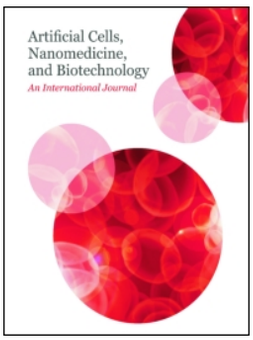

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MicroRNA-155 acts as a diagnostic and prognostic biomarker for oesophageal squamous cell carcinoma

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ABSTRACT

MicroRNA-155 is over-expressed in many human cancers, but researches on its association with malignant oesophageal squamous cell carcinoma (ESCC) are limited. The aim of the present study was to evaluate the potential value of *miR-155* as a biomarker for ESCC diagnosis and prognosis. In this study, we found that *miR-155* was significantly increased in ESCC tissues compared with the paired adjacent tissues and healthy normal controls ($p < .001$), according to qRT-PCR, which suggested that *miR-155* might act as an oncogene in ESCC. In addition, clinical features such as the depth of tumour invasion, tumour size, and TNM stage were all proved to impact the expression of *miR-155* ($p < .01$). Then, ROC curve analysis, reaching an AUC of 0.870, and a sensitivity and specificity of 83.5% and 77.5%, respectively, revealed that *miR-155* was a predictive factor for ESCC. As well, high expression of *miR-155* was associated with poor overall survival of the patients (log-rank test, $p = .004$), according to Kaplan-Meier analysis. *MiR-155* might be an independent predictor for overall survival in ESCC patients, manifested by Cox regression analysis (HR = 16.94, 95%CI = 3.33–86.12, $p = .001$). Taken together, *miR-155* could be an independent diagnostic and prognostic biomarker for ESCC.

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KEYWORDS
Oesophageal squamous cell carcinoma; MiR-155; diagnosis; prognosis

Introduction

Oesophageal cancer, an aggressive malignancy, is one of the leading causes of cancer-related mortality worldwide, showing a variable geographic distribution, while oesophageal squamous cell carcinoma (ESCC) is the most common histological type of this disease [1,2]. Despite remarkable advances in diagnostic and therapeutic techniques for the malignancy, like preoperative chemotherapeutic, radiotherapeutic and surgical treatment, the survival rate of ESCC patients has not been qualitatively improved [3,4], still witnessing poor prognoses. To improve the diagnosis and prognosis of ESCC patients, more predictive and useful tumour biomarkers need to be identified. Therefore, it is an important strategy to develop sensitive and specific screening technologies for patients with oesophageal cancer.

MiRNAs, about 18–25 nucleotides, are a large family of non-coding RNAs that are significant in regulating gene expressions at post-transcriptional level. They can play regulatory roles in cell development, metabolism, immunity, proliferation, differentiation, and apoptosis [5,6]. Moreover, miRNAs can improve mRNA degradation, prevent mRNA from being translated, and thus regulate gene expression by binding to complementary target mRNA [7]. In addition, earlier findings demonstrated that miRNAs were involved in regulating tumorigenesis, tumour development and progression [8], and clinical and quantitative studies confirmed that the expression levels of some miRNAs were associated with overall survival of patients with malignant tumours. Therefore, miRNAs may serve as potential diagnostic or prognostic biomarkers for cancers.

MicroRNA-155 (*MiR-155*) is a typical multifunctional miRNA involved in numerous biological processes including inflammation and immunity. Recent studies showed that *miR-155* expression might be associated with the diagnosis or prognosis of colorectal cancer, gastric cancer, or non-small cell lung cancer [9–11]. However, we still lack evidence for the prognostic role of *miR-155* expression in ESCC. Thus, the aim of the study was to investigate clinical significance of *miR-155* expression in ESCC, including its diagnostic and prognostic value in the malignancy.

Materials and methods

Patients and specimens

We collected tumour tissue samples from 96 ESCC patients who underwent surgery at the First Affiliated Hospital of

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Zhengzhou University, but received no preoperative treatments (chemotherapy or radiotherapy). In addition, paired adjacent normal tissues and 20 healthy specimens were used as controls. Clinicopathologic data of the patients were summarised in Table 1. Complete information from a follow-up for at least 5 years was available through telephone call and questionnaire letters to the patients or their relatives. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All patients agreed to the study procedure and signed consent forms. All specimens were anonymous and handled according to the ethical and legal standards.

