

Clinical value of combined detection of miR-1202 and miR-195 in early diagnosis of cervical cancer

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Abstract. The clinical value of the combined detection of miR-1202 and miR-195 in the early diagnosis of cervical cancer was studied. A retrospective analysis of 70 cervical cancer patients treated in the The Third Affiliated Hospital of Kunming Medical University and Yunnan Cancer Hospital from October 2015 to December 2017 was performed, and the lesion tissues were used as the experimental group. Normal cervical tissues from another 67 healthy females confirmed by physical examination at the same period were selected as the control group. The FIGO staging criteria were used for staging of the cervical cancer patients, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method was used for the detection of the expression of miR-1202 and miR-195 in different tissues, and the receiver operating curve (ROC) was used for the analysis of the application values of miR-1202 and miR-195 diagnosis alone and their combined diagnosis in early cervical cancer patients. The levels of miR-1202 and miR-195 in the experimental group were lower than those in the control group ($P < 0.05$). The differences were significant in the different stages of cervical cancer tissues ($P < 0.05$). The later the staging of cervical cancer tissues were, the lower the levels of miR-1202 and miR-195 were. The sensitivities and area under the curve (AUC) values of miR-1202 and miR-195 in the combined diagnosis of early cervical cancer were significantly higher than those of miR-1202 and miR-195 alone. The expression levels of miR-1202 and miR-195 in the cervical cancer patients are different in different stages. Guiding clinical treatment and prognosis according to the results of combined detection is beneficial for the development of treatment for cervical cancer patients and for prognostic judgement, worthy of popularization and application.

Introduction

Cervical cancer has the second highest incidence of female common tumors, with the highest mortality rate among female cancers. Approximately 300,000 people die of it every year around the world (1). In recent years, the incidence of cervical cancer has increased and tends to be younger in age, posing a serious threat to the health of females (2). Without obvious symptoms in early onset, once found, cervical cancer is in the middle and late stage, with a poor prognosis. Therefore, its early diagnosis is of great significance for its treatment and prognosis (3).

At present, the pathogenesis of cervical cancer is still not fully clarified. Studies have shown that the occurrence and development of cervical cancer is an extremely complex multi-stage process regulated by many factors. The regulation of nucleic acid level is closely related to the occurrence of cervical cancer. In particular, the expression of target genes of non-coding microRNA (miRNA) is influenced by its target regulation, thereby affecting the occurrence and development of cervical cancer (4,5). For example, miR-31 (6) has an impact on the biological function of cervical cancer cells by the target regulation of gene ARID1A.

In recent years, related studies have shown that miR-1202 can function in the occurrence and development of multiple tumors by inhibiting the proliferation of tumor cells. Among them, miR-1202 has been studied in breast cancer (7) and endometrial cancer (8). However, there is no related report on the expression and diagnostic value of it in cervical cancer. miR-195 is an important cancer suppressor gene (9). Studies have shown that it inhibits tumor cells in many tumors such as hepatocellular carcinoma (10), breast cancer (11) and non-small cell lung cancer (12). Yet, its expression and diagnostic value in cervical cancer is still not very clear.

Therefore, in this study, in order to provide a better plan for the early diagnosis of cervical cancer, the expression of miR-1202 and miR-195 in cervical cancer was explored. Thus, the clinical value of the combined detection of miR-1202 and miR-195 in the early diagnosis was investigated.

Patients and methods

General information. A retrospective analysis of 70 cervical cancer patients treated in The Third Affiliated Hospital of

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Kunming Medical University and Yunnan Cancer Hospital (Kunming, China) from October 2015 to December 2017 was performed, lesion tissues were used as the experimental group. The average age of the patients was 40.2 ± 3.7 years. According to the FIGO staging criteria, there were: stage I with 26 cases, stage II with 29 cases, stage III with 9 cases and stage IV with 6 cases. There were 47 cases of cervical squamous cell carcinoma and 23 cases of cervical adenocarcinoma according to the pathological type. Normal cervical tissues from another 67 healthy females confirmed by physical examination at the same period were selected as the control group. The average age was 41.1 ± 2.8 years. All the tissues were taken out and quickly stored at -80°C . There was no significant difference in age and BMI between the two groups of patients ($P > 0.05$; Table I).

Inclusion and exclusion criteria. Inclusion criteria were: i) the experimental group included patients confirmed with cervical cancer by pathology, and ii) the control group included females confirmed as healthy by physical examination. Exclusion criteria were: i) patients having undergone radiotherapy and chemotherapy prior to taking specimens; ii) patients with severe other organ diseases; iii) patients who did not cooperate with the examination. and iv) patients with cognitive and communication impairment. The participants and their family members signed an informed consent form to cooperate with the medical staff to complete relevant medical treatment and the study was approved by the Ethics Committee of The Third Affiliated Hospital of Kunming Medical University and Yunnan Cancer Hospital.

