

Original Article

Serum levels of miR-21, miR-31 and let-7 significantly predict response to radiochemotherapy and prognosis of patients with locally advanced ESCC in Chinese Han population

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Abstract: Objective: To investigate the relationships between serum levels of microRNA21 (miR-21), microRNA31 (miR-31) and lethal-7 (let-7) and clinical response to radiochemotherapy for patients with locally advanced esophageal squamous cell carcinoma (ESCC) in Chinese Han population. Methods: Serum miR-21, miR-31 and let-7 expressions in 86 Chinese Han ethnic patients with locally advanced ESCC (case group) and 82 healthy controls (control group) were respectively detected by quantitative real-time polymerase chain reaction (qRT-PCR). All patients were treated with radiochemotherapy and its efficacy was observed. Results: Compared with control group, miR-21 and miR-31 expression increased while let-7 expression decreased in case group (all $P < 0.05$). Correlation analysis revealed a positive correlation between miR-21 and miR-31 expressions, and miR-31 and let-7 expressions (all $P < 0.05$). The expressions of miR-21, miR-31 and let-7 were correlated with ESCC tumor-node-metastasis staging, degree of pathological differentiation, lymph node metastasis, and clinical response to radiochemotherapy (all $P < 0.05$). The median progression free survival of patients with low miR-21, miR-31 expressions and high let-7 expression was higher than that of those with high miR-21, miR-31 expressions and low let-7 expression (all $P < 0.05$). Conclusion: The differential expressions of miR-21, miR-31 and let-7 may associate with radiochemotherapy efficacy in patients with locally advanced ESCC, and miR-21, miR-31 and let-7 may be potential biomarkers for radiochemotherapy efficacy.

Keywords: MicroRNA21, microRNA31, lethal-7, advanced esophageal squamous cell carcinoma, radiochemotherapy, efficacy

Introduction

Esophageal cancer, arising from the esophagus, is a serious malignancy concerning mortality and prognosis, affecting more than 450,000 people worldwide with rapidly increased incidence, and the 5-year survival rate ranges from 15% to 20% [1]. As a common histological type of esophageal cancer worldwide, esophageal squamous cell carcinoma (ESCC) has a higher incidence in developing nations, although a continually declined incidence is demonstrated in the USA [2, 3]. The burden of esophageal cancer remained high in China, especially for males in rural areas, and ESCC is still predominant histologic type accounting for more than

90% of esophageal cancer cases [4, 5]. ESCC is often diagnosed at a locally advanced stage, and preoperative radiochemotherapy therapy is currently considered as a promising strategy for advanced ESCC, but the response rate to radiochemotherapy is widely various in individual patient [2, 6]. Consequently, it is important to find potential biomarkers affecting individual difference to radiochemotherapy, and growing evidence demonstrated the role of distinct microRNA (miR) expression profiles between responders and nonresponders [7, 8].

Accumulating evidence revealed significant roles of miRs in various human cancers by modulating several biological and pathologic pro-

cesses, such as differentiation, growth, proliferation, and apoptosis in cells, as well as angiogenesis, invasion and metastasis in tumors [9-11]. In addition, results provide insight into the potential mechanisms of miR in ESCC pathogenesis through modulating the phenotype of epithelial cells towards epithelial-mesenchymal transition (EMT), and miRs may target molecular pathways involved in cell survival after chemotherapy [8, 12]. Specifically, identified dysregulated miRs in esophageal adenocarcinoma (EAC) and ESCC include up-regulated miR-21, miR-192, miR-194, miR-106-25 polycistron, miR-10b, miR-151, and miR-93, and down-regulated miR-375, miR-203, miR-205, miR-145, miR-27b, miR-100, miR-125b, let-7c, etc. [13]. Hiyoshi Y et al. suggested that miR-21 targets PDCD4 at the posttranscriptional level and regulates cell proliferation and invasion in ESCC [14]. It has been further revealed that the upregulation of miR-21 may predict poor survival in ESCC patients receiving radiochemotherapy [15]. Further, miR-31 expression was indicated to be related to histological differentiation in ESCC, and can promote ESCC colony formation, migration and invasion by regulating tumor suppressor genes; patients with high-levels of serum miR-31 also had a poorer prognosis [16, 17]. It has been suggested that miR let-7 may be involved in the chemosensitivity of cancer cell lines in vitro, significantly reduced expressions of let-7b, c, d, e were found in gemcitabine-resistant pancreatic cancer cells [18]. The miR let-7a represses chemoresistance and tumorigenicity in head and neck cancer [19]. Importantly, it has been revealed that Let-7 expression may be potential for predicting response to cisplatin-based chemotherapy in ESCC through regulating IL-6/STAT3 pathway [20]. Although significantly higher serum levels of miR-21 and let-7c were detected in esophageal cancer patients, there was no significant relationship between chemosensitivity and the levels of miR-21 or let-7c [21]. Therefore, we aimed to investigate the relationship between the levels of serum miR-21, miR-31 and let-7c and response to chemotherapy and prognosis of patients with locally advanced ESCC receiving radiochemotherapy in Chinese Han population in order to develop novel and clinically useful markers to distinguish patients with poor prognosis.