Table 1. Relationship between *miR-155* expression and clinicopathological characteristics.

		miR-155 level		
Variable	N	High	Low	*p* Value
Age				.081
≧60	45	20	25	
<60	51	31	20	
Sex				.444
male	43	22	21	
female	53	29	24	
Tumour size (mm)				.001**
≧50	53	36	17	
<50	43	15	28	
Depth of tumour invasion				.002**
T1, T2	48	18	30	
T3, T4	48	33	15	
Lymph node metastasis				.576
Absent	47	25	22	
Present	49	26	23	
Distant metastasis				.322
Absent	46	26	20	
Present	50	25	25	
TNM Stage				.000**
I, II	48	16	32	
III, IV	48	35	13	

**$p < .01$

Reverse transcription and qRT-PCR for miR-155

To detect miRNA level adopting real-time PCR, total RNA was purified from all ESCC samples and 20 normal control specimens using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA samples only reaching an OD A260/A280 ratio close to 2.0 were adopted, which indicated that the RNA was pure. *miR-155* and U6 specific cDNAs were synthesised with TaqMan MicroRNA Reverse Transcription Kit, according to the manufacturer's protocol. Then reverse transcription products were amplified and detected through quantitative real-time PCR (qRT-PCR) using TaqMan microRNA assay. *MiR-155* expression levels were analysed as relative quantities, *via* normalised to endogenous control (U6). Primer sequences were as follows: *miR-155* RT:5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTT-GAGACCCCTAT-3′; *miR-155* F: 5′-ACACTCCAGC TGGGTTAATGCTAATCGTGAT-3′, R: 5′-TGGTGTCGTGGAGT CG-3′. U6: F: 5′-CTCGCTTCGGCAGC ACA-3′, R: 5′-AACGCTTCACGAATTTGCGT-3′.

Statistical analysis

Statistical analyses were performed with SPSS 13.0 software (SPSS, Chicago, IL). Student's *t*-test was used to analyse difference in *miR-155* expression between ESCC tissues and the two groups of control specimens. Malignant samples were divided into high and low level groups, using their median *miR-155* expression level as a cut-off value. Associations between *miR-*

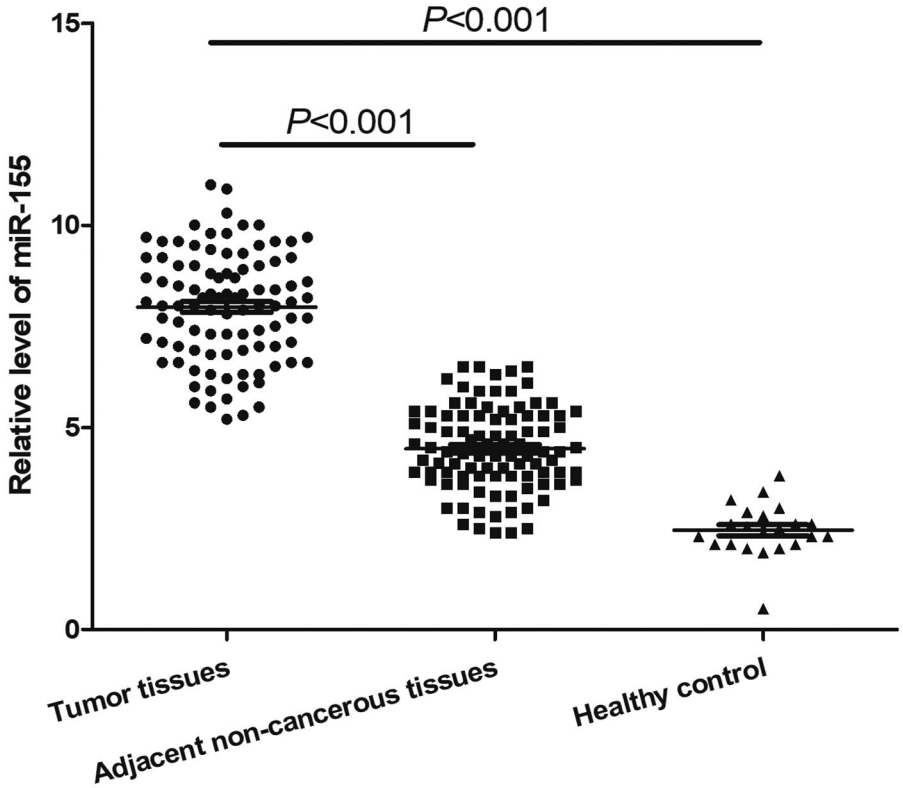

Figure 1. Relative *miR-155* expression levels. The expression of *miR-155* in ESCC tissues was significantly higher than that in matched adjacent samples ($p < .001$) and healthy control specimens ($p < .001$).

