Digestive and Liver Disease 51 (2019) 149–156

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Oncology

MiR-139-5p regulates VEGFR and downstream signaling pathways to inhibit the development of esophageal cancer

Wenpeng Jiao[a], Jinyan Zhang[b], Yuanyuan Wei[a], Junhua Feng[b], Ming Ma[b], Hongzheng Zhao[b], Lihong Wang[b], Wenjing Jiao[b,*]

[a] Dept. of Radiation Oncology, Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei Province, China
[b] Clinical Laboratory, Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei Province, China

ARTICLE INFO

Article history:
Received 12 February 2018
Received in revised form 20 July 2018
Accepted 23 July 2018
Available online 27 August 2018

Keywords:
Biomarker
High-risk area
miR-139-5p
Oesophageal cancer
Prognostic

ABSTRACT

Background: MiR-139-5p plays a significant role in tumorigenesis, metastasis and recurrence, suggesting that it may potentially be used as a promising biomarker for esophageal cancer diagnosis, prognosis and therapy. This study aimed to investigate the role and the mechanism of miRNA-139-5p in esophageal cancer.

Methods: This study included 11 patients from an area with a high incidence of esophageal cancer. The expression levels of miRNA-139-5p in esophageal cancer tissues and para-carcinoma tissues of 11 patients were measured. We examined the expression of miR-139-5p in serum obtained from 92 consecutive patients from Cixian, which is a region in Hebei Province with a high rate of histologically confirmed esophageal cancer. The expression of miR-139-5p in esophageal cancer cell lines was detected. In the KYSE150 cell line with the lowest expression level of miR-139-5p, we transfected a plasmid to upregulate the expression level and examined the role of miR-139-5p in esophageal squamous cell carcinoma proliferation, migration and invasion. We conducted a gene profiling study using miR-139-5p cell lines to detect the expression of significant genes related to tumor progression, including cyclinD1, E-cadherin and VEGFR-1. We then constructed luciferase reporters containing miR-139-5p, which contained wild-type (WT) or mutated-type (Mut) VEGFR-1 binding sites to investigate the target.

Results: MiRNA-139-5p expression levels in esophageal cancer tissues from 11 patients were significantly higher than those in para-carcinoma tissues. MiR-139-5p expression in the serum of 92 patients with esophageal cancer was associated with gender ($P = 0.039$) and TNM stage ($P = 0.015$). Factors that were not correlated with miR-139-5p expression were age ($P = 0.293$), smoking history ($P = 0.397$), length of tumor ($P = 0.309$), width of tumor ($P = 0.296$), depth of tumor ($P = 0.724$), lymphoma metastasis ($P = 0.531$) and postoperative therapy ($P = 0.884$). MiR-139-5p ($P = 0.013$) correlated significantly with observed survival rates. The lymphoma metastasis ($P = 0.005$) and TNM stage ($P = 0.000$) were significantly associated with observed survival rates. However, no significant relationships were found between the miR-139-5p and patient characteristics including gender, age, smoking history, tumor size and postoperative therapy. In the KYSE150 cell line, the expression level of miR-139-5p was the lowest. We transfected a plasmid to upregulate the expression level and found that the cell proliferation, metastasis and invasion abilities decreased. Upregulation of miR-139-5p inhibited the expression of Cyclin D1 and VEGFR-1 and increased the expression of E-cadherin. For further confirmation, we constructed luciferase reporters containing miR-139-5p, which contained wild-type (WT) or mutated-type (Mut) VEGFR-1 binding sites for target investigation. The results show that the corresponding VEGFR-1-Mut construct no longer suppressed miR-139-5p.

Conclusions: MiR-139-5p may be a novel therapeutic target and prognostic biomarker of esophageal cancer.

* Corresponding author.
E-mail addresses: jiaowenpeng163@163.com (W. Jiao), jinyanzhang66@163.com (J. Zhang), jysy0101@126.com (Y. Wei), 13833478260@163.com (J. Feng), maming19830419@163.com (M. Ma), 56178603@qq.com (H. Zhao), wlh.19630704@163.com (L. Wang), wenjing-79@163.com (W. Jiao).

https://doi.org/10.1016/j.dld.2018.07.017
1590-8658/

1. Introduction

According to GLOBOCAN [1], esophageal cancer is the eighth most common cancer worldwide, with an estimated 456,000 new cases in 2012; it is the sixth most common cause of death from cancer with an estimated 400,000 deaths. Approximately 80% of the cases worldwide occur in less developed regions. In China, there are approximately 223,000 new esophageal cancer cases and 197,200 esophageal cancer deaths [2]. Cixian is one of the highest risk areas for esophageal cancer in the world. The incidence rate of esophageal cancer in Cixian is 20 times higher than that of the world [2]. According to histopathological classification, esophageal cancer can be divided into squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma. Esophageal squamous cell carcinoma accounts for 80% of all esophageal cancers worldwide. It is the most common histological type of esophageal carcinoma in low-resource countries. Esophageal squamous cell carcinoma is prevalent in Asian countries, accounting for more than 95% of esophageal cancers [3]. The prognosis of esophageal cancer is poor with an approximately 20% overall 5-year survival rate [4].

