4787-3p expression and clinical characteristics (P >0.05). ROC analyses revealed that the AUCs of serum exosomal miR-4787-3p for lymph node metastasis prediction was 0.787. The Cox regression analysis found that high expression serum exosomal miR-4787-3p were correlated with poor prognoses (for OS, HR=2.68, 95% CI: 1.02~7.04; for DFS, HR = 2.65, 95% CI: 1.05~6.68). Nevertheless, no association between tissue miR-4787-3p expression and ESCC prognosis. In addition, upregulated expression of miR-4787-3p could promote migration and invasion in vitro.

**Conclusions:** Serum exosomal miR-4787-3p can be promising biomarkers for ESCC metastasis and prognosis


# Background

Esophageal cancer, a global public health problem, remains as one of the common digestive system cancers in the world [1]. Esophageal squamous cell carcinoma (ESCC) is the major histological types of esophageal cancer, especially in China [2]. Because of the lack of specific symptoms or signs, most of ESCC have exhibited lymph node metastasis as the first diagnosis. Lymph node metastasis is a risk factor for recurrence and a poor prognosis of ESCC [3], with survival rates decreasing from ~70 to ~18% when lymph node metastasis occurs [4]. Such outcomes highlight the urgency to explore novel non-invasive biomarkers, which can be applied for the diagnosis lymph node metastasis.

Recent studies have confirmed the vital roles of microRNA (miRNAs) in the progression of various cancers [5–7], including ESCC. The potential use of miRNAs in the ESCC lymph node metastasis and prognosis has been widely reported. For instance, some specific miRNAs, such as serum miR-548k, miRNA-21, miR-1246, and miR-655, which play an essential role in lymph node metastasis and poor prognosis, have been identified in ESCC [8–11]. Moreover, miRNAs could adjust the expression of gene by binding to complementary nucleotide sequences in 3' untranslated regions of mRNAs [12]. Lu et al.

observed that the serum miR-10b-3p aberrant expression in ESCC is correlated with lymph node metastasis and poor prognosis. Mechanistically, this study demonstrated that miR-10b-3p increased FOXO3 expression via targeting the 3'-untranslated region [13]. Those studies demonstrated that miRNAs could be used as markers for ESCC lymph node metastasis and prognosis. Nevertheless, several studies have found that circulation miRNAs have low concentration and poor stability in body fluids [14, 15]. Therefore, it is necessary to identify more stable serum miRNAs biomarkers that can be applied for the prediction of lymph node metastasis and prognosis.

Exosomes contain miRNA, nucleic acids, and proteins, and their cargo often varies under various physiological and pathological processes [16, 17], being reflective of the state of the originating host cells. Studies have found that tumor-derived exosomes contain more miRNAs than healthy cells [18], and they are protected by the lipid bilayer structure [19]. Previous studies have demonstrated that tumor derived exosomes miRNAs can be delivered into the tumor microenvironment or spread away through the circulation [20, 21], which could trigger tumor metastasis in consequence. So far, only two studies investigated the role of exosomal miRNAs in ESCC lymph node metastasis. Our previous study showed that upregulated expression of serum exosomal miRNA-766-3p was association with lymph node metastasis and short overall survival rate [22]. Another study explored a novel serum four exosomal miRNAs model for the identification of lymph node metastasis cases in ESCC [23]. Hence, serum exosomal miRNAs may be useful as potential biomarkers of ESCC lymph node metastasis and prognosis.

In this study, the miRNA array was performed to detect the ESCC serum exosomal miRNAs expression. The relative expression of miR-4787-3p was calculated from ESCC serum and tissue samples by qRT-PCR. Cell experiments were conducted to assess the role of miR-4787-3p on cell migration, invasion, and proliferation in ESCC cells. This study aimed to demonstrate miR-4787-3p expression in ESCC serum and tissue, to clarify the role of serum exosomal miR-4787-3p for ESCC lymph node metastasis and prognosis.

# Methods

### Patients and samples

From December 2014 to August 2017, seventy-nine patients with a ESCC were recruited in the Fujian Cancer Hospital & Fujian Medical University Cancer Hospital. All participants provided written informed consent. ESCC were diagnosed through histological examination, and the tumor stage was determined based on the American Joint Committee on Cancer seventh edition of TNM classification. No patients had any anti-cancer treatment before surgery. The ESCC with lymph node metastasis was defined according the previous study [22]. Based on histopathology, 79 ESCC were divided into two groups: 42 ESCC with lymph node metastasis and 37 ESCC without lymph node metastasis. The supplement table 1 presented the clinical characteristics of the ESCC patients. Besides, 88 ESCC tissue samples were collected at the same hospital from December 2014 to August 2017. The follow-up data were acquired at

the end of November 2019, with a median observation time of 22 months. The study protocol was reviewed and approved by the Fujian Medical University ethics committee (No. 2014095).

### Serum Exosomes isolation

Exosomes from the serum samples was extracted with a ExoQuick™ (SBI, USA) according to the instructions. Furthermore, transmission electron microscopy and western blot were used to confirm the existence of exosomes.

### Serum Exosomal miRNA and protein isolation

According to the instructions, the exosomal miRNAs and protein were extracted from serum with the Total TRIzol Reagent and Protein Isolation Kit (Life Technologies, USA). The concentration of the RNA was analyzed using NanoDrop ND-1000 system (NanoDrop, UK). Besides, the concentration of exoaomal protein was detected by BCA kit (Zhuangmeng, Beijing, China).

### MicroRNA microarray analysis

MicroRNA microarray analysis was conducted as described in our previous study [22].

Our microRNA microarray analysis revealed that miR-4787-3p, and miR-766-3p were selected. In this study, we explored the relationship between the expression of serum exosomal miR-4787-3p and ESCC.