155 expression and clinicopathological characteristics were analysed *via* Chi-square test. Receiver operating characteristic (ROC) curve was used for diagnosis analysis. Survival curves were constituted using the Kaplan–Meier method with log-rank test. Univariate and multivariate analyses were performed with Cox regression analysis. All data were shown as mean ± SD, and $p < .05$ was considered as statistically significant level.

Results

The expression of miR-155 was increased in ESCC

In this study, qRT-PCR was used to measure the expression levels of *miR-155* in ESCC tissues, adjacent normal tissues and healthy controls (The expression level of every specimen was displayed in Supplementary_materials). The final results showed that after normalised to U6, the relative expression of *miR-155* in ESCC specimens and matched adjacent normal tissues were 7.98 ± 1.35 and 4.47 ± 1.04, respectively, such figure in 20 normal control specimens was 2.55 ± 0.51 (Figure 1). The differences in *miR-155* level between the ESCC samples and two groups of controls were both statistically significant ($p < .001$), indicating that *miR-155* expression was increased in ESCC. The results demonstrated that *miR-155* might play an oncogenic role in ESCC.

Table 2. Multivariate analysis of prognostic factors in ESCC.

Parameters	HR (95%CI)	*p*
Depth of tumour invasion	6.06 (1.62–22.67)	.007**
High *miR-155* level	16.94 (3.33–86.12)	.001**
Low *miR-155* level	–	

**$p < .01$

Relationship between miR-155 and clinicopathological characteristics of ESCC patients

To explore correlation between *miR-155* expression and pathological characteristics in ESCC, we manually divided ESCC specimens into two groups: high group with a relative expression level of *miR-155* higher than 7.98 (mean value for the expression) and low group with *miR-155* level lower than 7.98. Accordingly, 51 cases were classified into high expression group while 45 in low expression group. Then we investigated the association of *miR-155* expression with clinicopathological data of the patients. Results showed that *miR-155* level was significantly associated with tumour size ($p = .001$), depth of tumour invasion ($p = .002$) and TNM stage ($p = .000$). However, there was no association between *miR-155* expression and age, sex, lymph node metastasis or distant metastasis (all, $p > .05$, Table 2).

In addition, we also compared the expression levels of *miR-155* among ESCC patients based on their age, tumour size, tumour invasion and TNM stages. As displayed in Figure 2, patients with large tumour size, deep tumour invasion and advanced TNM stages exhibited significantly high levels of *miR-155* ($p < .05$ for all), but there was no obvious association between age and *miR-155* expression ($p = .094$).

Diagnostic value of miR-155 in ESCC

ROC curve analysis was performed to distinguish ESCC patients from healthy controls with an area under the curve (AUC) of 0.870 (95%CI = 0.797–0.943, $p < .001$, Figure 3) accompanied by a sensitivity of 83.5% and a specificity of 77.5% at the cut-off of 7.75. The results showed that *miR-155* was an useful marker in diagnosing ESCC.