Many esophageal cancer patients are already local advanced and advanced at the time of diagnosis, and esophageal cancer is susceptible to recurrence and metastasis, which are the main reasons for poor prognosis of esophageal cancer. Although there are many factors influencing the prognosis of esophageal cancer, additional influential factors need to be studied further. It is necessary for a new index to enable more accurate predictions of prognoses and to provide the best preoperative counseling to patients. Many studies have shown that miRNA plays an important role in the progression of cancers [5–8]. MicroRNAs (miRNAs) are short non-coding RNAs that regulate the expression of protein-coding genes by partially binding to specific target sites of mRNAs. miRNAs perform important functions in complicated cellular biological processes and can inhibit esophageal squamous cell carcinoma progression [6,9]. As we know, the miR-21 has been identified commonly as the miRNA overexpressed in cancer of the esophagus. One research study indicated that miR-21 promoted cell proliferation, migration, and resistance to apoptosis through PTEN/PI3K/AKT signaling pathway in esophageal cancer [10,11]. Conversely, miRNA can also inhibit the development of cancer of the esophagus. MiR-375 can inhibit cancer cell migration and invasion in esophageal squamous cell carcinoma by regulation of MMP13 [12]. We have reviewed many studies through TCGA, NCBI and other databases and found that miRNA-139-5p plays an important role in the development of cancer, but the mechanism of miRNA-139-5p in esophageal cancer is not clear. To obtain more information regarding the characteristics and survival prospect of patients with esophageal squamous cell carcinoma, we focused on the influence of miRNA-139-5p on the abnormal expression of genes or proteins. Among the miRNAs, differential expression of miRNA-139-5p serves a significant role in tumorigenesis, metastasis and recurrence, thus suggesting that it may potentially be used as a promising biomarker for cancer diagnosis, prognosis and therapy [13]. MiR-139-5p was expected to serve as a biomarker to eventually be implemented in a clinical setting. In addition, our research found miR-139-5p regulates VEGFR and its downstream primers signaling pathway to inhibit the development of esophageal cancer.

In this study, we examined the expression levels of miR-139-5p in 11 cases of esophageal carcinoma and para-carcinoma tissues and analyzed the associations between miR-139-5p levels and survival in 92 patients from an area with a high incidence of esophageal cancer to explore the possible utility of miR-139-5p as a prognostic biomarker for esophageal cancer.

2. Materials and methods

2.1. Patients and samples

This study included 11 patients Cixian, an area of high incidence of esophageal cancer. The patients were new cases in 2017. All patients were surgically treated at the Fourth Hospital of Hebei Medical University. The inclusion criterion was that the patient must have received a pathological diagnosis of primary esophageal cancer. Surgical samples were obtained within 30 min postoperation from each patient and immediately stored in liquid nitrogen. The expression levels of miRNA-139-5p in esophageal cancer tissues and para-carcinoma tissue of the 11 patients were measured.

These data were obtained from 96 consecutive patients from Cixian, which is a region in Hebei Province with a high rate of histologically confirmed esophageal cancer. All patients were also surgically treated at the Fourth Hospital of Hebei Medical University from January 1, 2009, to December 31, 2010. The inclusion criterion was the same to the above rules. A total of 96 cases that were admitted met the inclusion criterion and only 92 cases with completed follow-up evaluations were included in the study. The follow-up rate was 95.83%. Among these 92 patients, 50 died of esophageal cancer, whereas 42 were alive on December 31, 2015. The follow-up period ranged from 1 to 60 months, and the median follow-up period was 35 months. Overall, 65 patients were male (70.7%), and 27 patients were female (29.3%). The median age was 58 years old (range 43–78 years old). This study was approved by the Institutional Human Ethics Committee, and prior Informed Consent was obtained from all patients. The patient and tumor characteristics are shown in Table 1.

2.2. Follow-up studies

Follow-up evaluations were performed according to the standard follow-up system of the hospital every 6 months after patients were discharged. The deadline for follow-up evaluations was December 31, 2015. All patients were followed from the date of histological diagnosis until death or last day of follow-up and were followed for at least 20 months. The survival period was measured from the date of admission to the date of death or to the date of the follow-up deadline.

2.3. CCK-8 assays

Esophageal cancer cells were seeded in 96 well plates (5×10^3 cells/well). Cell proliferation was assessed at 12, 24, 36, and 48 h. Then, 20 μL of CCK-8 reagent was added to each well, incubated at 37 °C and 5% CO_2 for 2–4 h, and then transduced with 150 μL of DMSO. Absorbance was recorded at 450 nm with a universal microplate reader.

2.4. Transwell assays

For the transwell migration assay, 2×10^4 cells were plated in the top chamber with a noncoated membrane. For the invasion assay, 2.5×10^4 cells were plated in the top chamber with a Matrigel-coated membrane. In both assays, the cells were plated in medium without serum, and medium supplemented with serum was used as a chemoattractant in the lower chamber. The cells were incubated for 24 h, and the cells that did not migrate or invade through the pores were gently removed by a cotton swab. Cells on the lower surface of the membrane were fixed and stained with Giemsa crystal violet solution and counted under light microscopy magnification.

Table 1
Patient and tumor characteristics.

Cohort	Cases	%	Deaths	%
Gender				
Male	65	70.70%	37	56.90%
Female	27	29.30%	13	48.10%
Age				
<65	74	80.40%	38	51.40%
≥65	18	19.60%	12	66.70%
Smoking history				
Yes	54	58.70%	32	59.30%
No	38	41.30%	18	47.40%
Length				
<3 cm	8	8.70%	5	62.50%
3–8 cm	73	79.30%	39	53.40%
>8 cm	11	12.00%	6	54.50%
Width				
<2.5 cm	12	13.00%	8	66.70%
2.5–6 cm	74	80.40%	40	54.10%
>6 cm	6	6.50%	2	33.30%
Depth				
<2 cm	11	12.00%	5	45.50%
2–4 cm	75	81.50%	41	54.70%
>4 cm	6	6.50%	4	66.70%
N				
Lymphoma metastasis	49	53.30%	32	65.30%
No lymphoma metastasis	43	46.70%	18	41.90%
TNM stage				
I	2	2.20%	0	0.00%
II	43	46.70%	13	30.20%
III	47	51.10%	37	78.70%
Postoperative therapy				
Radiotherapy	4	4.30%	3	75.00%
Chemotherapy	57	62.00%	30	52.60%
Chemoradiotherapy	6	6.50%	5	83.30%
No	25	27.20%	12	48.00%