### Western blot

The protein was analyzed by western blot as previously described [22]. The primary antibodies used in this study were as follows: anti-CD81(ABclonal, Wuhan, China), anti-CD63(ABclonal, Wuhan, China).

### Quantitative RT-PCR (qRT-PCR)

qRT-PCR was performed to assess the expressions of miR-4787-3p. U6 was used as internal controls. The primers were shown as follows: miR-4787-3p: CACTGCCCCGCGCAAA; U6: TCGTAAGCGTTCCATATTTTTAA. The conditions of the amplification reaction were as follows: 95°C for 10 mine, 95°C for 10 s, 40 cycles of 66°C. The relative expression of miR-4787-3p was estimated by $2^{-\Delta CT}$.

### Cell lines and cell culture

The KYSE150, TE-1, and ECA109 were obtained from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). All cell lines were cultured in Dulbecco's modified Eagle's medium mixture with 10% fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere with 5% $CO_2$.

### Cell transfection

The miR-4787-3p inhibitor, miR-4787-3p NC, and miR-4787-3p mimics plasmid were purchased from Genepharma (Shanghai, China). Lipofectamine 2000 was used to transfect cells.

### Cell migration and invasion

Transwell chamber was used for transwell invasion and invasion assay (the chamber without matrigel was used for cell migration, and transwell chamber with matrigel was used for cell invasion). In brief, after 24 hours of starvation, cells passed into the bottom chamber was fastened in 5% glutaraldehyde and stained with 0.1% crystal violet. The pictures of migration and invasive cells were obtained by an inverted microscope (OLYMPUS, Japan) and cell number was counted in four fields. The experiment was carried out in triplicate.

### Cell proliferation assay

Cells ($3 \times 10^3$~$6 \times 10^3$ cells) were plated and transfected as above mentioned. Cell counting kit-8 (CCK-8) assay was assessed at 0, 24, 48, and 72 hours. CCK-8 reagent (10 μL) was added to each well. After a 2-hour incubation at 37˚C, the optical density was read at 450 nm by an enzyme immunoassay instrument (Denovix). The experiments were repeated in three times.

### Functional Enrichment Analysis

TargetScan (http://www.targetscan.org/vert_72/) was applied for predicting the potential target genes of miR-4787-3p. Using the DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/) to perform GO and KEGG pathway analyses according to the target genes. The significantly GO terms and KEGG pathway were presented based on a P value <0.05.

### The Cancer Genome Atlas and GEO database analysis

ESCC patient's microRNA profiling array was downloaded from the TCGA (https://portal.gdc.cancer.gov/) and GEO database

(GSE43732, https://www.ncbi.nlm.nih.gov/).

### Statistical analyses.

The chi-square test was applied to explore the relationship between clinical factors and miR-4784-3p expression. A receiver operating characteristic (ROC) curve and the area under the curve was constructed to determine the predictive ESCC with lymph node metastasis efficacy of serum exosomal miR-4784-3p. OS and DFS curves were estimated using the Kaplan-Meier method with a log-rank test. Hazard ratios and 95% confidence intervals for ESCC prognosis were calculated by the Cox regression analysis. All statistical analyses were performed with R software (R 3.5.3). A P value < 0.05 was indicative of statistical significance for two-sided tests.

# Results

### Screening of serum exosomal miR-4787-3p

The characterization of serum exosomes and screening serum exosomal miRNAs profiles was reported by our previous study [22]. In this study, we selected the candidate serum exosomal miRNAs according to the previous experimental findings that serum exosomal miR-4787-3p was associated with lymph node metastasis.

### Serum exosomal miR-4787-3p was associated with lymph node metastasis

Initially, serum exosomal miR-4787-3p expression was detected in 79 ESCC. The results of qRT-PCR indicated that serum exosomal miR-4787-3p was higher with lymph node metastasis than that in ESCC without lymph node metastasis (P =0.011, supplement figure 1). Next, we divided ESCC into low expression group (n = 40) and high expression group (n = 39) on the basis of the median values of serum exosomal miR-4787-3p expression in ESCC. As presented in supplement table 2, high expression of serum exosomal miR-4787-3p significantly increased the ESCC lymph node metastasis with OR of 3.93 (95% CI:1.23-12.55).

### Serum exosomal miR-4787-3p and tissues miR-4787-3p expression levels correlated with the clinical characteristics of ESCC

As showed in table 1, the serum exosomal miR-4787-3p expression was significantly correlated with histologic grade (P = 0.010), and TNM stage (P = 0.033). No significant association between serum exosomal miR-4787-3p and other clinical characteristics. In addition, we detected the tissue miR-4787-3p expression from 88 ESCC tissue samples.

the expression of tissue miR-4787-3p was detected in 88 ESCC patients. The showed that no significant relationship between the expression of tissue miR-4787-3p and clinical characteristics (supplement table 3).

### The value of serum exosomal miR-4787-3p for diagnosis lymph node metastasis

As presented in figure 1, the AUC of combining serum exosomal miR-4787-3p expression and clinical factors (sex, age, tumor size, and histologic grade) (AUC = 0.787; 95% CI, 0.678~0.897, model 1), was higher than only included clinical factors (AUC = 0.709; 95% CI, 0.588~0.829, model 2).

### The relationship between the expression of serum exosomal miR-4787-3p and survival outcome in ESCC

In this study, the 1-year, and 3-year survival rates were 88.70% (95% CI, 81.30-96.10), and 57.00% (95% CI, 43.70-70.30), respectively. Compared with serum exosomal miR-4787-3p expression group, the log-rank test indicated that ESCC patients with high serum exosomal miR-4787-3p had lower overall survival (P <0.05, figure 2) and disease-free survival rate (P <0.05, figure 2). After adjusted sex, age, tumor size, histologic grade, adjuvant chemotherapy, and serum exosomal miR-4787-3p expression (Because there

was correlated with serum exosomal miR-4787-3p and TNM (rs=0.274, P =0.015), we did not include TNM in multivariate Cox regression analysis), multivariate Cox regression analysis found that high expression serum exosomal miR-4787-3p were correlated with poor prognosis (for OS, HR=2.68, 95% CI: 1.02~7.04; for DFS, HR = 2.65, 95% CI: 1.05~6.68, table 2).