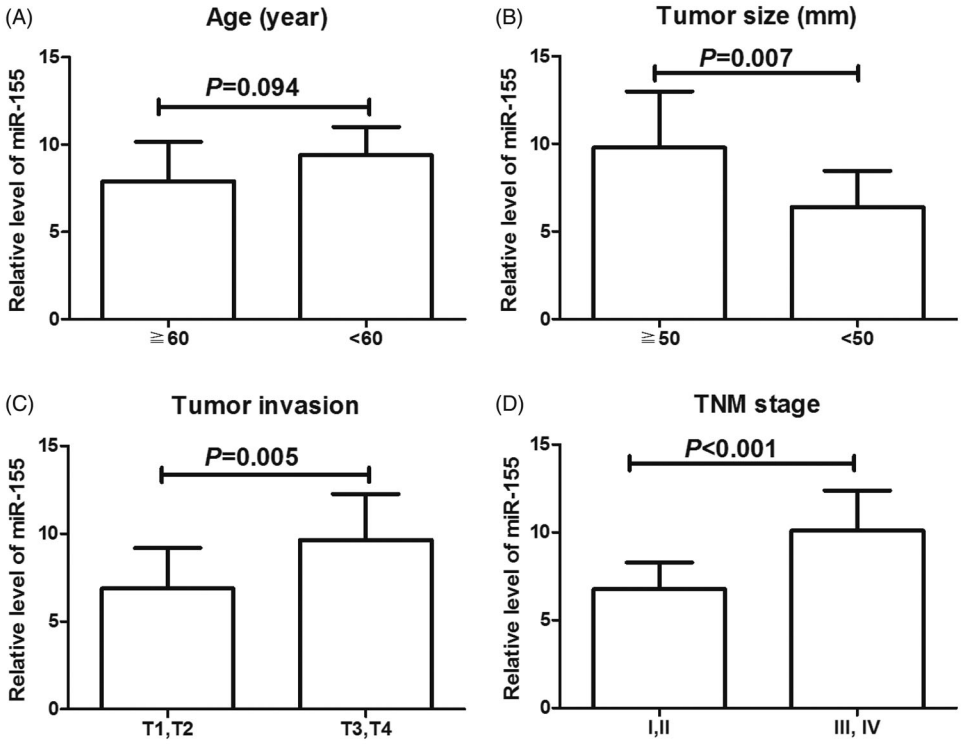

Figure 2. The comparison of *miR-155* expression in malignant tissues between ESCC patients with different ages (A), tumour sizes (B), tumour invasions (C) and TNM stages (D).

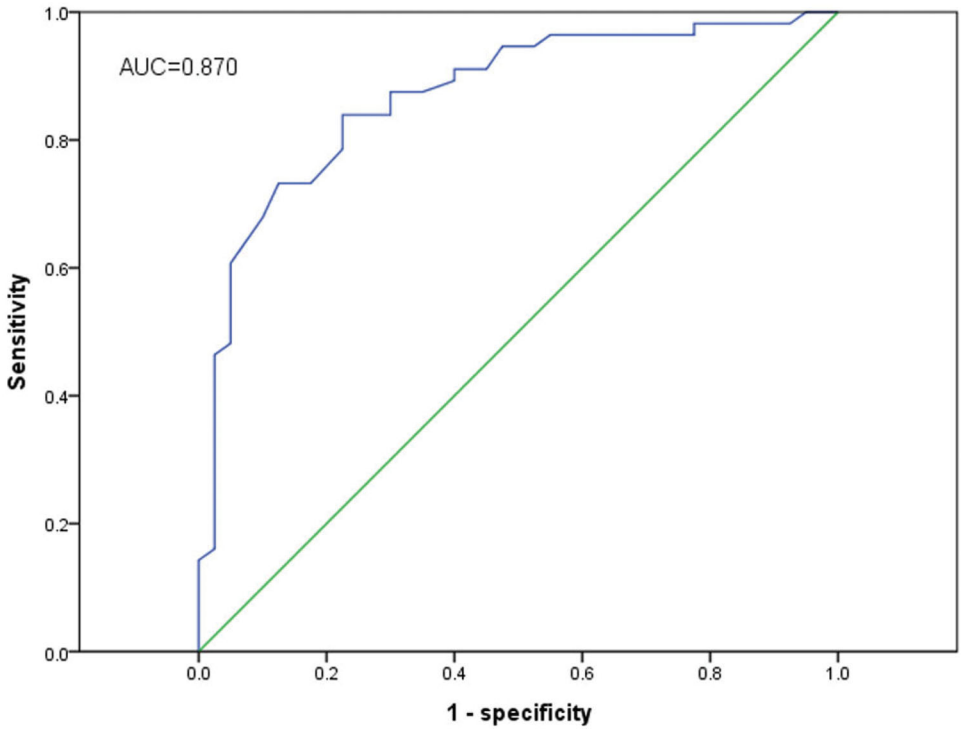

Figure 3. Receiver operating characteristics (ROC) curve analysis for the diagnostic value of *miR-155* in ESCC. The AUC (the areas under the ROC curve) was 0.870 (95%CI = 0.797–0.943, $p < .001$), with a sensitivity of 83.5% and a specificity of 77.5%, respectively.