2.5. Quantitative real-time RT-PCR

Total miRNAs were isolated from frozen tissues using a mirVana miRNA isolation kit (Ambion), according to the manufacturer's instructions. The first strand cDNA was synthesized using RevertAid first strand cDNA synthesis kit (Fermentas), which was then amplified using TaqMan gene expression master mix (Applied Biosystems) and Applied Biosystems 7500 Real Time PCR system following the manufacturer's instructions. The qRT-PCR specific primers were purchased from Ambion. RNU6B served as endogenous control. Data were presented as fold differences based on calculations of $2^{-\Delta\Delta Ct}$.

2.6. Western blot analysis

Cells were lysed in RIPA buffer (Santa Cruz Biotechnology, standard protocol). Equal amounts of protein were separated by 10% SDS-PAGE and then transferred to polyvinylidene difluoride membranes (Amersham Biosciences, Buckinghamshire, UK). The membranes were incubated at room temperature for 1 h with 10% nonfat milk, followed by incubation with rabbit or mouse antibodies (anti-STAT3, 1:1500, Abcam, USA; anti-E-cadherin, 1:1500, Abcam, USA; anti-BTG2, 1:1500, Abcam, USA; anti-GAPDH, 1:2500, SIGMA, USA) at 4 °C overnight. After washing, blots were treated with the appropriate HRP-conjugated secondary antibody at room temperature for 1 h and then developed by enhanced chemiluminescence according to the manufacturer's protocol.

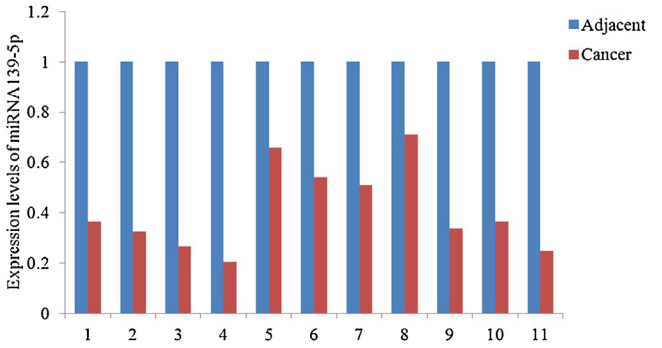

Fig. 1. Expression levels of miRNA139-5p in oesophageal squamous cell carcinoma.

2.7. Statistical analysis

The SPSS 21.0 and Excel software were used for the statistical analysis. We used χ^2 tests or the Fisher's exact probability method to determine the relationship between the miR-139-5p and patient information variables (i.e., gender, age, family history, personal history, TNM stage, and postoperative therapy). Observed survival rates were calculated using the Kaplan–Meier method. The observed survival rates of the patients in different groups were compared with the log-rank χ^2 test (inspection level 5, 0.05). Univariate and multivariate Cox regression models were used to determine the factors that influenced observed survival rates. All results were repeated three times and taken the mean to make sure the results were more accurate.

3. Results

MiRNA-139-5p expression level in esophageal cancer tissues was significantly lower than that in para-carcinoma tissue

We tested the miR-139-5p expression level in 11 cases of esophageal cancer tissue and para-carcinoma tissue in Cixian. The expression level of para-carcinoma tissue was selected as comparison. It showed that the expression level of miR-139-5p in esophageal tissue level decreases significantly (Fig. 1).

3.1. Association of the miR-139-5p with patient characteristics

Correlation between miR-139-5p and patient characteristics was assessed using χ^2 tests or Fisher's exact tests. The results showed that miR-139-5p expression was associated with gender ($P = 0.039$) and TNM stage ($P = 0.015$). The same test was used to analyze the correlations between miR-139-5p levels and age ($P = 0.293$), smoking history ($P = 0.397$), length of tumor ($P = 0.309$), width of tumor ($P = 0.296$), depth of tumor ($P = 0.724$), lymphoma metastasis ($P = 0.531$) and postoperative therapy ($P = 0.884$). None of the patient characteristics were correlated with miR-139-5p expression (Table 2).

3.2. Prognostic value of miR-139-5p in esophageal squamous cell carcinoma

A univariate analysis was used to show that miR-139-5p ($P = 0.013$) was significantly correlated with observed survival rates. The lymphoma metastasis ($P = 0.005$) and TNM stage ($P = 0.000$) were also shown to be significantly associated with the observed survival rates (Table 3). The univariate analysis showed that when evaluating the observed survival rates, better outcomes were observed in esophageal squamous cell carcinoma patients with high miR-139-5p expression (Fig. 2). In the univariate analysis, no significant relationship was found between the miR-139-5p

