Diagnostic Values of miR-21, miR-124, and M-CSF in Patients With Early Cervical Cancer

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Abstract
Objective: This study aimed to investigate the diagnostic values of microRNA-21, microRNA-124, and macrophage colony-stimulating factor in patients with cervical cancer. Methods: A total of 68 patients with cervical cancer admitted in our hospital (cervical cancer group) and 57 healthy individuals undergoing physical examinations (healthy group, also control group) were enrolled in this study. The expression of serum microRNA-21 and microRNA-124 was detected by quantitative reverse transcription polymerase chain reaction. The expression of serum macrophage colony-stimulating factor was detected by enzyme-linked immunosorbent assay. The diagnostic values of microRNA-21, microRNA-124, and macrophage colony-stimulating factor in cervical cancer were analyzed. The correlations between the expression of microRNA-21 and microRNA-124 with that of macrophage colony-stimulating factor were also analyzed. Results: Compared to those in the healthy group, patients in the cervical cancer group had a higher expression of microRNA-21 and macrophage colony-stimulating factor ($P < .05$) but lower expression of microRNA-124 ($P < .05$). The expression of microRNA-21, microRNA-124, and macrophage colony-stimulating factor in the patients correlated with the tumor size, tumor node metastasis (TNM) staging, tumor differentiation, and the presence or absence of lymph node metastasis and human papillomavirus infection ($P < .05$). According to the receiver operating characteristic curves, the area under the curve of microRNA-21 for diagnosing cervical cancer was 0.723, the specificity was 58.82%, and the sensitivity was 91.23%. The area under the curve of microRNA-124 was 0.766, the specificity was 94.12%, and the sensitivity was 57.89%. The area under the curve of macrophage colony-stimulating factor was 0.754, the specificity was 64.71%, and the sensitivity was 87.72%. Pearson correlation analysis showed that the expression of microRNA-21 positively correlated with that of macrophage colony-stimulating factor ($r = 0.6825$, $P < .001$), and the expression of microRNA-124 negatively correlated with that of macrophage colony-stimulating factor ($r = -0.6476$, $P < .001$). Conclusion: MicroRNA-21, microRNA-124, and macrophage colony-stimulating factor may be involved in the development and progression of cervical cancer. The detection of serum microRNA-21, microRNA-124, and macrophage colony-stimulating factor has good sensitivity and specificity in the diagnosis of cervical cancer.

Keywords
miR-21, miR-124, M-CSF, cervical cancer, diagnostic value

Abbreviations
AUC, area under the curve; M-CSF, macrophage colony-stimulating factor; miRNA, microRNA; miR-21, microRNA-21; miR-124, microRNA-124; HPV, human papillomavirus; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; ROC, receiver operating characteristics; TCT, ThinPrep cytology test.

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Introduction
Cervical cancer is the second most common cancer after breast cancer.\textsuperscript{1} Its mortality in the low- and middle-income countries is reported to be 18 times higher than that in the high-income countries.\textsuperscript{2} One of the major risk factors for cervical cancer is persistent high-risk human papillomavirus (HPV) infection.
Clinically, most patients with cervical cancer are in the middle and advanced stages when treated, and thus, they miss the best time window for treatment. Therefore, molecular biomarkers related to the early diagnosis of cervical cancer should be evaluated, and the regulatory mechanisms of molecular signals during the development and progression of the disease should be explored. Moreover, therapeutic targets and biomarkers that affect the development and progression should be discovered. All of these are important for improving the early diagnosis and treatment of the disease as well as for improving the prognosis of the disease.