### Correlation between tissue miR-4787-3p and the prognosis of ESCC

Further, the relationships between tissues miR-4787-3p and ESCC prognosis were explored. The log-rank test revealed that no correlation between the expression tissue miR-4787-3p and ESCC prognosis (for OS, P =0.897; for DFS, P =0.914, supplement figure 2). A multivariate Cox regression analysis showed that there was no relationship between tissue miR-4787-3p expression level and OS (HR =0.88, 95% CI: 0.44~1.77, supplement table 4) and DFS (HR =0.88, 95% CI: 0.45~1.73, supplement table 4). Furthermore, the ESCC miRNA expression microarray data from TCGA database (n=96) and GEO database (n=119) were analyzed. No significant association between the expression of tissue miR-4787-3p and overall survival in TCGA database (P =0.211, supplement figure 3A) and GEO database (P =0.492, supplement figure 3B).

### Expressions of miR-4787-3p in ESCC cells after transfection

As showed in figure 3, in three different ESCC cells, compared with the NC group, the miR-4787-3p expression was increased in the miR-4787-3p mimics (P <0.05), and the miR-4787-3p expression was decreased in the miR-4787-3p inhibitors (P <0.05), indicating miR-4787-3p vector could be successfully transfected into the ESCC cells.

### Overexpression miR-4787-3p promotes migration and invasion in vivo

As shown in figure 4, compared with NC group, the results showed that overexpression of miR-4787-3p significantly increased migration of KYSE150 cells (P <0.05), but not in TE-1, and ECA109 cells (P >0.05). In addition, knockdown miR-4787-3p inhibited the migration ability of KYSE150, TE-1, and ECA109 cells (all P <0.05). For transwell invasion assay, compared with NC group, the results found that overexpression of miR-4787-3p significantly increased invasion of KYSE150, TE-1, and ECA109 (P <0.05, figure 5). Also, the miR-4787-3p inhibitor group had lower numbers of KYSE150, TE-1, and ECA109 cells than the NC group (P <0.05, figure 5). Therefore, our results demonstrated upregulated expression of miR-4787-3p could promotes migration and invasion in ESCC cells.

### Effects of miR-4787-3p on the proliferation of ESCC Cells

CCK-8 assay revealed that no difference in cells proliferation among the groups transfected with miR-4787-3p inhibitor, NC, or miR-4787-3p mimic (P > 0.05) in either KYSE150, TE-1 or ECA109 cells (Figure 6).

### Potential targeted genes of miR-4787-3p

A total of 581 targeted genes were identified in all datasets by using targetscan. To reveal biological functions based on targeted genes, we used the DAVID for gene ontology (GO) analysis and pathway enrichment analysis. GO term enrichment analysis results were enriched in transcription DNA-templated, cytosol, and protein binding. In addition, the KEGG pathway was mainly related to the endocytosis pathway, and purine metabolism pathway (Supplement figure 4).

# Discussion

Accumulating studies have reported that lymph node metastasis is associated with poor prognosis in ESCC. To diagnose ESCC lymph node metastasis at an early stage and improve prognosis, the identification of novel non-invasive screening molecular biomarker is important. In this study, we firstly reported that high expression of serum exosomal miR-4787-3p was association with ESCC lymph node metastasis and poor prognosis. In addition to that, we found overexpression of miR-4787-3p increases cell migration and invasion. This study demonstrated that serum exosomal miR-4787-3p can be promising biomarkers for ESCC metastasis and prognosis.

At present, computed tomography is often conducted to diagnose lymph node metastasis preoperatively, however, due to its limited accuracy [24, 25], which often leads to false diagnosis. Therefore, it is necessary to explored more sensitive and accurate molecular markers to diagnose lymph node metastasis. Recently, emerging evidence demonstrated that exosomal miRNAs modulate the tumor lymph node metastasis, which is favorable for tumor growth and invasion, by remodeling extracellular matrix, promoting tumor angiogenesis, inducting inflammation and immune suppression [26–28]. Previous studies indicated that serum exosomal miR-766-3p level was association with lymph node metastasis, which cloud predict ESCC lymph node metastasis (AUC = 0.778; 95% CI, 0.672–0.884) [22]. Another study pointed that four exosomal miRNAs model identified lymph node metastasis metastases with an AUC value of 0.865 [23]. Collectively, exosomal miRNAs played a crucial role in ESCC lymph node metastasis. Our present study reported that serum exosomal miR-4787-3p was higher in ESCC with lymph node metastasis than that in ESCC without lymph node metastasis. Besides, serum exosomal miR-4787-3p expression could predict lymph node metastasis with AUC of 0.787. We suspected that serum exosomal miR-4787-3p may be an important factor in the reception of metastasis by distal tissues and their release can promote lymph node metastasis. Our finding indicated that upregulated serum exosomal miR-4787-3p might be a new potential biomarker for diagnosing lymph node metastasis.