Materials and methods

Study subjects

According to the tumor-node-metastasis (TNM) esophageal cancer staging system published by the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) [22], 86 Chinese Han ethnic patients, who were pathologically diagnosed with locally advanced ESCC (III-IV) in the First Affiliated Hospital of Xinjiang Medical University between March 2012 and March 2014, were grouped into case group. Inclusion criteria were: (1) patients with an expected survival of over 6 months and without complications of esophageal fistula; (2) all patients with essentially normal renal, hepatic and cardiac function, and normal results for routine blood, urine and stool tests; (3) Chinese Han ethnic patients with pathologically diagnosed primary ESCC; (4) patients without preoperative surgery, chemotherapy or radiotherapy before sampling; (5) patients aged 30~65 years with good physical condition; (6) patients without any major organ dysfunctions or other illnesses requiring hospitalization. Exclusion criteria included: (1) Chinese Han ethnic patients with secondary ESCC; (2) patients with preoperative surgery, chemotherapy or radiotherapy; (3) patients complicated by other malignant tumors. In addition, 82 healthy controls confirmed by physical examination were randomly selected from the same region as control group. This study was approved and supervised by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. All study participants provided written informed consent before the experiments, and they were endowed with the right to know in the whole process at the same time.

Sample collection and storage

Before radiochemotherapy, venous blood (5 ml) was drawn from the case group and control group, allowed to clot at 37°C for 1 to 2 hours in the absence of anticoagulant, and then placed in a refrigerator overnight at 4°C to permit full clot retraction. When serum was precipitated naturally, samples were centrifuged at 3000×g for 10 min at 4°C to isolate serum, and the precipitate was discarded. Each sample of serum in clean test tubes was stored in an

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Table 1. Primers of miR-21, miR-31 and let-7 used for qRT-PCR

Genes	Primers
miR-21	F: 5'-TCCGAAGTTGTAGTCAGACT-3' R: 5'-GTGCAGGGTCCGACCT-3'
miR-31	F: 5'-AGGCAAGATGCTGGCATAGCT-3' R: 5'-GCGAGCACAGAATTAATACGAC-3'
let-7	F: 5'-GACATGTGAGGTAGTAGTTGT-3' R: 5'-GTGCAGGGTCCGAGGT-3'
U6	F: 5'-ATTGGAACGATACAGAGAAGATT-3' R: 5'-GGAACGCTTACGAATTTG-3'

U6 was introduced as internal reference; miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7; qRT-PCR, quantitative real-time polymerase chain reaction; F, forward; R, reverse.

ultra-low temperature freezer at -80°C for later use. The exposure duration of samples was supposed to be as short as possible during serum sample collection and storage to prevent RNA degradation.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Frozen serum (200 μl) was obtained for total RNA extraction by using miRNeasy Mini Kit (Qiagen, Germany). RNA samples (5 μl) diluted in RNA enzyme-free ultrapure water at 1:20. Absorption (OD) value at the wavelength of 260 nm and 280 nm were measured to determine the purity and concentration of RNA. OD260/OD280 ratio between 1.7~2.1 indicated a high degree of purity of RNA, suggesting that the RNA samples could be used in subsequent experiments. RT reaction was performed in a PTC-100TM PCR amplifier (MJ Research Inc., USA) to synthesize cDNA templates, and qPCR was conducted on an ABI 7500 Fast instrument (Applied Biosystems, Inc., Foster City, CA, USA) under the conditions as follows: predenaturation at 95°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, anneal at 55°C for 30 s and extension at 72°C for 30 s. U6 was introduced as internal reference, and the reaction primers were seen in **Table 1**. PCR outcomes were analyzed by Opticon Monitor analysis software version 3.1 (Bio-Rad Laboratories, Hercules, CA, USA). The cycle threshold (Ct) values of the reaction tubes were acquired by manually selecting the lowest points in the parallel rise of each logarithmic amplification curve. All data were analyzed by adopting $2^{-\Delta\Delta\text{Ct}}$ method, with $2^{-\Delta\text{Ct}}$ demonstrating the relative expression ratios of the target gene of the case