Correlation between miR-155 expression and prognosis in ESCC patients

Studies revealed that *miR-155* expression in ESCC was significantly associated with tumour progression. Thus, we evaluated its prognostic value in ESCC patients. Kaplan-Meier analysis with the log-rank test was utilised to evaluate difference in overall survival between high and low expression groups. Compared to those with low *miR-155* expression, patients with high expression had significantly worse overall survival rates (log-rank test, $p < .001$, Figure 4). In addition, Cox regression analysis was performed to evaluate the prognostic value of *miR-155* and clinicopathological features. The results showed that patients with high levels of *miR-155* expression had a 5.87-fold higher risk of death (95%CI = 1.606–21.445, $p < .001$) , while clinicopathological characteristics of TNM stage($p = .004$), tumour size ($p = .017$) and depth of tumour invasion ($p = .007$) were also associated with overall survival among ESCC patients. However, sex, age, lymph node metastasis and distant metastasis had no prognostic value regarding to overall survival.

Then, multivariate analysis was used to evaluate whether *miR-155* could serve as an independent prognostic marker for patients with ESCC. *MiR-155* expression was found to be independently associated with overall survival (HR = 16.94, 95%CI = 3.33–86.12; $p = .001$), indicating that high *miR-155* expression was correlated with decreased overall survival in ESCC patients.

Discussion

As a main histological type of oesophageal carcinoma, ESCC shows horrendous invasive capacity in gastrointestinal system, with a five-year survival rate less than 20% despite remarkable advances in its treatments [12,13]. In addition, delay in diagnosis is also a key reason for its poor survival outcomes. Therefore, it is essential to discover effective biomarkers that could predict the malignancy progression and improve its prognosis.

In previous studies, *miR-155* has been found to facilitate tumour invasion and migration in many tumours, acting as a mediator of EMT [14]. Evidences from abundant studies have indicted that the up-regulation of *miR-155* played oncogenic function in solid tumours [15], such as breast cancer [16], Burkitt lymphoma [17], pancreatic ductal adenocarcinoma [18,19], hepatocellular carcinoma [20], primary mediastinal, diffuse large-cell lymphoma [21], lung cancer [22], thyroid carcinoma [23], and cervical cancer [24]. Furthermore, *miR-155* was also over-expressed in haematopoietic malignancies inducing early B cell polyclonal proliferation, followed by high-grade lymphoma-pre-B leukaemia [25]. Relevant researches showed that the oncogenic properties of *miR-155* were related to its anti-apoptotic function through blocking caspase-3 activity or suppressing proapoptotic genes such as TP53BP1 [26,27]. Moreover, *miR-155* reduced PTEN and PDCD4 or SHIP1 with the up-regulation of phosphorylated AKT in NK-cell lymphoma/leukaemia, and promoted cell proliferation by down-regulating the suppressor of cytokine signalling 1 gene [28,29]. All these evidences revealed *miR-155* might function as an oncogenic miRNA in human cancers and could be a valuable diagnostic or prognostic biomarker in malignant diseases. *MiR-155* expression could serve as a prognostic marker in lung cancer [30], breast cancer, pancreatic ductal adenocarcinomas, hepatocellular carcinomas, and adult T-cell leukaemia [16,19,20].