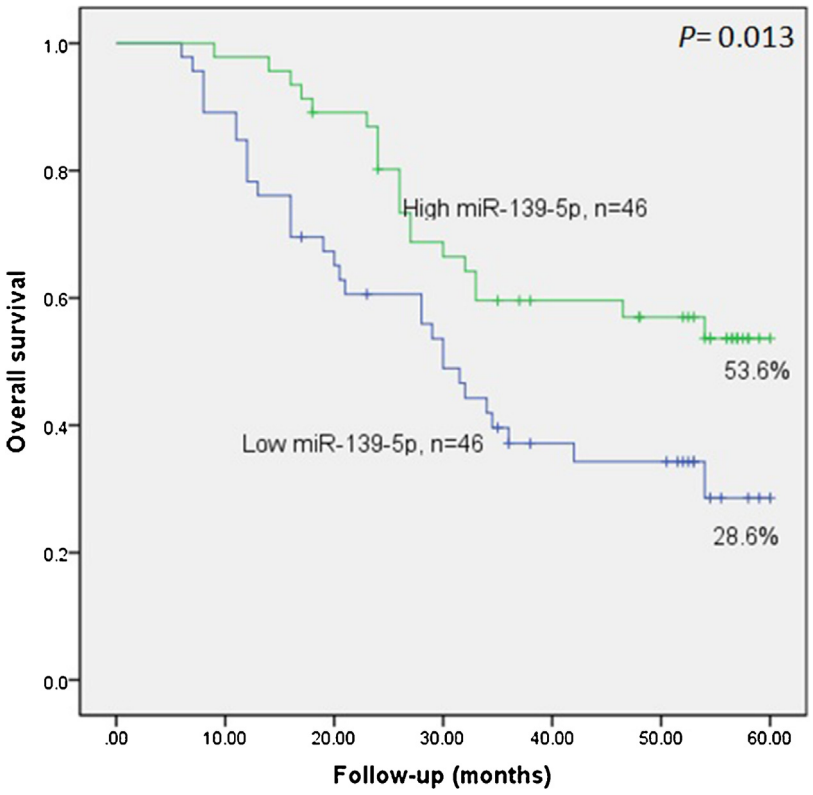

Fig. 2. Overall survivals in oesophageal squamous cell carcinoma patients with miR-139-5p expression ($p = 0.013$).

and patient characteristics including gender, age, smoking history, tumor size and postoperative therapy. Hence, in the multivariate analysis the lymphoma metastasis, TNM stage, the expression levels of miR-139-5p were included. The multivariate results indicate that there is a significant association between high expression levels of miR-139-5p and improved observed survival rates in esophageal cancer patients (RR: 0.497, 95%CI: 0.281–0.877). In addition, the lymphoma metastasis (RR: 2.260, 95%CI: 1.262–4.049) and TNM stage (RR: 4.503, 95%CI: 2.401–8.444) were found to be correlated with the observed survival rates (Table 4).

Upregulation of miR-139-5p inhibits the proliferation, migration and invasion of esophageal cancer cells

We examined the expression of miR-139-5p in 4 types of esophageal cancer cell lines (all of 4 types of esophageal cancer cell lines are esophageal squamous cell carcinoma), and the expression levels were low in all of them, with the expression levels in KYSE150 being the lowest (Fig. 3A). In the KYSE150 cell line with the lowest expression level of miR-139-5p, we transfected the plasmid, upregulated its expression level, and found that the cell proliferation, metastasis and invasion ability decreased (Fig. 3B). To investigate the mechanism which miR-139-5p inhibited cancer metastasis, we examined the role of miR-139-5p in esophageal squamous cell carcinoma proliferation, migration and invasion. The proliferation of miR-139-5p cell lines was dramatically decreased by 42% compared with that of the KYSE150 cell lines, according to the CCK-8 experimental results (Fig. 3C). We tested cellular migration and invasion levels using transwell chambers coated with Matrigel or without Matrigel. It was found that upregulating miR-139-5p significantly decreased the cell migration and invasion abilities of the miR-139-5p cell lines by 49.4% and 29.0%, respectively, compared with those of the KYSE150 cell lines (Fig. 3D, E) ($^*p < 0.05$).

Upregulation of miR-139-5p inhibits the expression of Cyclin D1 and VEGFR-1 and increased the expression of E-cadherin

We conducted a gene profiling study using miR-139-5p cell lines to detect the expression of significant genes related to tumor progression, including cyclinD1, E-cadherin and VEGFR-1 (GAPDH as inner reference) using Western blot. The results showed that the expression of E-cadherin was significantly upregulated in the miR-139-5p compared with the KYSE150 cells and the expression of cyclinD1 and VEGFR-1 was significantly down-regulated in miR-139-5p. We did not find differences in the expression levels of other genes (Fig. 4) ($^*p < 0.05$).

3.3. *MiR-139-5p regulates VEGFR-1 by targeting VEGFR-1*

VEGFR-1 may play a role in dysregulation of miR-139-5p. For further confirmation, we constructed luciferase reporters containing miR-139-5p, which contained wild-type (WT) or mutated (Mut) VEGFR-1 binding sites, for target investigation. We found that the corresponding VEGFR-1-Mut construct no longer suppressed miR-139-5p (Fig. 5A and B), indicating that miR-139-5p is a VEGFR-1-targeting miRNA ($^*p < 0.05$).

4. Discussion

Substantial research had been devoted to esophageal cancer pathogenesis, mechanism and prognosis in Cixian in order to prevent and control esophageal cancer. We searched microRNA associated with esophageal squamous cell carcinoma in PubMed, EMBASE, and the Central Registry of Controlled Trials of the Cochrane Library and so on, and finally found that miR-139-5p was closely related to the progression of esophageal squamous cell carcinoma. The first step was to verify miR-139-5p expression level in the esophageal cancer tissues. The results showed that the expression level of miR-139-5p in esophageal tissue level decreases significantly, which suggested that miR-139-5p may inhibit the development of esophageal cancer. However, some esophageal