group to the control group ($\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{case group}} - \Delta\text{Ct}_{\text{control group}}$, $\Delta\text{Ct} = \text{Ct}_{\text{miRNA}} - \text{Ct}_{\text{U6}}$). This experiment was repeated 3 times.

Radiochemotherapy and efficacy evaluation

Radiochemotherapy were utilized, with a radiotherapy dose of 60~64 Gy/30~32 fractions (fr) and a chemotherapy regimen of platinum-based combination chemotherapy, including FP regimen [5-fluorouracil (5-FU) + cis-dichlorodiammine platinum (cis-DDP)] and TP regimen (paclitaxel/docetaxel + platinum). Breast computed tomography (CT) or positron emission tomography (PET) with fluorine-18 fluorodeoxyglucose (^{18}F -FDG) was performed 4-6 weeks after radiochemotherapy to evaluate recent efficacy. According to Response Evaluation Criteria in Solid Tumors (RRECIST) guidelines (version 1.0) [20], recent efficacy was confirmed as complete remission (CR), partial remission (PR), stable disease (SD) or progressive disease (PD). CR + PR were considered as therapy-sensitive group, and SD + PD as therapy-resistant group.

Follow-up

Follow-up was performed by telephone survey, clinic and medical record review till June 2015 (duration, 3~36 months), with 6 patients lost and a follow-up rate of 93.0%. Progression free survival (PFS) was defined as the time from treatment to the time of cancer recurrence or patient's death.

Statistical analysis

The SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was applied for statistical data analysis. The *t* test was employed for data analysis with normal distribution, and a non-parametric rank sum test was applied with non-normal distribution. Ranked data were also statistically analyzed by the non-parametric rank sum test. Survival rate was calculated and analyzed by the Kaplan-Meier method and tested by the Log-rank test. Receiver operating characteristic (ROC) curve was used to estimate predictive significances of serum miR-21, miR-31 and Let-7 expression. $P < 0.05$ was considered as statistically significant.

Results

General data

There were 86 patients (49 males and 37 females) in the case group with a mean age of

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Table 2. Comparison of general data between case group and control group

Variables	Case group (n = 86)	Control group (n = 82)	t/ χ^2	P value
Mean age	51.7 \pm 3.5	52.1 \pm 3.2	0.772	0.441
Gender (M/F)	49/37	42/40	0.560	0.454
Smoking history (Yes/No)	35/51	29/53	0.506	0.477
Drinking history (Yes/No)	37/49	35/47	0.002	0.965

M: male; F: female.

51.7 \pm 3.5 years, and 82 patients (42 males and 40 females) in the control group with a mean age was 52.1 \pm 3.2 years. No statistical significance in age, gender stratification, smoking history or drinking history was examined between those two groups (all $P > 0.05$) (**Table 2**).

Serum miR-21, miR-31 and let-7 expression

The relative expression of serum miR-21 in the case group was obviously increased compared with the control group (10.57 \pm 3.19 vs. 6.12 \pm 1.48, $P < 0.01$) (**Figure 1A**). The relative expression of serum miR-31 in the case group was apparently higher than that in the control group (2.24 \pm 1.11 vs. 1.04 \pm 0.29, $P < 0.01$) (**Figure 1B**). The relative expression of serum let-7 in the case group was evidently lower than that in the control group (0.41 \pm 0.17 vs. 9.12 \pm 1.86, $P < 0.01$) (**Figure 1C**).

Correlation analyses

Correlation analyses revealed a positive correlation between serum miR-21 and miR-31 expressions ($r = 0.781$, $P < 0.01$; **Figure 2A**) and between serum miR-31 and let-7 expressions ($r = 0.229$, $P = 0.034$; **Figure 2C**) in locally advanced ESCC patients. A slight positive correlation between serum miR-21 and let-7 expressions was also found in locally advanced ESCC patients, but without statistical significance ($r = 0.201$, $P = 0.064$; **Figure 2B**).

