Original Article
Decreased expression of *microRNA-124* is an independent prognostic factor in patients with cervical cancer

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Abstract: Purpose: Recently, *microRNA-124* (*miRNA-124* or *miR-124*) has been demonstrated as a potential tumor suppressor in several types of cancers. However, the role of *miR-124* in cervical cancer remains unclear. This study was aimed at investigating the prognostic significance of *miR-124* in cervical cancer. Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to analyze *miR-124* expression in 127 cervical cancers tissues and matched adjacent normal tissues. The expression of *miR-124* was assessed for the correlation with clinicopathologic characteristics with chi-square test. The overall survival of patients with different level of *miR-124* expression was analyzed by the Kaplan-Meier analysis. The influences of clinicopathologic characteristics and *miR-124* in the prognosis of cervical cancer were estimated via Cox regression analysis. Results: The expression of *miR-124* was lower in cervical cancer tissues compared with adjacent normal tissues according to qRT-PCR ($P<0.001$). Low expression of *miR-24* was closely correlated with FIGO stage ($P=0.041$), vascular invasion ($P=0.021$) and lymph node metastasis ($P=0.020$). Patients with low *miR-124* expression had a significantly shorter overall survival than those with high *miR-124* expression ($P<0.05$). Multivariate analysis revealed that low *miR-124* expression could be an independent bio-marker in the prognosis of cervical cancer ($P=0.044$, HR=2.759, 95% CI=1.027-7.413). Conclusions: *MiR-124* was decreased and might play a certain role in the development of cervical cancer. The down-regulation expression of *miR-124* may be an independent prognostic factor in patients with cervical cancer.

Keywords: *microRNA-124*, prognosis, cervical cancer

Introduction

According to WHO reports, cervical cancer is the third most common cancer in the women all over the world [1]. The main cause of cervical cancer is a persistent infection with high-risk (hr)-human papillomavirus (HPV) [2]. And HPV-based strategies may be a promising therapeutic methods [3]. Meanwhile, the radiotherapy, chemotherapy and surgery were used as standard treatment modalities for patients with cervical cancer recently. However, as there are many factors such as hormonal contraceptive, smoking, parity, number of sexual partners, and molecular alterations seem to be promoter to this cancer [4-7]. Therefore, it is necessary to find a new effective prognostic markers and therapeutic strategies to improve the treatment of cervical cancer.

MicroRNAs (*miRNAs*) are a class of small non-coding RNAs with 18-23 nucleotides in length. It can regulate the stability and expression efficiency of mRNAs at the post-transcription level mainly by binding to 3'-UTR of target mRNAs, leading to mRNA degradation or translation inhibition [8, 9]. miRNAs can not only act as an oncogene but also can serve as tumor suppressor in various cancers. And depending on the targets of the miRNAs, which may provide insights into the functional detection of human malignancies [10]. *MicroRNA-124* (*miR-124*) is one of the most abundant miRNAs in the central nervous system and has been shown to play a key role in the pathogenesis of various cancers [11-17]. In previous studies, *miR-124* had been confirmed to repress vasculogenic mimicry, migration and invasion in cervical cancer by targeting amotL1 in vitro and its down-
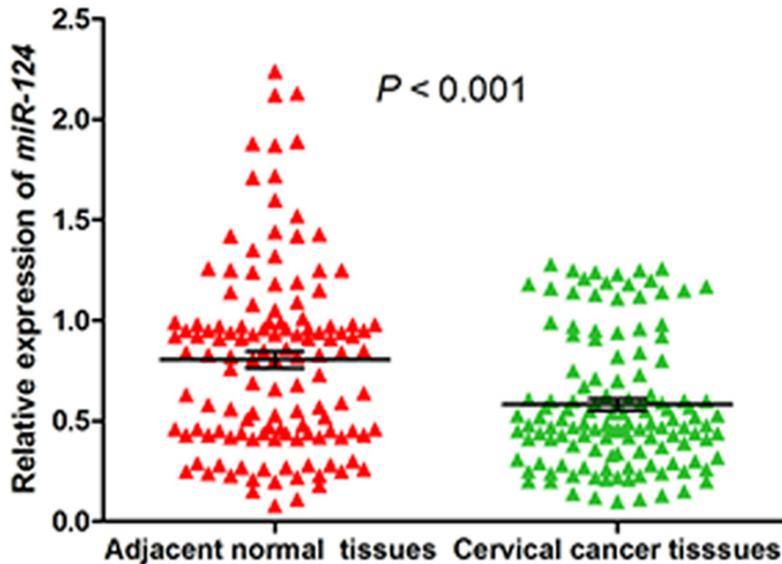
Figure 1. The expression level of *miR-124* in the cervical cancer tissues and adjacent normal tissues. The *miR-124* expression was lower in cancer tissues than adjacent normal tissues (*P*<0.001).