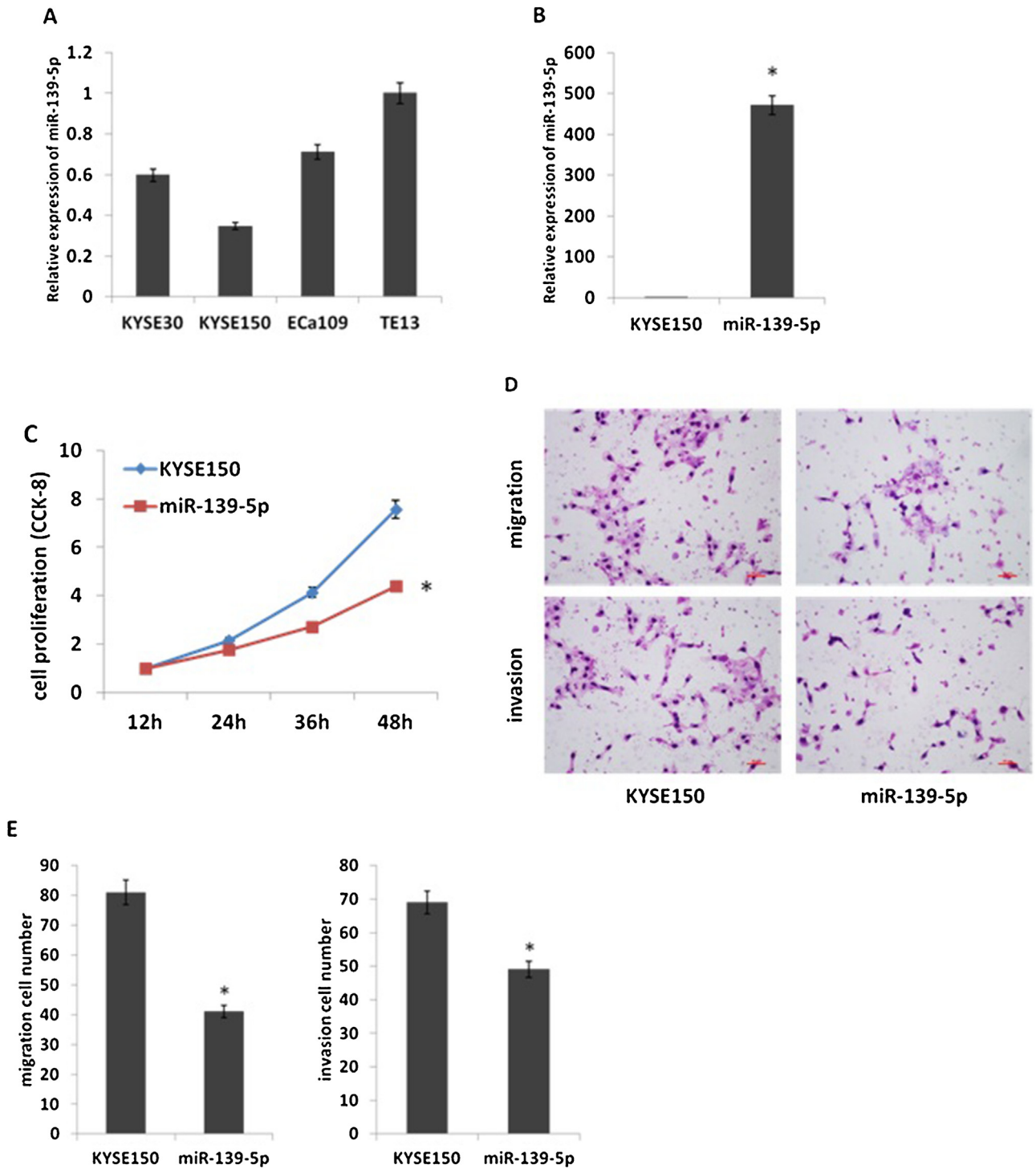

Fig. 3. A: The expression of miR-139-5p in four types of oesophageal cancer cell lines. B: the relative miR-139-5p expression was measured according to the standard value of KYSE150 cells, which was 1. C: the proliferation of miR-139-5p cells was compared with that of KYSE150 cells using CCK-8 assays. D and E: the migration and invasion of miR-139-5p cells was compared with that of KYSE150 cells using a transwell assay (*$p < 0.05$).

cancer patients did not undergo surgery and esophageal cancer tissue is not easy to obtain. It is not suitable as a tumor marker in esophageal cancer tissues. Therefore, we further examined the expression of miR-139-5p in serum and analyzed the correlation between miR-139-5p levels and the prognosis of esophageal cancer. The results showed that miR-139-5p expression was associated with TNM stage and survival time. Low expression of miR-139-5p is associated with poor prognosis of esophageal cancer. To further assess the function of miR-139-5p in esophageal cancer, we upregulated miR-139-5p and found it inhibited the proliferation, migration and invasion of esophageal cancer cells. It indicated that the miR-139-5p plays a regulatory role to inhibit the development of esophageal cancer. To explore the mechanism of miR-139-5p inhibition of esophageal cancer progression, we up-regulated miR-139-5p and found that it inhibited the expression of Cyclin D1 and VEGFR-1 and increased the expression of E-cadherin. MiR-139-5p inhibits target gene expression by binding to target gene mRNA

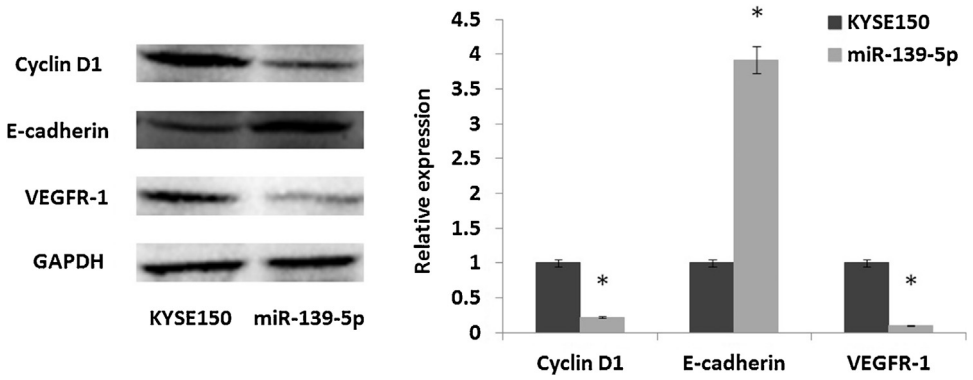

Fig. 4. Up-regulation of miR-139-5p inhibits the expression of Cyclin D1 and VEGFR-1 and increased the expression of E-cadherin (*$p < 0.05$).