Serum miR-21, miR-31 and let-7 expression and clinicopathological features

As shown in **Table 3**, observable relationships between expression of serum miR-21, miR-31 and let-7 and TNM staging, degree of pathological differentiation, lymph node metastasis (LNM) were detected (all $P < 0.01$). However, no relationship was found between expression of

serum miR-21, miR-31 and let-7 with gender or age (all $P > 0.05$).

Serum miR-21, miR-31 and let-7 expression and radiochemotherapy efficacy

There were 50 patients in the therapy-sensitive group, including 28 males and 22 females, with a mean age of 51.4 \pm 3.1 years. In the therapy-resistant group ($n = 36$), 21 patients were male, and the rest 15 were female; the mean age was 51.9 \pm 3.7 years. There was no statistical significance in age or gender between these two groups (both $P > 0.05$). The respective mean relative expression was regarded as the boundary to divide high expression and low expression groups. The high expression rates of miR-21 and miR-31 in the therapy-sensitive group were lower than those in the therapy-resistant group, respectively (miR-21, 26.0% vs. 66.7%; miR-31, 12.0% vs. 69.4%, both $P < 0.05$). The low expression rate of let-7 in the therapy-sensitive group was lower than that in the therapy-resistant group (34.0% vs. 88.9%; $P < 0.05$) (**Table 4**).

Serum miR-21, miR-31 and let-7 expression in predicting radiochemotherapy efficacy

The area under curve (AUC), sensitivity and specificity of miR-21 expression in predicting the efficacy of radiochemotherapy in ESCC were separately 0.809, 72.2% and 82.0% (**Figure 3A**); the AUC, sensitivity and specificity of miR-31 expression in predicting the efficacy of radiochemotherapy in ESCC were separately 0.928, 91.7% and 90.0% (**Figure 3B**); the AUC, sensitivity and specificity of let-7 expression in predicting the efficacy of radiochemotherapy in ESCC were separately 0.838, 69.4% and 90.0% (**Figure 3C**).

Serum miR-21, miR-31 and let-7 expression and prognosis of ESCC

The median PFS of all patients was 27.5 months. The median PFS of the patients with low miR-21 expression was higher than those with high miR-21 expression (29.00 months vs. 21.00 months, $P < 0.01$). The median PFS of the patients with low miR-31 expression was higher than that of those with high miR-31

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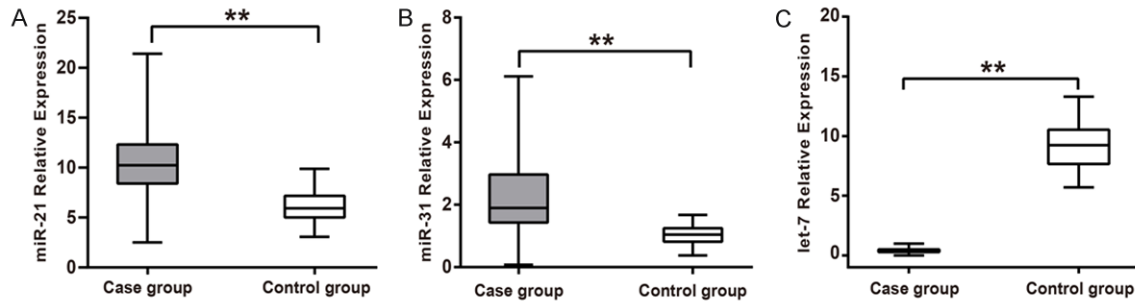


Figure 1. Relative expressions of miR-21 (A), miR-31 (B) and Let-7 (C) in case group and control group. Obviously increased relative expressions of serum miR-21 and miR-31, and evidently lower relative expression of serum let-7 were found in the case group compared with the control group. Note: miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7.