MicroRNAs (miRNAs) are widely present in eukaryotic cells; they are highly conserved and endogenous noncoding, 19- to 25-nucleotide-long transcripts. Some miRNAs are closely related to the development and progression of cervical cancer. For instance, Tan et al have found that miR-378 enhances the migration of cervical cancer by directly targeting the autophagy-related protein 12. After overexpressing miR-375 in the human cervical cancer cells, SiHa and CaSki, Wang et al discovered that miR-375 inhibits the migration of the cells through the target gene SP1. Hu et al have shown that miRNA-200a and miRNA-9 inhibit the metastasis of cervical cancer cells and predict the survival time of patients with cervical cancer. These studies indicate that miRNAs may play an important role in the development and progression of cervical cancer and can be used as biomarkers for the early diagnosis of this disease. MicroRNA-21 (miR-21) is highly expressed in gastric cancer, prostate cancer, and breast cancer; therefore, it is considered a carcinogenic miRNA. Previous studies have confirmed that microRNA-124 (miR-124) has a low expression in malignant tumors, such as gastric cancer, and is associated with the formation and malignant progression of some tumors, such as breast cancer. According to Sun et al, low expression of miR-124 correlates with the poor prognosis of pancreatic cancer. As a special cytokine existing in the bone marrow cavity, macrophage colony-stimulating factor (M-CSF) promotes macrophage colony formation and regulates the proliferation and differentiation of macrophages. A study has shown that M-CSF plays an important role in the tumors and is involved in the proliferation of tumor cells and tumor angiogenesis. However, the roles of miR-21, miR-124, and M-CSF in cervical cancer have been rarely studied.

At present, the gold standard for the diagnosis of cervical cancer is a cervical biopsy, which has difficulties in obtaining specimens and arriving at a timely diagnosis. The acquisition of the blood tumor markers is relative simple, of which the operation is easy to automate, the detection results are quantitative data, and the test price is low; hence, it is easy to be applied in the early screening. Therefore, the expression of serum miR-21, miR-124, and M-CSF in patients with cervical cancer was explored in this study. The diagnostic values of these markers in cervical cancer, as well as the correlation among these 3 biomarkers, were analyzed to provide a reference for the application of these potential molecular biomarkers in cervical cancer.

Materials and Methods

General Information

A total of 68 patients with cervical cancer admitted to our hospital (cervical cancer group) and 57 healthy individuals undergoing physical examinations (healthy group, also control group) from May 2014 to January 2015 were enrolled in this study. The patients in the cervical cancer group were aged between 45 and 59 years, with a mean age of 47.57 ± 8.19 years. The individuals in the healthy group were aged between 43 and 61 years, with a mean age of 48.39 ± 10.17 years. The inclusion criteria were patients confirmedly diagnosed with cervical cancer by liquid-based ThinPrep cytology test (TCT), HPV testing, and B-mode ultrasound of the reproductive system. The healthy individuals underwent physical examinations in the physical examination center of our hospital and had normal examination results. All of them had no other tumors or cardiac, hepatic, and renal diseases. Their family members had no history of cancer. The exclusion criteria were pregnant women, patients with thyroid diseases and immunological diseases, patients who were bedridden for a long time, patients with severe hypertension and diabetes, patients who had taken glucocorticoids and antibiotics in the preceding 2 weeks, and patients with mental and cognitive disorders. All patients did not receive radiotherapy, chemotherapy, or other treatment before blood collection. The study was approved by the ethics committee of Affiliated Hospital of Jining Medical College. All study participants provided written informed consent before participating in the study.

Main Instruments and Reagents

Macrophage colony-stimulating factor enzyme-linked immunosorbent assay (ELISA) kit (YM-QP10199; Shanghai YuanMu Biological Technology Co., Ltd., Shanghai, China), a multifunctional ELISA microplate (Infinite 200 PRO; Coslan scientific LTD, Guangzhou, China), Light Cycler real-time fluorescence quantitative PCR instrument (Roche, Basel, Switzerland), total RNA extraction kit (Solarbio R1200; Shanghai Hengfei Refrigeration Engineering Equipment Co., Ltd., Shanghai, China), M-MLV reverse transcription kit (Vazyme, Nanjing, China), ultraviolet spectrophotometer (Multiskan Sky; Thermo Fisher, Shanghai, China), qReal-time PCR kit (Invitrogen, Grand Island, New York), and SYBR Green qPCR Master Mix kit (Thermo Fisher) were obtained. The primers for miR-21, miR-124, and the internal reference (U6) were synthesized by Sangon Biotech Co, Ltd. (Shanghai, China, Table 1).

Detection Method

Fasting venous blood samples (5 mL) were obtained from the study participants belonging to the 2 groups in the morning. The samples were placed in vacuum blood collection tubes and centrifuged at 3000g per minute for separation. Macrophage colony-stimulating factor was detected by ELISA, while miR-21 and miR-124 were detected by quantitative reverse
transcription polymerase chain reaction (qRT-PCR) strictly according to the kit instructions. The optical density values at 450 nm were measured within 30 minutes.