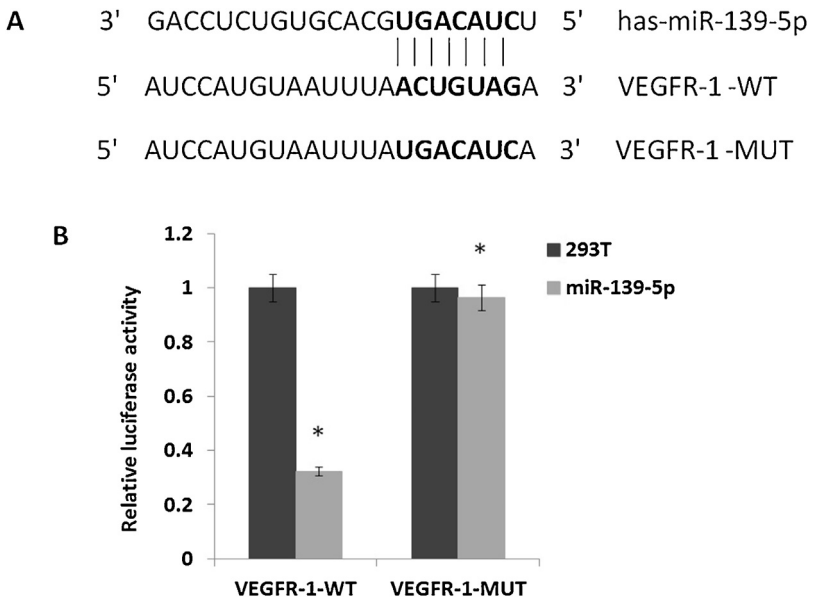

Fig. 5. A: Putative miR-139-5p-binding sequence of VEGFR-1 RNA. Mutation was generated on the VEGFR-1 RNA sequence in the complementary site for the seed region of miR-139-5p. B: 293T and miR-139-5p cells were transfected with vector, wild-type VEGFR-1 (VEGFR-1-WT) or mutant VEGFR-1 (VEGFR-1-Mut) with a mutation of the miRNA binding site; 48 h after transfection, the luciferase activity was measured using a dual-luciferase reporter gene assay system (*$p < 0.05$).

3′-UTR of VEGFR-1 to suppress the proliferation and metastasis of esophageal cancer.

As mentioned above, microRNAs (miRNAs) regulate the expression of protein-coding genes by partially binding to specific target sites of mRNAs. They perform an important function in complicated cellular biological processes. There are many studies about the regulatory mechanism of miRNAs in esophageal cancer, but there is little research on miR-139-5p acting upon esophageal cancer. This study demonstrates that miR-139-5p regulates VEGFR and downstream targets in the signaling pathway to inhibit the development of esophageal cancer.

MiR-139-5p is located within the second intron of the phosphodiesterase 2A gene on chromosome 11q13.4 [14,15]. The miR-139-5p gene is transcribed by the RNA polymerase II to produce a long RNA molecule, primary-miR-139-5p (pri-miR-139-5p) in the nucleus. Pri-miR-139-5p is processed into a hairpin-shaped stem-loop precursor (pre-miR-139-5p) by the action of a RNase III endonuclease, Drosha and a cofactor, DGCR8/Pasha [16,17]. Pre-miR-139-5p is cleaved again in the cytoplasm by Dicer, a second RNase III endonuclease. Subsequently, mature forms of miR-139-5p are formed. Mature miRNAs are incorporated into the RNA-induced silencing complex (RISC) and guide the RISC to binding the 3′-UTR of the target gene mRNA, leading to the degradation or translational inhibition of target mRNA [17,18]. Studies have indicated there was the possibility for a single miRNA to regulate hundreds of potential targets [19,20].

Accurate and reliable results of diagnosis serve a critical role in guiding treatment and estimating prognosis. MiR-139-5p could potentially be used as a biomarker for screening detection in cancer. Research has shown that reduced levels of miR-139-5p at terminal stage of esophageal squamous cell carcinoma imply a latent capacity to improve early diagnosis [21]. Conditional logistic regression analysis also demonstrated a close correlation between reduced miR-139-5p expression and increased risk for esophageal cancer (odds ratio = 2.024) [22]. Reports have suggested that miR-139-5p possible targets, including the oncogenic nuclear receptor subfamily 5 group A member 2 (NR5A2), topoisomerase II α (TOP2a), insulin-like growth factor 1 receptor (IGF-1R) and Rho-kinase 2 (ROCK2), are involved in cancer progression and metastasis. miR-139-5p exerts a growth- and invasion-suppressing function in human esophageal squamous cell carcinomas by targeting the oncogenic nuclear receptor subfamily 5 group A member 2 (NR5A2) [22]. NR5A2, also known as liver receptor homolog-1, enhances cell cycle progression through the G1 phase and cell proliferation by inducing the expression of cyclins D1 and E1, and prevents cells from apoptosis [23,24]. A previous study demonstrated that its

Table 2
Association of the miR-139 with patient characteristics.