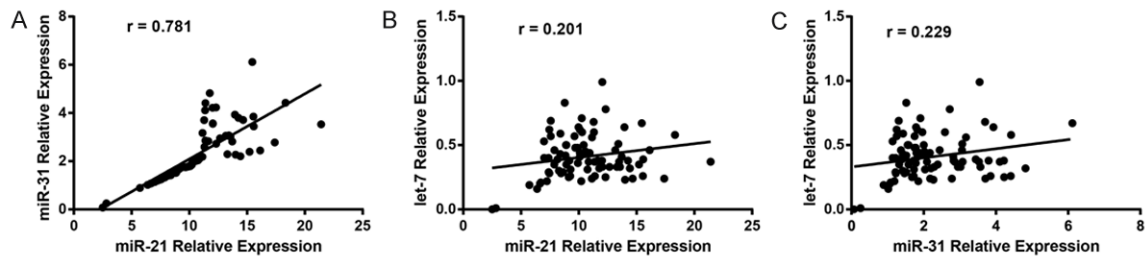


Figure 2. Correlations among relative expressions of miR-21, miR-31 and Let-7 in locally advanced ESCC patients. Correlation analyses revealed a positive correlation between serum miR-21 and miR-31 expressions and between serum miR-31 and let-7 expressions. A slight positive correlation between serum miR-21 and let-7 expressions was also found, but without statistical significance. Note: A. Correlation analysis of relative expressions of miR-21 and miR-31; B. Correlation analysis of relative expressions of miR-21 and let-7; C. Correlation analysis of relative expressions of miR-31 and let-7; miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7; ESCC, esophageal squamous cell carcinoma.

expression (29.00 months vs. 17.0 months, $P < 0.01$). The median PFS of the patients with low let-7 expression was lower than those with high let-7 expression (17.00 months vs. 21.00 months $P = 0.031$) (Figure 4).

Discussion

The up-regulation of miR-21 and miR-31 and down-regulation of let-7 have been identified in patients with locally advanced ESCC compared with the healthy volunteers, suggested that supporting the important roles of differentially expressed miR-21, miR-31 and let-7 in the pathogenesis of ESCC. Consistent with our findings, previous studies revealed significantly up-regulated miR-21 and miR-31 and down-regulated let-7c in patients with ESCC, indicating that altered expressions of miR-21 and miR-31 and let-7c may play oncogenetic functions and may be considered as biomarker for initial diag-

nosis of ESCC [13, 17, 23, 24]. Numerous miRs have been categorized into tumor suppressor miRs and oncogenic miRs (oncomiRs), and the oncogenic functions of up-regulated miR-21 and miR-31 and down-regulated let-7 in ESCC may be resulted from the correlations of over-expressed miR-21 and miR-31 and lowly expressed let-7 with inactivation of tumor-suppressor target genes [25, 26]. Furthermore, we found that the significantly higher expressions of miR-21 and miR-31 and significantly lower let-7 expression were observed in patients with stage IV ESCC, poorly differentiated ESCC and positive lymph node metastasis. These findings share similar evidence with previous study, Hiyoshi Y et al. revealed that patients with lymph node metastasis or venous invasion showed significantly high expression of miR-21 [14]; Hummel R et al. suggested that alterations in the expression of miR-21 correlate with tumor location and lymph node status patients

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Table 3. Correlations of serum expression levels of miR-21, miR-31 and let-7 with clinicopathological features in ESCC

Variables	n	miR-21	P	miR-31	P	let-7	P
Age (years)							
≤ 50	45	10.94 ± 3.43	0.260	2.20 ± 1.12	0.740	0.40 ± 0.19	0.592
> 50	41	10.15 ± 2.89		2.28 ± 1.10		0.42 ± 0.15	
Gender							
M	49	10.30 ± 3.13	0.376	2.08 ± 1.00	0.115	0.38 ± 0.15	0.594
F	37	10.92 ± 3.27		2.46 ± 1.20		0.35 ± 0.17	
LNM							
Yes	48	11.19 ± 2.78	0.041	2.48 ± 1.12	0.023	0.36 ± 0.13	0.003
No	38	9.78 ± 3.53		1.94 ± 1.02		0.47 ± 0.20	
Degree of pathological differentiation							
Stage I	35	11.61 ± 3.53	0.016	2.63 ± 1.23	0.009	0.35 ± 0.14	0.008
Stage II	28	10.37 ± 2.70		2.01 ± 1.01		0.41 ± 0.17	
Stage III	23	9.21 ± 2.65		1.93 ± 0.84		0.50 ± 0.18	
Position of ESCC							
Upper	40	10.42 ± 3.35	0.208	2.33 ± 0.38	0.437	0.43 ± 0.03	0.647
Middle	20	9.77 ± 2.48		2.24 ± 0.31		0.44 ± 0.05	
Lower	26	11.41 ± 3.33		2.12 ± 0.33		0.46 ± 0.08	
Clinical staging							
Stage III	39	9.81 ± 2.64	0.045	1.87 ± 0.89	0.002	0.45 ± 0.17	0.028
Stage IV	47	11.19 ± 3.49		2.61 ± 0.17		0.37 ± 0.16	

miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7; M: male; F: female; ESCC, esophageal squamous cell carcinoma; LNM, lymph node metastasis.