regulation had also been observed cervical which may be caused by DNA methylation-based silencing [13, 18]. Nevertheless, the clinical and prognostic significance of *miR-124* expression in cervical cancer has not been determined yet.

In this study, we aimed to investigate the expression of *miR-124* in cervical cancer and further explore the prognostic significance of *miR-124* in cervical cancer.

Methods and materials

Samples collection

The tumor tissues and adjacent normal tissues were obtained from 127 patients who were diagnosed as cervical cancer. All patients recruited in this study were not subjected to preoperative radiotherapy and/or chemotherapy. This study was approved by the Ethical Committee at the affiliations, and the written consents were obtained from all these participants in advance.

Tumor specimens and corresponding adjacent normal tissues were collected and frozen by liquid nitrogen immediately, then stored at -80°C for RNA extraction. Clinicopathologic characteristics including age, tumor size, histology type, FIGO stage, differentiation, lymph node metas-

tases and vascular invasion were recorded in a database. A 5-years' follow-up was conducted and the information was updated through a telephone or questionnaire. The overall survival of patients was defined as the day of surgery to the day of death. Patients died from unexpected events or other disease was excluded in our study.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tumor tissues and adjacent normal tissues using the TRIzol reagent (Invitrogen, CA, USA). The expression of *miR-124* was determined by RT-PCR with SYBR Premix Ex Taq II kit (Takara, Dalian City, Liaoning Province, China) according to the manufacturer's instructions. *RNU6B* was taken as the endogenous control. The PCR primers for *miR-124* or *RNU6B* were designed as follows: miR-124 forward, 5'-GATACTCATAAGGCACGCGG-3' and reverse, 5'-GTGCAGGGTCCGAGGT-3'. *RNU-6B* forward, 5'-TGCGGGTGCTCGCTTCGGCAGC-3' and reverse 5'-CCAGTGCAGGGTCCGAGGT-3'. The relative expression level of *miR-124* was calculated by the $2^{-\Delta\Delta Ct}$ method. The experiment was operated in triplicate.

Statistical analysis

The software of SPSS version 18.0 for Windows was used for statistical analysis. Comparisons of *miR-124* levels between cervical cancer tissues and adjacent normal tissues were performed using T-test. The correlation between *miR-124* expression and clinicopathologic characteristics of patients with cervical cancer was evaluated by X^2-test. Association of *miR-124* expression with overall survival was estimated by Kaplan-Meier analysis, and the resulting curves were compared using the log-rank test. The multivariate Cox proportional hazard regression analysis was used to evaluate the prognostic factors including miR-124 and clinicopathologic characteristics of patients with

Table 1. Correlation between *miR-124* expression and clinicopathologic characteristics in cervical cancer patients

| Parameters | Case (n) | miR-124 expression | | X^2 | *P* value |
		High *miR-124* expression (n)	Low *miR-124* expression (n)		
All	127	57	70		
Age (years)					
<50	52	23	29	0.036	0.849
≥50	75	34	40		
Tumor size (cm)					
≤4	49	22	27	0.000	0.998
>4	78	35	43		
Histology					
Squamous	91	44	47	1.562	0.211
Adenocarcinoma	36	13	23		
FIGO stage					
Ib-IIa	63	34	29	4.172	0.041
IIb~IIIa	64	23	41		
Differentiation					
Well + moderate	82	35	47	0.452	0.501
Poor	45	22	23		
Lymph nodes metastasis					
Yes	85	32	53	5.438	0.020
No	42	25	17		
Vascular invasion					
Yes	52	17	35	5.289	0.021
No	75	40	35		