Factor	Low miR-139 (n = 46)		High miR-139 (n = 46)		χ^2	*P*
	No.	%	No.	%		
Gender					4.246	0.039
Male	28	43.10%	37	56.90%		
Female	18	66.70%	9	33.30%		
Age					1.105	0.293
<65	39	52.70%	35	47.30%		
≥65	7	38.90%	11	61.10%		
Smoking history					0.717	0.397
Yes	25	46.30%	29	53.70%		
No	21	55.30%	17	44.70%		
Length					2.59	0.309
<3 cm	4	50.00%	4	50.00%		
3–8 cm	39	53.40%	34	46.60%		
>8 cm	3	27.30%	8	72.70%		
Width					2.747	0.296
<2.5 cm	6	50.00%	6	50.00%		
2.5–6 cm	39	52.70%	35	47.30%		
>6 cm	1	16.70%	5	83.30%		
Depth					0.907	0.724
<2 cm	5	45.50%	6	54.50%		
2–4 cm	39	52.00%	36	48.00%		
>4 cm	2	33.30%	4	66.70%		
N					0.393	0.531
Lymphoma metastasis	23	46.90%	26	53.10%		
No lymphoma metastasis	23	53.50%	20	46.50%		
TNM stage					7.721	0.015
I	1	50.00%	1	50.00%		
II	15	34.90%	28	65.10%		
III	30	63.80%	17	36.20%		
Postoperative therapy					0.999	0.884
Radiotherapy	2	50.00%	2	50.00%		
Chemotherapy	30	52.60%	27	47.40%		
Chemoradiotherapy	2	33.30%	4	66.70%		
No	12	48.00%	13	52.00%		

overexpression resulted in the posttranslational truncation of E-cadherin and increased expression of MMP-9 [25], consequently contributing to cancer motility and invasion.

In this study, we found that miR-139-5p is a VEGFR-1-targeting miRNA. Blocking angiogenesis by inhibiting VEGF represents an established therapeutic strategy in many cancers. However, the role of VEGFR-1 in tumor biology has remained elusive [26]. One study reported that VEGFR1 or VEGFR2 blocking can significantly attenuate VEGF-induced Src and Erk signaling, as well as the proliferation and migration of $VEGFR1^+$ and $VEGFR2^+$ bone marrow cells and their pro-invasion effect on cancer cells. In vivo data of this study showed for the first time that systemic blocking of $VEGFR1^+$ or $VEGFR2^+$ nontumor cells with neutralizing antibodies is sufficient to significantly suppress esophageal tumor growth, angiogenesis and metastasis in mice [27]. Another research study showed that VEGF expression correlates significantly with the coexpression of its receptors, VEGFR-1 and VEGFR-2; however, these receptors did not appear to contribute directly to tumor progression. Nevertheless, VEGF and its receptors represented a logical target for antiangiogenic therapy for esophageal SCC [28]. MiR-139-5p is a VEGFR-1-targeting miRNA that plays a key role in inhibition of esophageal cancer.

The clinical significance of miR-139-5p is that it can predict and analyze survival time of esophageal cancer. MiR-139-5p is a marker of esophageal cancer and can play a key role to detect the serum level of miR-139-5p in esophageal cancer patients. If the expression of serum levels of miR-139-5p is low, it may represent a poor prognosis for esophageal cancer patients. The poor prognosis should be given appropriate treatment, such as VEGFR-targeted drugs.

Table 3
Prognostic factors according to the univariate analysis.

Factor	Case	OS rate (%)			*P*
		1 year	3 years	5 years	
Gender					0.265
Male	65	83.1	47.7	37.9	
Female	27	88.9	54.2	49.7	
Age					0.139
<65	74	89.2	51.3	45	
≥65	18	83.3	35.9	29.9	
Smoking history					0.296
Yes	54	88.9	43.8	35.1	
No	38	86.8	54.6	50.4	
Length					0.825
<3 cm	8	75	37.5	37.5	
3–8 cm	73	91.8	50	41.5	
>8 cm	11	72.7	45.5	45.5	
Width					0.504
<2.5 cm	12	83.3	33.3	33.3	
2.5–6 cm	74	89.2	50	41.8	
>6 cm	6	83.3	62.5	62.5	
Depth					0.486
<2 cm	11	90.9	54.5	54.5	
2–4 cm	75	89.3	48.6	40.8	
>4 cm	6	50	33.3	33.3	
N					0.005
Lymphoma metastasis	49	81.6	37.2	22.7	
No lymphoma metastasis	43	95.3	60.4	57.9	
TNM stage					0
I	2	100	100	100	
II	43	95.3	71	68.2	
III	47	80.9	25.2	15	
Postoperative therapy					0.117
Radiotherapy	4	50	25	25	
Chemotherapy	57	89.5	48.7	44	
Chemoradiotherapy	6	83.3	16.7	16.7	
No	25	84	59.3	49.4	
miR-139					0.013
High	46	95.7	59.6	53.6	
Low	46	78.3	37.1	28.6	

Table 4
Prognostic factors according to the multivariate analysis.

Factor	*β*	SE	*Wald*	*RR* (95% CI)	*P*
N	0.816	0.297	7.518	2.260 (1.262–4.049)	0.006
TNM stage	1.505	0.321	21.997	4.503 (2.401–8.444)	0.000
miR-139-5p	−0.700	0.290	5.823	0.497 (0.281–0.877)	0.016

Moreover, miR-139-5p expression levels can assist in selecting the suitable treatment option. For example, metastatic esophageal cancer patients should choose chemotherapy, not radiotherapy.

Taken together, miR-139-5p plays an important role in esophageal cancer regulatory mechanisms. It has substantial clinical significance and implications. This study indicates the tumor-suppressive role of miR-139-5p, reflecting the status of tumor growth and spread. MiR-139-5p is a novel therapeutic target and prognostic biomarker of esophageal cancer that acts by regulating VEGFR and the signaling pathways of its downstream primers to inhibit the development of esophageal cancer.

Conflict of interest

None declared.