Table 4. Serum miR-21, miR-31 and let-7 expressions in predicting radiochemotherapy efficacy in ESCC [case (%)]

Group	n	miR-21		miR-31		let-7	
		High expression	Low expression	High expression	Low expression	High expression	Low expression
Therapy-sensitive	50	13 (26.0)*	37 (74.0)	6 (12.0)*	44 (88.0)	33 (66.0)	17 (34.0)*
Therapy-resistant	36	24 (66.7)	12 (33.3)	25 (69.4)	11 (30.6)	4 (11.1)	32 (88.9)

miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7; ESCC, esophageal squamous cell carcinoma; *, compared with therapy-resistant group, $P < 0.05$.

with locally advanced ESCC undergoing esophagectomy [27]; Lin RJ et al. indicated that miR-31 expression may be correlated to histological differentiation in ESCC [16]; Liu Q et al. demonstrated that Let-7 could inhibit cell proliferation and lower expressed in ESCC, and there was a correlation between let-7 lower expression and lymph node metastasis in ESCC patients [28].

Radiochemotherapy is important for local tumor control, but tumor resistance to radiochemotherapy is a substantial clinical problem. Increasing evidence supports a role for miR in histopathologic response to multimodal thera-

peutic treatment in ESCC by characterize miR profiles of responder and nonresponder [29-31]. We made further efforts to evaluate whether miR-21, miR-31 and let-7 can be used as potential biomarkers regarding the response to radiochemotherapy and prognosis of patients with locally advanced ESCC to distinguish nonresponders for better individualized treatment. Results in the present study indicated that high expression of miR-21 and miR-31 and low expression of let-7 were significantly correlated with poor response to radiochemotherapy, and miR-21, miR-31 and let-7 may be used as biomarkers to predict the radiochemotherapy

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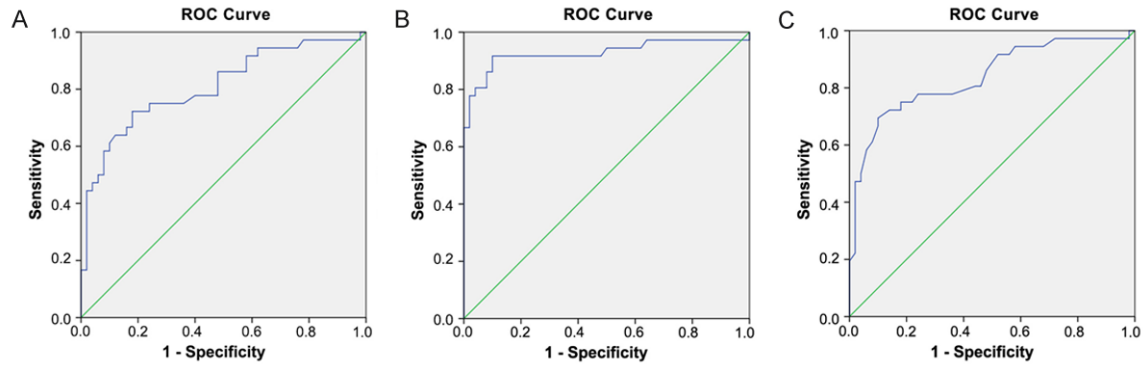


Figure 3. ROC curves of miR-21 (A), miR-31 (B) and let-7 (C) in predicting the efficacy of radiochemotherapy in ESCC. The area under curve (AUC), sensitivity and specificity of miR-21, miR-31 and let-7 expressions in predicting the efficacy of radiochemotherapy in ESCC were 0.809, 72.2% and 82.0%, 0.928, 91.7% and 90.0% and 0.838, 69.4% and 90.0%, respectively. Note: ROC, receiver operating characteristic; ESCC, esophageal squamous cell carcinoma; miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7.