**Quantitative Reverse Transcription Polymerase Chain Reaction.** The total RNA was extracted from the serum according to the instructions mentioned in the TRIzol extraction kit. The synthesized cDNA was stored at −20°C for later use. The system (20 μL in total) was as follows: 10 μL of PCR Premix, 2 μL of upstream primer (10×; concentration of 10 μmol/L), 2 μL of downstream primer (10×; concentration of 10 μmol/L), and double-distilled water (RNase- and DNase-free, finally added to make up to 20 μL). The ABI PRISM 7500 (Shanghai PuDi Biotech Co., Ltd., Shanghai, China) fluorescence quantitative PCR instrument manufacturer software was used to analyze the amplification data. The results were expressed by 2^ΔΔCT.20

**Statistical Methods**

SPSS 21.0 was used for the statistical analyses. The categorical variables data were expressed by n (%). The continuous variables were expressed by mean ± standard deviation. The continuous variables between the groups were compared by t test, whereas the categorical variables were compared by χ² test. Receiver operating characteristic (ROC) curves were plotted to assess the diagnostic values of miR-21, miR-124, and M-CSF in cervical cancer. Pearson correlation coefficient was used to analyze the correlations between miR-21, miR-124, and M-CSF. P < .05 was considered statistically significant.

**Results**

**General Information**

There were no statistically significant differences between the cervical cancer and healthy groups in terms of age, body mass index, history of smoking, history of drinking, past medical history, exercise habits, place of residence, blood glucose, alanine aminotransferase, aspartate aminotransferase, hemoglobin, red blood cell count, and platelet count (P > .05; Table 2).

**Comparison of Expression of miR-21, miR-124, and M-CSF**

Compared to individuals in the healthy group, patients in the cervical cancer group had higher expression of miR-21 and M-CSF (P < .05) but lower expression of miR-124 (P < .05; Table 3).

**Diagnostic Values of miR-21, miR-124, and M-CSF**

According to the ROC curves, the area under the curve (AUC) of miR-21 for diagnosing cervical cancer was 0.723 (95% confidence interval [CI]: 0.631-0.815), the specificity was 58.82%, the sensitivity was 91.23%, and the cutoff value was 3.855. The AUC of miR-124 was 0.766 (95% CI: 0.677-0.856), the specificity was 94.12%, the sensitivity was 57.89%, and the cutoff value was 1.67. The AUC of M-CSF was 0.754 (95% CI: 0.666-0.841), the specificity was 64.71%, the sensitivity was 87.72%, and the cutoff value was 382.70 pg/mL (Table 5, Figure 2).

**Correlations Between miR-21 and miR-124, and M-CSF With the Clinicopathological Features**

The expression of miR-21, miR-124, and M-CSF in patients with cervical cancer correlated with the tumor size, TNM staging, tumor differentiation, and the presence or absence of lymph node metastasis and HPV infection (P < .05). However, it did not correlate with age, place of residence, and tumor types (P > .05; Table 3).

**Correlations Between miR-21 and miR-124, and M-CSF**

According to the Pearson correlation analysis, the expression of miR-21 positively correlated with that of M-CSF (r = 0.6825, P < .001) and the expression of M-CSF increased with that of miR-21. The expression of miR-124 was negatively correlated with that of M-CSF (r = −0.6476, P < .001), and the expression of M-CSF decreased with that of miR-124 (Figure 3).

**Discussion**

Cervical cancer is a disease related to viral infection.21 Although the incidence of cervical cancer in the developed countries have reduced due to the screening of the disease and use of HPV vaccines, the incidence of the disease in the underdeveloped countries has not decreased and the overall survival rate has not increased.22,23 Therefore, it is of great significance for the prevention and treatment of cervical cancer to understand the mechanisms of development and progression of the disease.24 With progress in research, it has been found that HPV infection alone cannot cause the malignant transformation of the cervical cells and that changes in the functions and expression of other genes are also involved in the pathological process of cervical cancer.25,26 According to a recent study, as a new research hotspot in tumor biology, miRNA has been closely