Lymph node metastasis is association with poor prognosis in esophageal cancer. Recent studies indicated that cancer cell-derived exosomal miRNAs could affect prognosis by influencing tumor microenvironment (such as promoting lymph angiogenesis, suppressing immune) [29, 30]. In the present study, Cox regression analysis was used to explore the expression of serum exosomal miR-4787-3p in ESCC and its effect on prognosis. The results indicated that serum exosomal miR-4787-3p overexpression was strongly related with poor prognosis. In addition, the overexpression of miR-4787-3p increases the ability of cell migration and invasion in vitro. We hypothesized that serum exosomal miR-4787-3p might contribute to poor prognosis via promoting lymph node metastasis. In currently studies,

there were two studies explored the association between serum miRNAs expression level and ESCC prognosis. Takeshita et al. [31] found that elevated levels of serum exosomal miR-1246 indicate poor prognosis in ESCC (HR = 4.03, 95% CI: 1.27 ~ 12.73). Our previous study demonstrated that upregulated serum exosomal miR-766-3p was association with poor prognosis (HR = 2.21, 95% CI: 1.00 ~ 4.87) [22]. These findings are consistent with our study result. Thus, overexpression of serum exosomal miR-4787-3p might be a useful biomarker for ESCC prognosis. Nevertheless, the studies of miR-4787-3p have not yet been reported, and its related mechanism of action needs to be further investigated.

Interestingly, our results found that serum exosomal miR-4787-3p was not consistent with tissue miR-4787-3p expression, where miR-4787-3p expression was higher in serum exosomal than that in ESCC tissue sample (Z = 3.258, P = 0.001, supplement Fig. 5), However, no significantly correlation between serum exosomal miR-4787-3p and tissue miR-4787-3p (rs= -0.131, P = 0.524, supplement Fig. 5). Previous studies have reported the inconsistency of miRNA expression in blood and tissues samples [32, 33], and the potential reasons can be explained as follows. Firstly, exosomes can selectively package cancer tissue miRNAs, resulting in its high expression in blood samples [33]. Besides, circulating miRNA may originate from various tissues and cell types in the body [34]. No significant correlations were observed between tissue miRNA-4787-3p and lymph node metastasis. Furthermore, we also investigated the association between tissue miR-4787-3p and ESCC prognosis, while there was no association observed. To validate the results of this study, we further explored the association between miR-4787-3p and ESCC prognosis in ESCC tissues from TCGA and GEO databases. Consistent with our finding, no association between the expression of tissue miR-4787-3p and overall survival in TCGA and GEO database. Therefore, our findings revealed an association between serum exosomal miR-4787-3p and ESCC prognosis, but not in ESCC tissues miR-4787-3p.

For functional enrichment analyses, the KEGG pathway analysis indicated that endocytosis pathway is the most closely related pathway. Endocytosis pathway has been shown to participate in bringing ligands, and lipids into the cell and cleaning them from the cell surface [35], which plays vital role in cancer prognosis. As reported by the previous study, the endocytosis pathway has been shown in high miR-222-3p expression, which plays a crucial role in lung cancer prognosis [36]. Another study found that the association of has-miR-378c and has-miR-642a expression with cervical squamous cell carcinoma prognosis might be through its involvement in endocytosis pathways [37]. Thus, we hypothesized that miR-4787-3p activates the endocytosis pathway to promote the development of ESCC, while the potential mechanism needs to be further validated.

There are some limitations of our study needed to be addressed. First, the potential molecular mechanisms of the altered serum exosomal miR-4787-3p expression level may need further investigation. Besides, further research in a larger cohort is required to pursue the potential of serum exosomal miR-4787-3p as an ESCC lymph node metastasis and prognosis biomarker.

# Conclusions

In conclusion, we revealed that high expression level of serum exosomal miR-4787-3p were associated with lymph node metastasis and poor prognosis in ESCC for the first time. Overexpression of miR-4787-3p can increase the migration and invasion abilities of ESCC cells. Hence, serum exoaomal miR-4787-3p can also be a promising biomarker for metastasis and prognosis.

# Abbreviations

ESCC: esophageal squamous cell carcinoma, TNM: tumor node metastasis, OD: optical density, ROC: A receiver operating characteristic, TCGA: the cancer genome atlas, GEO: Gene Expression Omnibus database, OS: overall survival, DFS: disease-free survival, HR: hazard ratio, 95% CI: 95% confidence interval, qRT-PCR: quantitative real-time PCR, GO: gene ontology, KEGG: Kyoto encyclopedia of genes and genomes.

# Declarations

### Authors' contributions

HZJ conceived of the study, participated in its design and reviewed the manuscript. LS, and LZ designed the study, performed the data analysis and drafted the manuscript. ZZR, and RWQ performed drafted the manuscript. WJW, LZQ, ZQY, and LSS carried out the clinical sample collection and drafted the part of manuscript. WJW, and CYM performed pathological evaluation and interpretation of all samples included in the study. All authors read and approved the final manuscript.

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### Declarations

Ethics approval and consent to participate

Informed consent was obtained from participants, and the study was approved by the Institutional Review Board of Fujian Medical University (number: 201495).

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

### Availability of data and materials

The datasets generated during and/or analyzed during the current study are not publicly available due we are performing related research, but are available from the corresponding author on reasonable request.

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# Tables

Table 1 The association between serum exosomal miR-4787-3p expression and clinical characteristics factors

| Variable | Low expression group | High expression group | $\chi 2$ | *P*-value |
|---|---|---|---|---|
| Sex | | | 0.035 | 0.852 |
| Male | 29(72.5) | 29(74.4) | | |
| Female | 11(27.5) | 10(25.6) | | |
| Age (years) | | | 0.110 | 0.741 |
| ≤60 | 18(45.0) | 19(48.7) | | |
| >60 | 22(55.0) | 20(51.3) | | |
| Tumor size (cm) | | | 0.063 | 0.802 |
| ≤4 | 26(65.0) | 25(64.1) | | |
| >4 | 11(27.5) | 12(30.8) | | |
| Histologic grade | | | 6.605 | 0.010 |
| G3/G2 | 38(95.0) | 29(74.4) | | |
| G1 | 1(2.5) | 8(20.5) | | |
| TNM stage | | | 4.565 | 0.033 |
| I/II | 25(62.5) | 15(38.5) | | |
|  | 15(37.5) | 24(61.5) | | |
| Adjuvant chemotherapy | | | 0.107 | 0.744 |
| No | 23(57.5) | 21(53.8) | | |
| Yes | 17(42.5) | 18(46.2) | | |