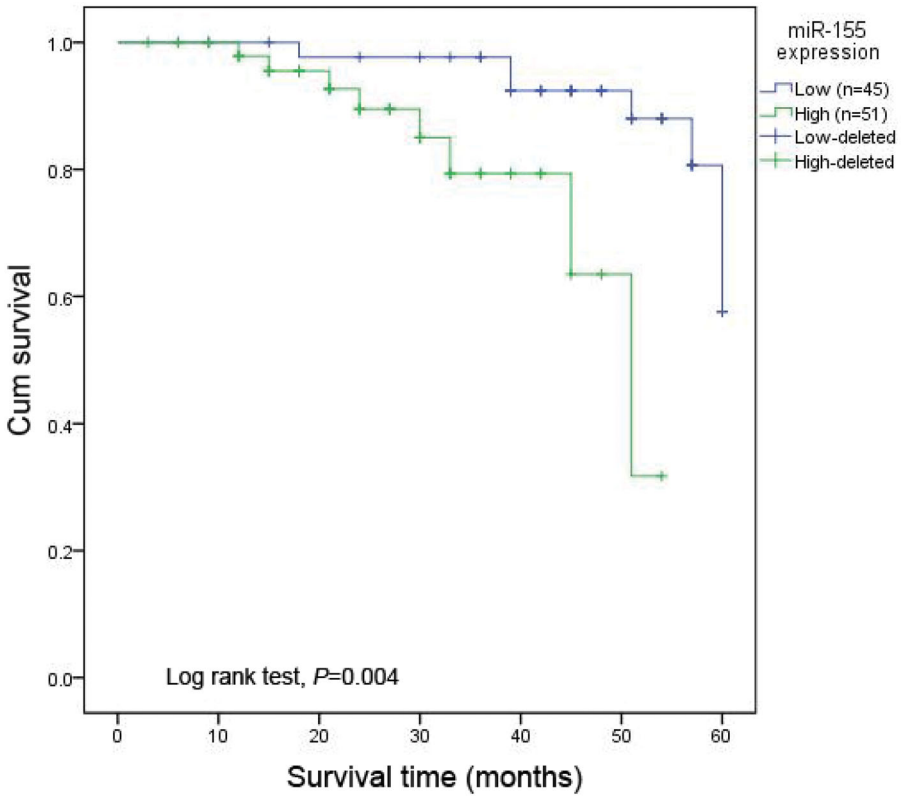

Figure 4. Kaplan-Meier analysis for the overall survival of patients with ESCC according to the expression of *miR-155*. Patients with high *miR-155* expression had shorter overall survival than those with low expression. Log rank test proved that the difference was significant ($p < .01$).

Previous studies revealed that *miR-155* was dramatically increased in ESCC tissues and could act as an oncogene through targeting TP53INP1 in the malignancy [31]. Meanwhile, a significant association between circulating *miR-155* level and oesophageal cancer development also revealed that *miR-155* might be a potential biomarker for the diagnosis of the disease [32]. However, whether *miR-155* could be a diagnostic and/or prognostic biomarker for ESCC has not been reported so far. With the aim of evaluating biological function of *miR-155*, we detected the expression of *miR-155* in ESCC cases, matched adjacent tissues and normal controls adopting qRT-PCR. The results revealed that *miR-155* expression was significantly increased, and its high expression reduced survival time of patients with ESCC. Moreover, ROC analysis with AUC (0.870) revealed that *miR-155* expression was a diagnostic indicator for ESCC. In addition, qRT-PCR is a sensitive, faster and affordable technology for gene detection [33]. Thus, miR-155 detection based on qRT-PCR technology might be a practical and economical approach for early screening ESCC. These evidences revealed that *miR-155* could be an independent diagnostic and prognostic factor for ESCC. However, the sample size was relatively small in our research that might influence the accuracy of our results. Second, the results obtained in our study were not verified in other populations. Lastly, the diagnosis specificity of *miR-155* was relatively low. Thus, applying *miR-155* in early detection of ESCC might cause diagnostic errors, so miR-155 might be employed as an auxiliary tool for such operation. Studies covering larger samples in different populations are necessary to recognise the clinical significance of *miR-155* in ESCC more clearer.

In addition, the expression level of *miR-155* was associated with tumour size, tumour grade, tumour stage and lymph node metastasis in patients with malignant tumours [16,34]. Therefore, we investigated the association of *miR-155* expression with the clinicopathological characters of ESCC patients. The results revealed that the expression of *miR-155* was positively correlated with some clinical features such as depth of tumour invasion, tumour size, and TNM stage. However, there was no association between *miR-155* expression and age, sex, lymph node metastasis or distant metastasis.

In conclusion, a significant relationship exists between increased expression of *miR-155* and the development of ESCC. *MiR-155* might serve as a new biomarker in the diagnosis and prognosis of ESCC.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary materials

The original data were loaded as Supplementary materials.

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