Experimental instruments and materials. Refrigerator at -80°C (Sanyo, Tokyo, Japan); reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (iQ5 Multicolor; Bio-Rad Laboratories, Inc., Hercules, CA, USA); TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA); RT-qPCR kit and minScript reverse transcription kit (Takara Biotechnology Co., Ltd., Dalian, China).

Methods. The lesion tissues and normal cervical tissues were taken out from the refrigerator at -80°C . Trizol reagent was added, tissue RNA was extracted, and $1 \mu\text{l}$ of total RNA was obtained. The specific stem ring primers were added, respectively. Reverse transcription cDNA was performed according to the manufacturer's instructions. Reaction temperature: at 42°C for 15 min and at 85°C for 5 sec. Primers for miR-1202 and miR-195 were amplified according to sequence design, with U6 as an internal reference control (Table II). Amplification conditions: denaturation at 95°C for 30 sec, annealing at 60°C for 10 sec, extension at 72°C for 20 sec, for a total of 40 cycles, and extension at 72°C for 5 min to stop the reaction. The expression of miR-1202 and miR-195, respectively, was calculated and analyzed using the $2^{-\Delta\text{Ct}}$ method (13).

Statistical analysis. Chi-square test was used for enumeration data. Measurement data were expressed as mean \pm standard deviation, using the t-test. SPSS19.0 (Shanghai Yuchuang Network Technology Co., Ltd., Shanghai, China) statistical software was used for analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of relative expression of miR-1202 and miR-195 between the two groups. The relative expression of miR-1202 in the experimental group was 0.45 ± 0.13 , lower than that in the normal control group 0.97 ± 0.32 ; $P < 0.05$, and that of miR-195 in the experimental group was 0.53 ± 0.15 , lower than that in the normal control group 0.96 ± 0.25 ($P < 0.05$; Table III).

Comparison of relative expression of miR-1202 and miR-195 in different stages. In the cervical cancer patients of early stage (I-II) and middle-late stage (III-IV), the expression levels of miR-1202 in the experimental group were 0.65 ± 0.22 and 0.31 ± 0.10 , respectively, and those of miR-195 were 0.78 ± 0.18 and 0.29 ± 0.16 , respectively. Based on statistical analysis, there was a significant difference in the overall level ($P < 0.001$), suggesting that the later the clinical staging is, the lower the expression levels of miR-1202 and miR-195 are (Table IV).

Diagnostic values of miR-1202 and miR-195 detection alone and their combined detection in early stage (I-II) of cervical cancer. Calculated with early patients and healthy population, in the diagnosis of early cervical cancer patients, the diagnosis standard of miR-1202 alone was < 0.845 . The sensitivity, specificity and area under the curve (AUC) value was 81.82%, 65.67% and 0.7225, respectively. That of miR-195 diagnosis alone was < 0.950 . The sensitivity, specificity and AUC value was 80.00%, 49.25% and 0.6544, respectively. The combined diagnosis of miR-1202 and miR-195 was 89.09%, 58.21% and 0.7544, respectively. Among them, the sensitivity and AUC value of the combined diagnosis were significantly higher than those of miR-1202 and miR-195 diagnosis alone (Table V and Fig. 1).

Discussion

Cervical cancer is one of the most common malignant tumors of the reproductive system in females, with the highest incidence that is still rising (14). Studies have reported (15) that early cervical cancer is not easy to be detected, but once found, it is often in the middle and late stage, with a higher recurrence, invasion and metastasis rate, leading to poor treatment and prognosis of cervical cancer patients. The pathogenesis of cervical cancer is a very complicated process, involving the abnormalities of the structures and expression of many coding and non-coding genes, from normal cervical epithelial cells to infiltration of cancer cells (16). As a single-stranded non-coding regulatory RNA, by participating in the regulation of target gene expression, miRNA plays a role in various biological processes such as cell proliferation, apoptosis, differentiation and migration (17). The biological function of miR-1202 is mainly related to cell proliferation and apoptosis. Studies of Du *et al* (18) showed that miR-1202 induced endoplasmic reticulum stress and apoptosis through the target regulation of Rab1A in glioma. It also inhibited the proliferation of glioma cells. Studies have shown (19) that as a cancer suppressor gene, miR-195 is closely related to the occurrence of breast cancer, bladder cancer, colorectal cancer and other tumors, through the regulation of target genes. In addition, related studies have reported that the expression of miR-195 is downregulated in the lesion tissues of cervical cancer. The downregulation is

Table I. Comparison of general information between the two groups of patients [n (%)].