Table 2 Univariate and multivariate analysis of overall survival and disease-free survival in ESCC for serum exosomal miR-4787-3p

| Variable | Overall survival | | Disease-free survival | |
|---|---|---|---|---|
| | Univariate | Multivariate* | Univariate | Multivariate* |
| x | | | | |
| Male | 1.00 | 1.00 | 1.00 | 1.00 |
| Female | 0.43(0.15,1.25) | 0.47(0.15,1.48) | 0.45(0.17,1.19) | 0.47(0.15,1.49) |
| e (years) | | | | |
| ≤60 | 1.00 | 1.00 | 1.00 | 1.00 |
| >60 | 1.83(0.82,4.11) | 1.67(0.62,4.44) | 2.12(0.96,4.65) | 1.87(0.72,4.87) |
| mor size (cm) | | | | |
| ≤4 | 1.00 | 1.00 | 1.00 | 1.00 |
| >4 | 2.13(0.97,4.70) | 1.62(0.62,4.27) | 2.50(1.17,5.33) | 1.88(0.74,4.73) |
| stologic grade | | | | |
| G3/G2 | 1.00 | 1.00 | 1.00 | 1.00 |
| G1 | 1.10(0.32,3.70) | 0.54(0.15,1.92) | 1.07(0.32,3.59) | 0.49(0.14,1.74) |
| juvant chemotherapy | | | | |
| No | 1.00 | 1.00 | 1.00 | 1.00 |
| Yes | 0.50(0.22,1.16) | 0.30(0.11,0.83) | 0.66(0.31,1.40) | 0.40(0.16,0.99) |
| rum exosomal R-4787-3p w expression oup | 1.00 | 1.00 | 1.00 | 1.00 |
| gh expression oup | 2.23(0.99,5.05) | 2.68(1.02,7.04) | 2.34(1.08,5.10) | 2.65(1.05,6.68) |

* Adjusted for sex, age, tumor size, histologic grade, adjuvant chemotherapy and serum exosomal miR-4787-3p.

## Figures

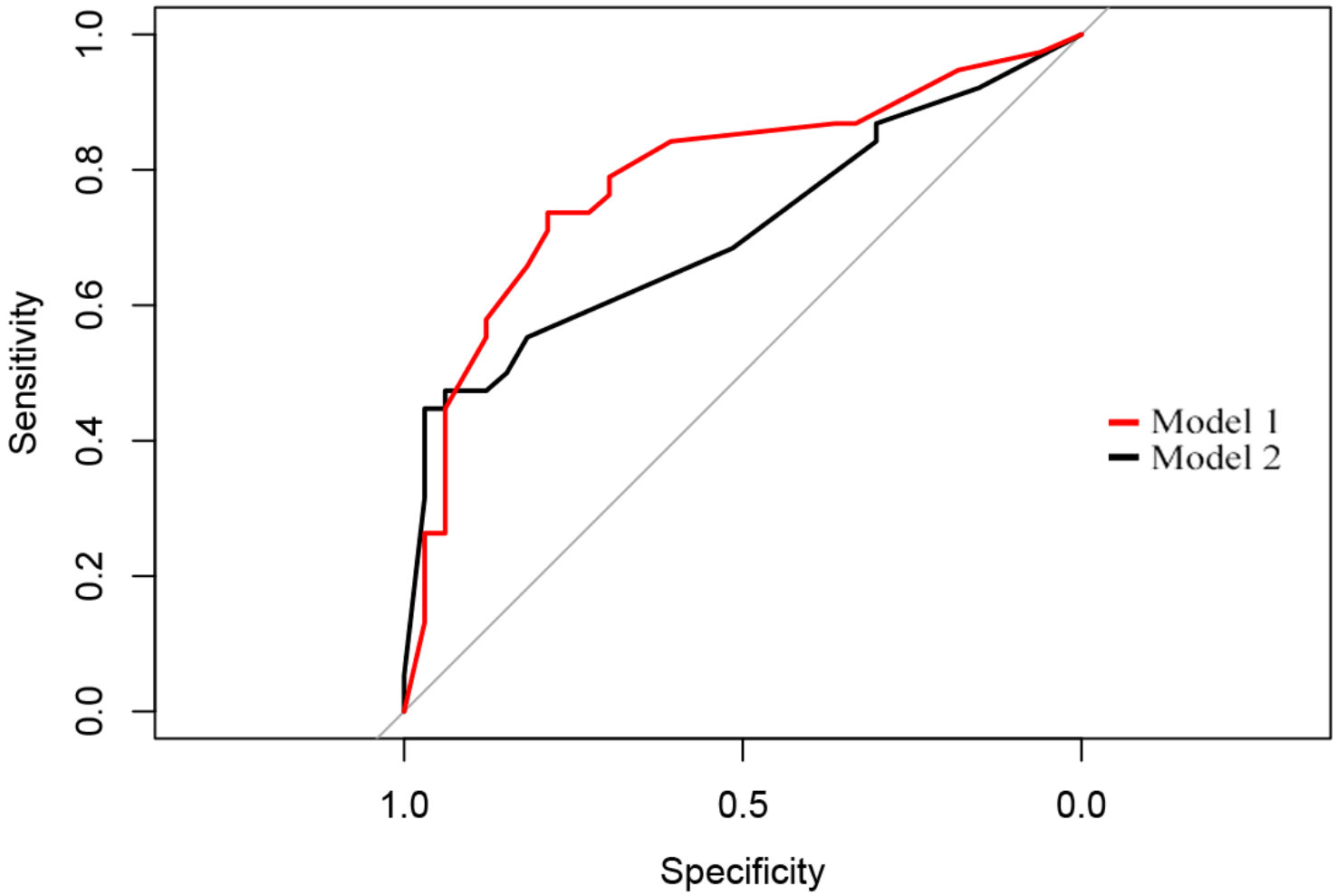



**Figure 1**

ROC curves were the predicted probability of detection with lymph node metastasis from the logistic regression model was calculated with serum exosomal miR-4787-3p and clinical characters (red roc curve) and only clinical characters (black roc curve).