lyzed by Chi-square test. As shown in **Table 1**, the expression of *miR-124* in cervical cancer was significantly associated with FIGO stage (*P*=0.041), vascular invasion (*P*=0.021) and lymph node metastasis (*P*=0.020). However, there were no correlation with other clinicopathologic characteristics such as age (*P*=0.849), tumor size (*P*=0.998), histology type (*P*=0.211) and differentiation (*P*=0.501). This might demonstrated that *miR-124* was related to the development of cervical cancer.

Associations between miR-124 expression and overall survival of patients with cervical cancer

cervical cancer. *P*<0.05 was considered that the difference was statistically significant.

Results

The expression of miR-124 in the cervical cancer tissues and adjacent normal tissues

We firstly examined *miR-124* expression level in 127 cervical cancer tissues and matched adjacent normal tissues by qRT-PCR. As shown in **Figure 1**, after normalization to *RNU6B* expression levels, the expression level of *miR-124* in cervical cancer tissues was significantly lower than that in adjacent normal tissues (0.5816 ± 0.3252 vs. 0.8046 ± 0.4625; *P*<0.001). The result indicated that *miR-124* might be a tumor suppressor in cervical cancer.

Correlations between miR-124 and clinicopathologic characteristics

The relationships between *miR-124* expression and clinicopathologic characteristics were ana-

The association between *miR-124* expression and overall survival of cervical cancer patients was investigated by Kaplan-Meier analysis and log-rank test. As shown in **Figure 2**, cervical cancer patients with low *miR-124* expression tend to have a shorter overall survival than those with high *miR-124* expression (log-rank test, *P*<0.05). Multivariate analysis using the Cox proportional hazards model for all variables showed that low *miR-124* expression was an important prognostic factor and might be an independent prognostic indicator for patients with cervical cancer (*P*=0.044, HR=2.759, 95% CI: 1.027-7.413, **Table 2**).

Discussion

Cervical cancer remains to be one of the leading causes of mortality among women [19]. The incidence of this disease is mostly high in developing countries in spite of the presence of screening programs [1]. Despite the early stage diagnosis and treatment over high-risk population is an effective method that can reduce the
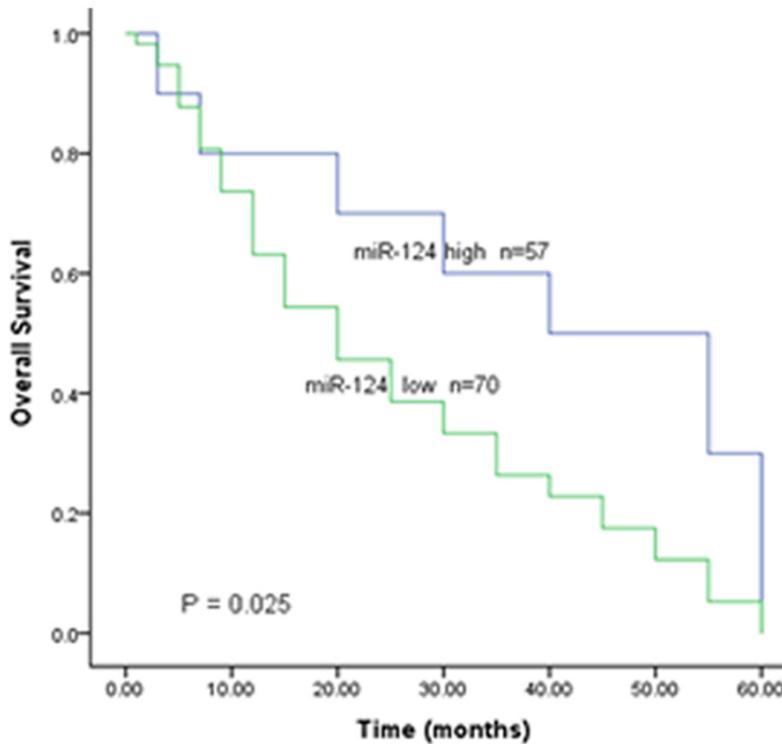
Figure 2. Kaplan-Meier analysis of *miR-124* expression which was used to estimate the association between it and overall survival of patients. Patients with low *miR-124* expression had a shorter overall survival than those with high *miR-124* expression (Log-rank test, *P*=0.025).