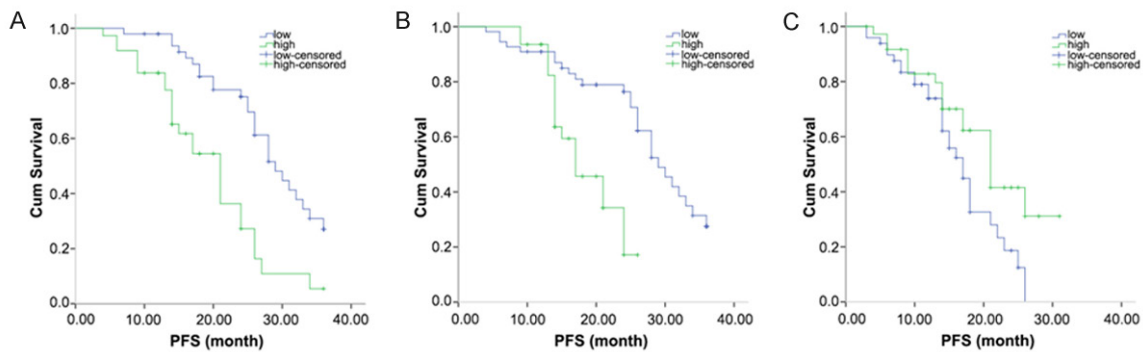


Figure 4. PFS of ESCC patients with different expression levels of miR-21 (A), miR-31 (B) and let-7 (C). The median PFSs of the patients with low miR-21 expression or low miR-31 expression were higher than those with high miR-21 expression or high miR-31 expression. The median PFS of the patients with low let-7 expression was lower than those with high let-7 expression. Note: PFS, progression free survival; ESCC, esophageal squamous cell carcinoma; miR-21, microRNA-21; miR-31, microRNA-31; let-7, lethal-7.

response in ESCC. Specifically, Kurashige J et al. reported that miR-21 levels were significantly reduced in ESCC patients who responded to chemotherapy, whereas no significant change was observed in the non-responders, concluding that miR-21 can be used as a response marker during chemotherapy for ESCC patients [23]. As previously described, Let-7 expression in ESCC can be potentially used to predict the response to cisplatin-based chemotherapy through the regulation of IL-6/STAT3 pathway [20]. The role of miR-31 was assessed in the development of chemoresistance to cisplatin, and over-expression of miR-31 is associated with increased resistance in ovarian cancer cells [32]. A previous study demonstrates a role of miR-31 in modulating radioresistance by

altering the expression of DNA repair genes involved in critical cellular defense against radiation-induced DNA damage, implying the potential role in of miR-31 as a predictive marker of response in EAC [33]. Therefore, further studies with regards to miR-31 and resistance to chemotherapy or radiotherapy in patients with ESCC are needed to confirm our results.

Results regarding the relationship between miR-21, miR-31 and Let-7 expression and prognosis of ESCC suggested that overexpressed miR-21 and miR-31, as well as lowly expressed Let-7 are associated with poor PFS. The prognostic value of miR-21 in ESCC has been replicated in independent studies, Hummel et al. suggested that miR-21 correlate with outcome

in patients with locally advanced ESCC undergoing esophagectomy (without neoadjuvant therapy) [27]; Li BX et al. showed that higher miR-21 level correlated significantly with PFS and overall survival (OS), predicting poor survival in ESCC receiving radiotherapy [15]. Patients with high-levels of serum miR-31 also had a poorer prognosis with regards to relapse-free survival and tumor-specific survival [17]. Low expression of let-7 might predict poor prognosis in patients with multiple cancers, and high let-7 expression is a prognostic factor for better OS and DFS in cancer patients, with particularly better DFS among the Asian populations [34, 35]. In patients with ESCC receiving cisplatin-based chemotherapy, the correlation of lowly expressed let-7c and poor prognosis has been confirmed [20]. The relationship of high expressions of miR-21 and miR-31, and low expression of let-7 with the poor prognosis of patients with ESCC receiving radiochemotherapy may result from the resistance to radiochemotherapy, and patients may not benefit from the radiochemotherapy as previously mentioned. However, we failed to further investigate the understanding mechanisms of these miRs with response to chemotherapy or radiotherapy or the relationship with prognosis.

In conclusion, miR-21, miR-31 and let-7 can be used as a response marker during radiochemotherapy for ESCC patient, and also related to with a poor prognosis in ESCC patients receiving radiochemotherapy, providing novel therapeutic targets in ESCC in Chinese Han population. Further studies are still needed to confirmed our findings and expound the understanding mechanisms.

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Disclosure of conflict of interest

None.

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