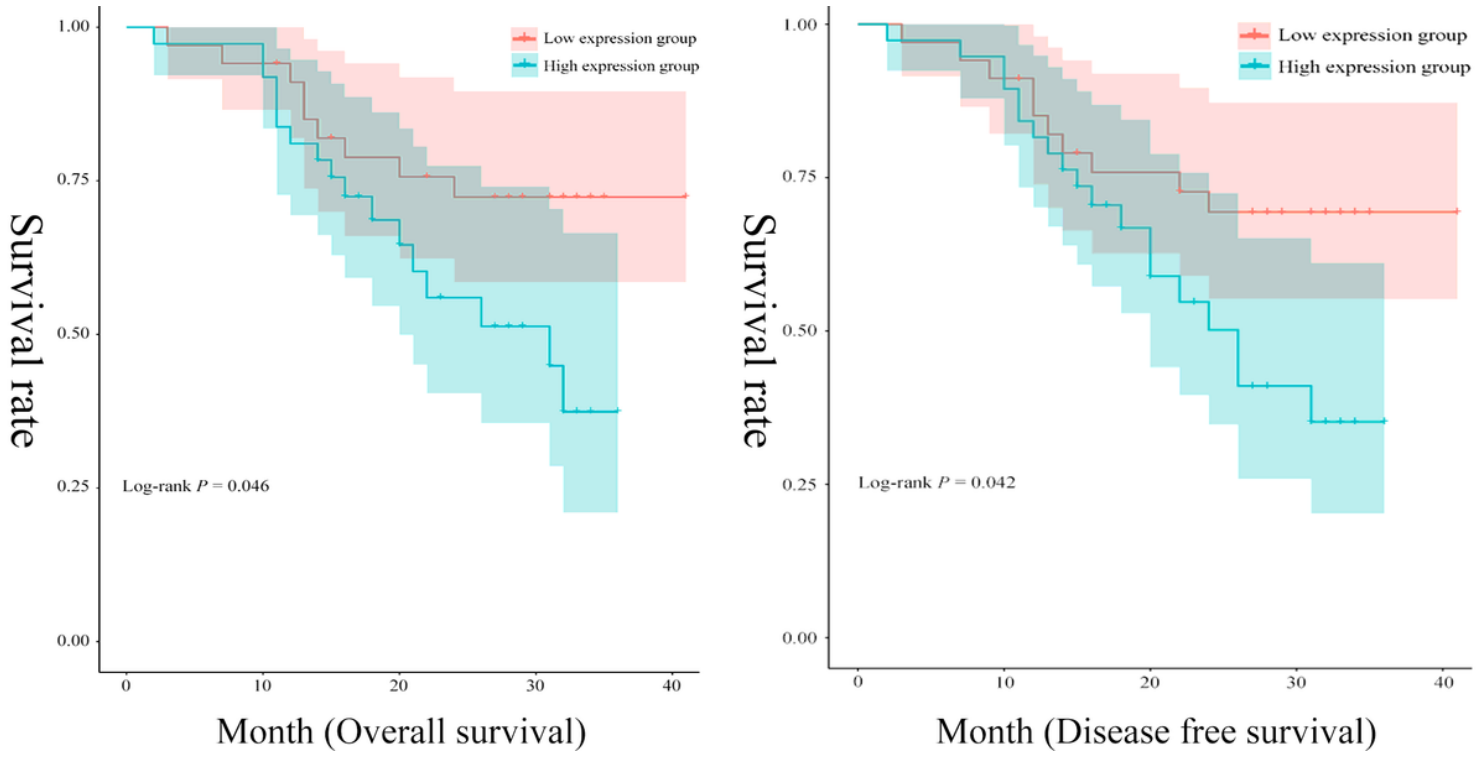



**Figure 2**

Kaplan-Meier survival curves showing the relationship between high or low serum exosomal miR-4787-3p expression levels with the overall survival and disease-free survival of ESCC.