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**Table 1. Primers for miR-21, miR-124, and U6.**

| Genes | Forward Primers | Reverse Primers |
|-------|-----------------|-----------------|
| miR-21 | 5′-GCTTGGCCCTAGCTTA | 5′-CAGTGCTGGGTCCAG |
|       | TCAGACT-3′       | AGTGA-3′         |
| miR-124 | 5′-GCTAAGGCACCGG | 5′-GTGCAGGGTCCAG |
|       | TG-3′           | GT-3′           |
| U6    | 5′-CTCGGTTCGCCG | 5′-AAGCGTTCAGAA |
|       | ACA-3′          | TTGCCT-3′       |

Abbreviations: miR-21, microRNA-21; miR-124, microRNA-124.
Table 3. Correlations of miR-21, miR-124, and M-CSF With the Clinicopathological Features.

| Factors                       | n   | miR-21 t/F | P Value | miR-124 t/F | P Value | M-CSF (pg/mL) t/F | P Value |
|-------------------------------|-----|------------|---------|------------|---------|------------------|---------|
| Age (years)                   |     |            |         |            |         |                  |         |
| ≤50                           | 38  | 4.12 ± 1.28| 1.02 ± 0.34| 419.14 ± 141.19| 0.318  | 0.751            |
| >50                           | 30  | 4.07 ± 1.46| 1.09 ± 0.28| 408.34 ± 135.84|        |                  |         |
| Place of residence            |     |            |         |            |         |                  |         |
| Countryside                   | 21  | 4.10 ± 1.34| 1.08 ± 0.34| 418.64 ± 144.51| 0.374  | 0.710            |
| City                          | 47  | 4.08 ± 1.44| 1.05 ± 0.28| 405.26 ± 132.59|        |                  |         |
| Tumor types                   |     |            |         |            |         |                  |         |
| Squamous cell carcinoma       | 32  | 4.11 ± 1.25| 1.12 ± 0.29| 408.48 ± 135.86|        |                  |         |
| Adenocarcinoma                | 25  | 4.08 ± 1.37| 1.04 ± 0.34| 414.15 ± 137.48|        |                  |         |
| Others                        | 11  | 4.10 ± 1.48| 0.97 ± 0.27| 412.33 ± 128.65|        |                  |         |
| Tumor size (cm)               | 6.833 | <0.001 | 4.658 | <0.001 | 5.829 | <0.001 |
| TNM staging                   | 8.769 | <0.001 | 5.935 | <0.001 | 6.857 | <0.001 |
| Stages I + II                 | 43  | 2.94 ± 1.15| 1.36 ± 0.36| 322.45 ± 112.62|        |                  |         |
| Stages III + IV               | 25  | 5.80 ± 1.52| 0.88 ± 0.24| 538.63 ± 144.95|        |                  |         |
| Tumor differentiation         | 3.515 | .001 | 3.706 | <0.001 | 4.418 | <0.001 |
| Moderate highly differentiated | 4.367 | ± 1.22 | 1.28 ± 0.36| 355.95 ± 134.67|        |                  |         |
| Poorly differentiated          | 24  | 4.97 ± 1.82| 0.96 ± 0.30| 511.26 ± 145.49|        |                  |         |
| Lymph node metastasis         | 4.919 | <0.001 | 5.855 | <0.001 | 5.537 | <0.001 |
| No                            | 40  | 3.41 ± 1.48| 1.37 ± 0.40| 346.36 ± 122.55|        |                  |         |
| Yes                           | 28  | 5.16 ± 1.39| 0.87 ± 0.25| 525.28 ± 142.62|        |                  |         |
| HPV infection                 | 4.119 | <0.001 | 4.577 | <0.001 | 4.385 | <0.001 |
| No                            | 25  | 3.65 ± 1.31| 1.31 ± 0.37| 357.26 ± 145.19|        |                  |         |
| Yes                           | 43  | 5.17 ± 1.55| 0.94 ± 0.29| 510.97 ± 135.94|        |                  |         |

Abbreviations: F, the statistical value of F test; HPV, human papillomavirus; M-CSF, macrophage colony-stimulating factor; miR-21, microRNA-21; miR-124, microRNA-124; t, the statistical value of t test; TNM, tumor node metastasis.