References

[1] GLOBOCAN. Estimated cancer incidence, mortality and prevalence worldwide in 2012; 2012. Available at: http://globocan.iarc.fr/Pages/fact sheets cancer.aspx.

[2] He J, Chen W. Chinese cancer registry annual report, 2012. Beijing: Press of Military Medical Sciences; 2012. p. 160–1.

[3] Anvari K, Sima HR, Seilanian Toussi M, et al. EGFR expression in patients with esophageal squamous cell carcinoma and its association with pathologic response to preoperative chemoradiotherapy: a study in Northeastern Iran. Arch Iran Med 2017;20(4):240–5.

[4] Zeng H, Zheng R, Guo Y, Zhang S, Zou X, Wang N, et al. Cancer survival in China, 2003–2005: a population-based study. Int J Cancer 2015;136:1921–30.

[5] Li BX, Yu Q, Shi ZL, et al. Circulating microRNAs in esophageal squamous cell carcinoma: association with locoregional staging and survival. Int J Clin Exp Med 2015;8:7241–50.

[6] Mao Y, Li L, Liu J, et al. MiR-495 inhibits esophageal squamous cell carcinoma progression by targeting Akt1. Oncotarget 2016;7(32):51223–36.

[7] Zhang W, Qian S, Yang G, et al. MicroRNA-199 suppresses cell proliferation, migration and invasion by downregulating RGS17 in hepatocellular carcinoma. Gene 2018;659:22–8.

[8] Zhou B, Sun C, Hu X, et al. MicroRNA-195 suppresses the progression of pancreatic cancer by targeting DCLK1. Cell Physiol Biochem 2017;44(5):1867–81.

[9] Huang LL, Huang LW, Wang L, et al. Potential role of miR-139-5p in cancer diagnosis, prognosis and therapy. Oncol Lett 2017;14(2):1215–22.

[10] Lu Z, Liu M, Stribinskis V, et al. MicroRNA-21 promotes cell transformation by targeting the programmed cell death 4 gene. Oncogene 2008;27(31):4373–9.

[11] Wu YR, Qi HJ, Deng DF, et al. MicroRNA-21 promotes cell proliferation, migration, and resistance to apoptosis through PTEN/PI3K/AKT signaling pathway in esophageal cancer. Tumour Biol 2016;37(9):12061–70.

[12] Osako Y, Seki N, Kita Y, et al. Regulation of MMP13 by antitumor microRNA-375 markedly inhibits cancer cell migration and invasion in esophageal squamous cell carcinoma. Int J Oncol 2016;49(6):2255–64.

[13] Pang C, Liu M, Fang W, et al. MiR-139-5p is increased in the peripheral blood of patients with prostate cancer. Cell Physiol Biochem 2016;39(3):1111–7.

[14] Corbetta S, Vaira V, Guarnieri V, et al. Differential expression of microRNAs in human parathyroid carcinomas compared with normal parathyroid tissue. Endocr Relat Cancer 2010;17:135–46.

[15] Shen K, Mao R, Ma L, et al. Post-transcriptional regulation of the tumor suppressor miR-139-5p and a network of miR-139-5p-mediated mRNA interactions in colorectal cancer. FEBS J 2014;281:3609–24.

[16] Liu X, Chen Z, Yu J, et al. MicroRNA profiling and head and neck cancer. Comp Funct Genomics 2009, 837514.

[17] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10:704–14.

[18] Qin C, Huang RY, Wang ZX. Potential role of miR-100 in cancer diagnosis, prognosis, and therapy. Tumour Biol 2015;36:1403–9.

[19] Saba R, Goodman CD, Huzarewich RL, et al. A miRNA signature of prion induced neurodegeneration. PLoS One 2008;3:e3652.

[20] Gu DN, Huang Q, Tian L. The molecular mechanisms and therapeutic potential of microRNA-7 in cancer. Expert Opin Ther Targets 2015;19:415–26.

[21] Yang M, Liu R, Sheng J, et al. Differential expression profiles of microRNAs as potential biomarkers for the early diagnosis of esophageal squamous cell carcinoma. Oncol Rep 2013;29:169–76.

[22] Liu R, Yang M, Meng Y, et al. Tumor-suppressive function of miR-139-5p in esophageal squamous cell carcinoma. PLoS One 2013;8(10):e77068.

[23] Wagner RT, Xu X, Yi F, et al. Canonical Wnt/β-catenin regulation of liver receptor homolog-1 mediates pluripotency gene expression. Stem Cells 2010;28:1794–804.

[24] Wang S, Lan F, Huang L, et al. Suppression of hLRH-1 mediated by a DNA vector-based RNA interference results in cell cycle arrest and induction of apoptosis in hepatocellular carcinoma cell BEL-7402. Biochem Biophys Res Commun 2005;333:917–24.

[25] Chand AL, Herridge KA, Thompson EW, et al. The orphan nuclear receptor LRH-1 promotes breast cancer motility and invasion. Endocr Relat Cancer 2010;17:965–75.

[26] Schultze A, Ben Batalla I, Riethdorf S, et al. VEGFR-1 expression levels predict occurrence of disseminated tumor cells in the bone marrow of patients with esophageal carcinoma. Clin Exp Metastasis 2012;29(8):879–87.

[27] Xu WW, Li B, Lam AK, et al. Targeting VEGFR1- and VEGFR2-expressing non-tumor cells is essential for esophageal cancer therapy. Oncotarget 2015;6(3):1790–805.

[28] Kato H, Yoshikawa M, Miyazaki T, et al. Expression of vascular endothelial growth factor (VEGF) and its receptors (Flt-1 and Flk-1) in esophageal squamous cell carcinoma. Anticancer Res 2002;22(6C):3977–84.