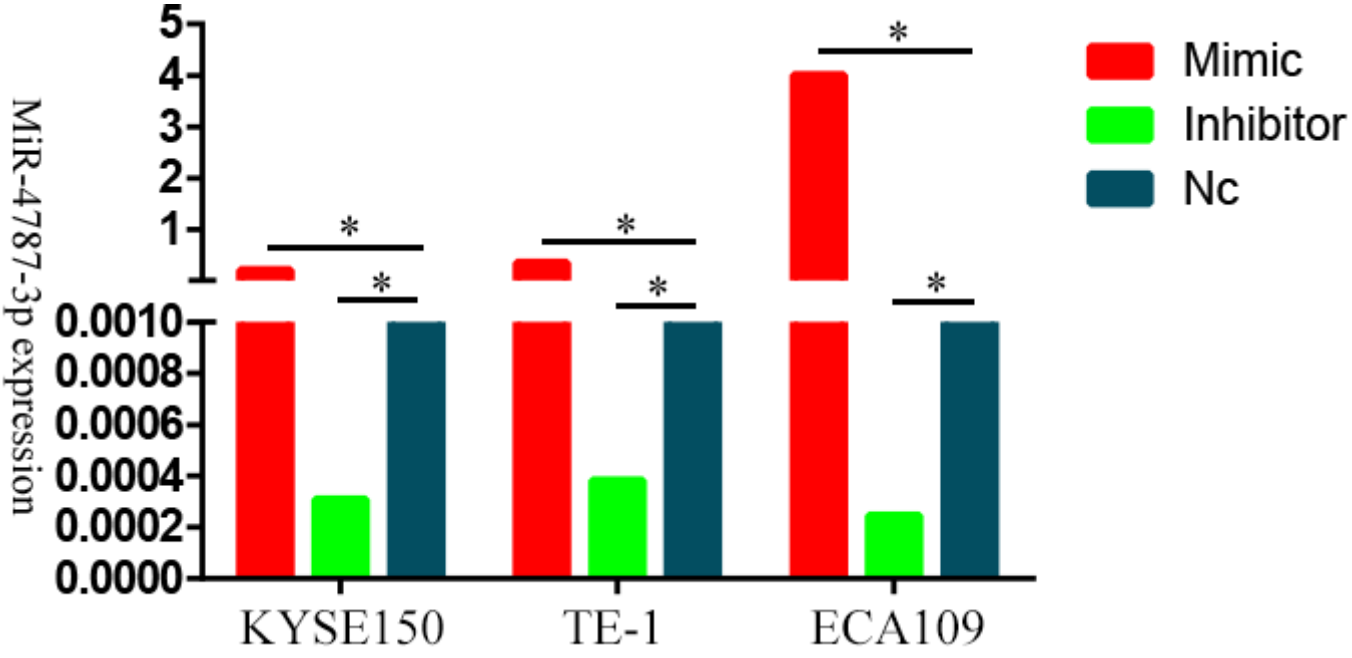
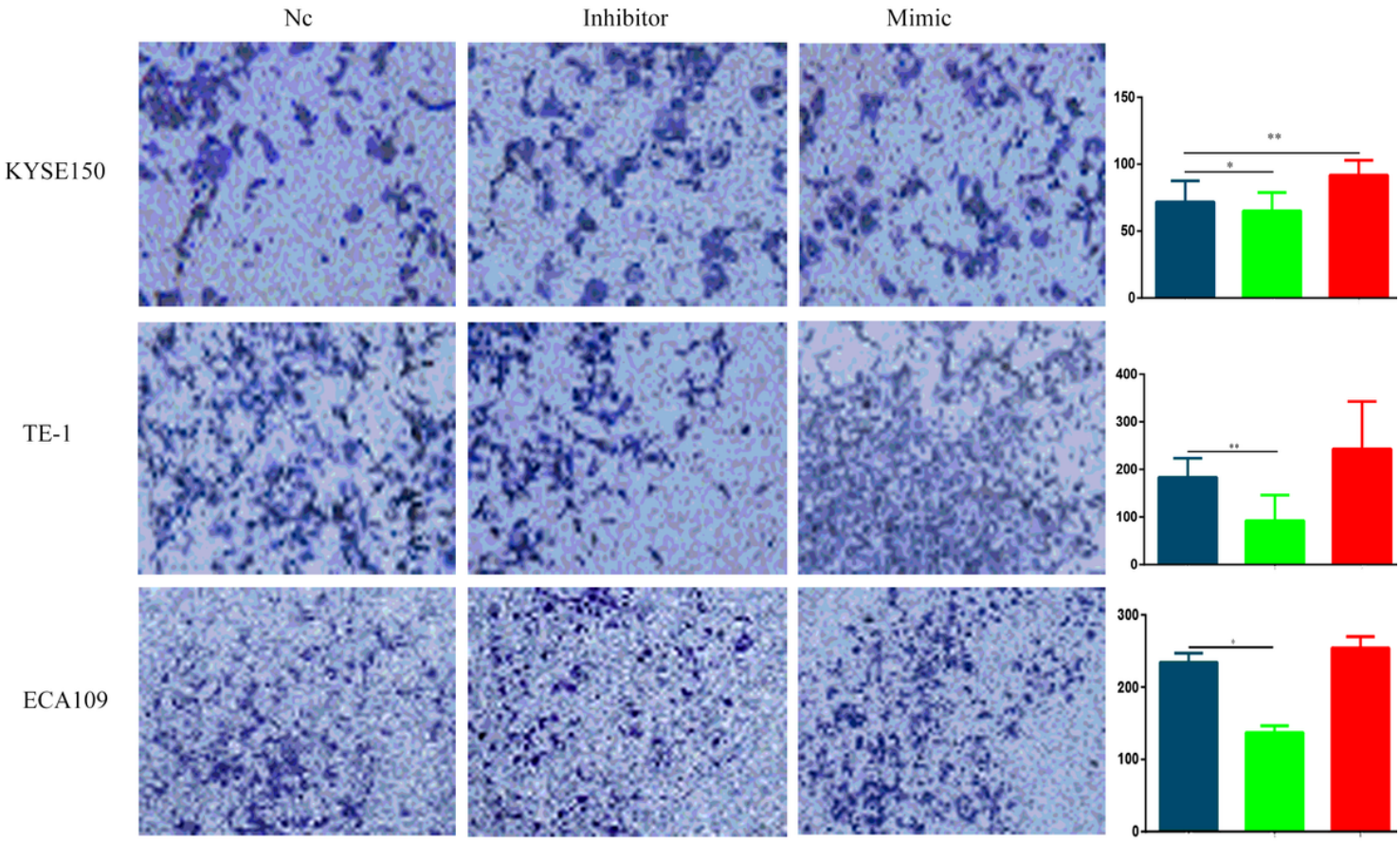



**Figure 3**

Expression of miR-4787-3p in ESCC cell lines after transfection with miR-4787-3p vector.




## Figure 4

The Migration of ESCC cells in vitro.