*Values are represented as mean ± standard deviation.
related to the development, progression, invasion, metastasis, and apoptosis of tumors, functioning as an oncogene or a tumor suppressor gene. Previous studies have shown that miR-21 and miR-124 are abnormally expressed in cervical cancer. After analyzing HPV16-positive CaSkI cells using clonal sequencing, Wang et al found that miR-21 is one of the miRNAs that has the most significant difference in expression. According to Wang et al, miR-124-3p inhibits the metastasis of cervical cancer cells by directly targeting IGF2BP, and its reduced expression is associated with advanced cervical cancer. However, previous studies on the expression of miR-21 and miR-124 in cervical cancer were limited to the exploration of cervical cancer cell lines cultured in vitro, and no studies were conducted on the expression of miR-21 and miR-124 in clinical patients with cervical cancer. Macrophage colony-stimulating factor is the major cytokine that regulates the differentiation and growth of monocyte–macrophage cell lines. Recent studies have found that its expression abnormally increases in many tumors and that circulating M-CSF in the serum has become a molecular marker for these tumors. These findings indicate that M-CSF plays an important role in the development and progression of many tumors. In this study, compared to those in the healthy group, patients in the cervical

| Groups            | n   | miR-21  | miR-124 | M-CSF (pg/mL) |
|-------------------|-----|---------|---------|---------------|
| Cervical cancer   | 68  | 4.09±1.43| 1.06±0.35| 413.65±140.77|
| Healthy group     | 57  | 3.20±0.50| 1.70±0.74| 312.23±68.51  |
| \( t \)           | -   | 4.473   | 6.339   | 4.966         |
| \( P \)           | -   | <.001   | <.001   | <.001         |

Abbreviations: M-CSF, macrophage colony-stimulating factor; miR-21, microRNA-21; miR-124, microRNA-124; \( t \), the statistical value of \( t \) test. \(^a\)Values are represented as mean ± SD.

Figure 1. Comparison of the expression of miR-21, miR-124, and M-CSF. The expression of miR-21 in the cervical cancer group was higher than that in the healthy group (\( P < .05 \)) (A). The expression of miR-124 in the cervical cancer group was lower than that in the healthy group (\( P < .05 \)) (B). The expression of M-CSF in the cervical cancer group was higher than that in the healthy group (\( P < .05 \)) (C). *\( P < .05 \) as compared to the healthy group. M-CSF indicates macrophage colony-stimulating factor; miR-21, microRNA-21; miR-124, microRNA-124.
Table 5. Diagnostic Values of miR-21, miR-124, and M-CSF.

| Indicators | AUC   | 95% CI      | Specificity (%) | Sensitivity (%) | Cutoff Value |
|------------|-------|-------------|-----------------|-----------------|--------------|
| miR-21     | 0.723 | 0.631-0.815 | 58.82 (40/68)   | 91.23 (52/57)   | 3.855        |
| miR-124    | 0.766 | 0.677-0.856 | 94.12 (64/68)   | 57.89 (33/57)   | 1.67         |
| M-CSF      | 0.754 | 0.666-0.841 | 64.71 (44/68)   | 87.72 (50/57)   | 382.70 pg/mL |

Abbreviations: AUC, area under the curve; CI, confidence interval; M-CSF, macrophage colony-stimulating factor; miR-21, microRNA-21; miR-124, microRNA-124.

Figure 2. Diagnostic values of miR-21, miR-124, and M-CSF. The AUC of miR-21 for diagnosing cervical cancer was 0.723 (95% CI: 0.631-0.815), the specificity was 58.82%, the sensitivity was 91.23%, and the cutoff value was 3.855. The AUC of miR-124 was 0.766 (95% CI: 0.677-0.856), the specificity was 94.12%, the sensitivity was 57.89%, and the cutoff value was 1.67. The AUC of M-CSF was 0.754 (95% CI: 0.666-0.841), the specificity was 64.71%, the sensitivity was 87.72%, and the cutoff value was 382.70 pg/mL. AUC indicates area under the curve; CI, confidence interval; M-CSF, macrophage colony-stimulating factor; miR-21, microRNA-21; miR-124, microRNA-124.
In summary, miR-21, miR-124, and M-CSF may be involved in the development and progression of cervical cancer. The detection of serum miR-21, miR-124, and M-CSF has good sensitivity and specificity in the diagnosis of cervical cancer.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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