Table 2. Multivariate analysis for factors influencing the overall survival rate of cervical patients

Variables	HR	95% CI	*P* Value
miR-124 (low/high)	2.759	1.027-7.413	0.044
FIGO stage	1.037	0.624-1.725	0.888
Lymph nodes metastasis	1.003	0.564-1.783	0.993
Vascular invasion	1.405	0.819-2.409	0.217

that various miRNAs were related to the occurrence, development and prognosis of cervical cancer. For instance, *miR-335* was found to be decreased and a useful prognostic marker in cervical cancer in the study of Wang et al [26]. Yang et al., confirmed that *miR-494* was an essential role in the carcinogenesis and progression of cervical cancer as it could promote cell proliferation by targeting with *PTEN* [27]. According to the study of Wang et al, the down-regulation of *miR-145* act as an important prognostic indicator in cervical cancer [28]. *MiR-124* was abnormal expressed in several cancers such as colorectal cancer, breast cancer, osteosarcoma, lung cancer, gastric cancer and so on [29-33]. Besides, *miR-124* family is hypermethylated to a high degree in high-grade cervical lesions and could repress the EMT process in cervical cancer [13, 18]. However, its prognostic value is unclear in cervical cancer. Therefore, identifying the function of *miR-124* may help to find a new bio-marker for the prognosis of this cancer which is enable deeper insight into the regulation of gene expression and the complexity of cancer progression. In this study, we investigated the expression of *miR-124* in cervical cancer tissues and adjacent normal tissues by qRT-PCR. The result demonstrated the down-regulation of miR-124 in cervical cancer which was consistent with the previous studies [34].

incidence and mortality of cervical cancer, the prognosis of cervical cancer is still poor [20-22]. Therefore, finding new molecular targets for its prognosis has the potential to improve the clinical strategies and outcomes of this disease.

More and more studies indicated that the molecular mechanisms of carcinogenesis are not only relevant to protein coding genes but also to miRNAs which are non-protein coding. Due to its various regulations on gene expression, miRNAs participate in multiple cellular functions, such as proliferation, apoptosis, differentiation, cancer carcinogenesis and progression [23-25]. Many reports had indicated

Based on the relative expression level analysis, the association of *miR-124* with clinicopathologic characteristics was analyzed to see whether it was involved in the development of cervical cancer. As a result, the expression of *miR-124* was proved to be influenced by FIGO

stage, vascular invasion, and lymph node metastasis significantly. However, *miR-124* expression was not associated with patient's age, tumor size, histology and differentiation. Combing with present results, it is thus speculated that *miR-124* may play a tumor suppresser role in cervical cancer progression.

We also estimated the prognostic value of *miR-124* for its specific expression in cervical cancer. Kaplan-Meier analysis and log-rank test manifested the overall survival was strengthened by high *miR-124* expression as patients with low *miR-124* expression lived much shorter than those with high *miR-124* expression. Then, Cox regression analysis adjusted for clinicopathologic characteristics showed that the *miR-124* expression could be an independent prognostic marker in patients with cervical cancer. These data indicated that *miR-124* expression play a crucial role in tumorigenesis, and progression of cervical cancer.

In conclusion, we discover that *miR-124* is down-regulated in cervical cancer tissues and closely correlation with tumor progression. Furthermore, *miR-124* is identified as an independent factor for predicting the clinical outcome of cervical cancer patients. The down-regulation of *miR-124* plays an important role in cervical cancer progression and a novel prognostic biomarker.

Disclosure of conflict of interest

None.

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