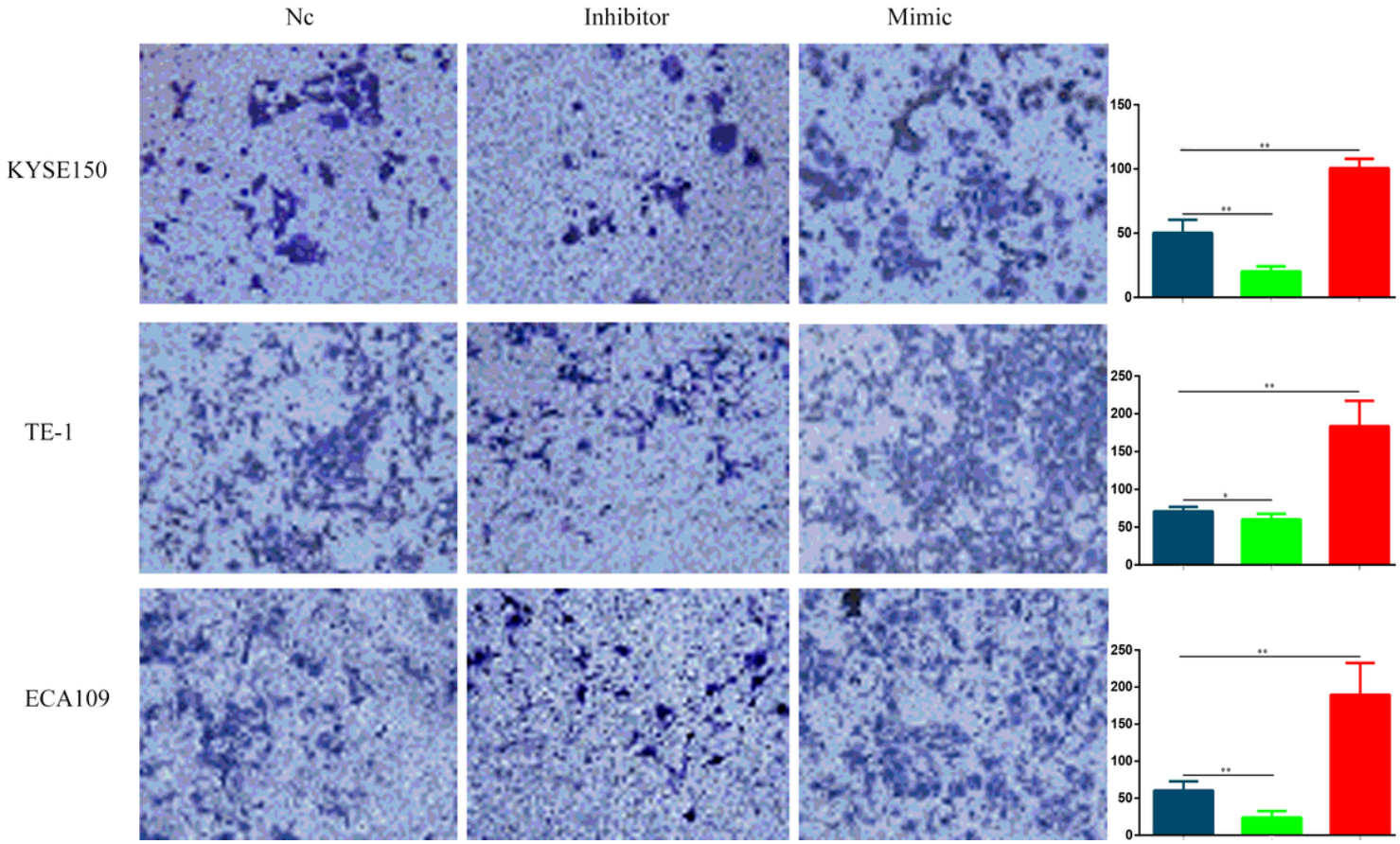



**Figure 5**

The invasion of ESCC cells in vitro.

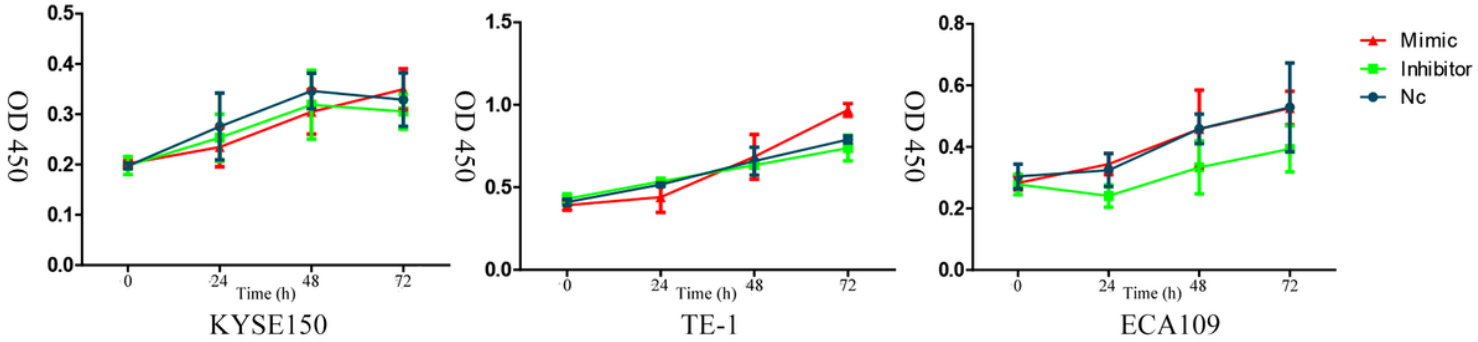



**Figure 6**

Cell proliferation curves of ESCC cells after transfection miR-4787-3p vector.

# Supplementary Files

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