Factors	Experimental group (n=70)	Control group (n=67)	χ^2	P-value
Age			0.002	0.966
≤40	41 (58.57)	39 (58.21)		
>40	29 (41.43)	28 (41.79)		
BMI			0.005	0.942
≤22	37 (52.86)	35 (52.24)		
>22	33 (47.14)	32 (47.76)		
Married or not			0.055	0.814
Yes	51 (72.86)	50 (74.63)		
No	19 (27.14)	17 (25.37)		
Fertile or not			0.165	0.685
Yes	49 (70.00)	49 (73.13)		
No	21 (30.00)	18 (26.87)		
Family history			-	-
Yes	23 (32.86)	-		
No	47 (67.14)	-		
Staging			-	-
Stage I	26 (37.14)	-		
Stage II	29 (41.43)	-		
Stage III	9 (12.86)	-		
Stage IV	6 (8.57)	-		
Pathological types			-	-
Cervical squamous cell carcinoma	47 (67.14)	-		
Cervical adenocarcinoma	23 (32.86)	-		

Table II. RT-qPCR reaction miRNA-related primers.

Factors	Upstream primer sequences	Downstream primer sequences
miR-1202	5'-ATCCAGTGCGTGTCGTG-3'	5'-TGCTGTGCCAGCTGCAGT-3'
miR-195	5'-TAGCAGCA-CAGAAATATTGGC-3'	5'-TGCTGTGCCAGCTGCAGT-3'
U6	5'-GCTTCGGCAGCACATATACTA-AAAT-3'	5'-CGCTTCACGAATTTGCGTGTTCAT-3'

Table III. Comparison of relative expression of miR-1202 and miR-195 in the two groups.

Factors	Experimental group (n=70)	Control group (n=67)	t	P-value
miR-1202	0.45±0.13	0.97±0.32	12.56	<0.001
miR-195	0.53±0.15	0.96±0.25	12.27	<0.001

Table IV. Comparison of relative expression of miR-1202 and miR-195 in different stages.

Staging	miR-1202	miR-195
Stage I-II (n=55)	0.65±0.22	0.78±0.18
Stage III-IV (n=15)	0.31±0.10	0.29±0.16
t	5.800	9.554
P-value	<0.001	<0.001

Table V. Diagnostic values of miR-1202 and miR-195 detection alone and their combined detection in early cervical cancer.

Diagnostic methods	Sensitivity (%)	Specificity (%)	AUC
miR-1202	81.82	65.67	0.7225
miR-195	80.00	49.25	0.6544
miR-1202 combined with miR-195	89.09	58.21	0.7544

AUC, area under the curve.

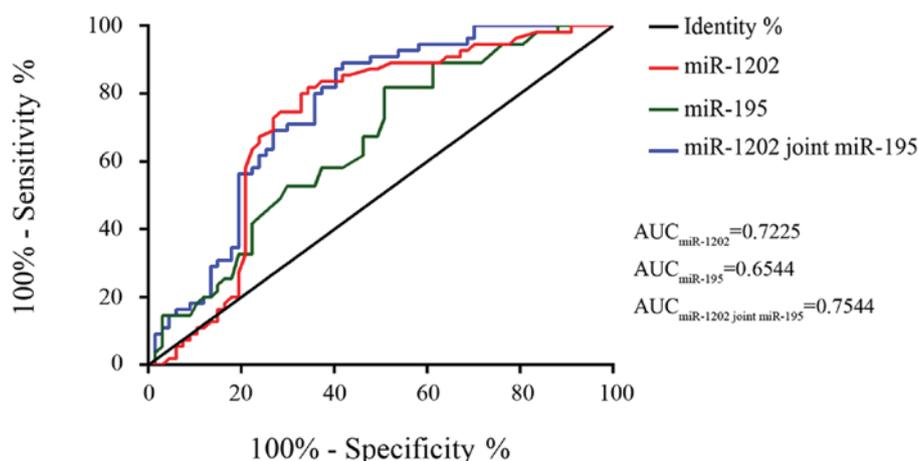


Figure 1. ROC curve of miR-1202 and miR-195 detection alone and their combined detection in early cervical cancer. Calculated with early patients and healthy population, in the diagnosis of early cervical cancer patients, the diagnosis standard of miR-1202 diagnosis alone was <0.845 , and that of miR-195 diagnosis alone was <0.950 . The AUC value of the ROC curve detected by miR-1202 alone was 0.7225, and that detected by miR-195 alone was 0.6544. That detected by miR-1202 combined with miR-195 was 0.7544, significantly higher than that detected by miR-1202 and miR-195 alone. AUC, area under the curve; ROC, receiver operating curve.

associated with deep interstitial infiltration and lymph node metastasis in cervical cancer patients (20).

In order to clarify the diagnostic values of miR-1202 and miR-195 in cervical cancer, in this study, their expression in lesion and normal cervical tissues was compared. It was found that the expression in the experimental group was lower than that in the control group ($P < 0.05$). Then, the relative expression of miR-1202 and miR-195 in different stages was compared. The results showed that the later the clinical staging was, the lower the expression levels were ($P < 0.05$). The study of Wipperman *et al* (21) showed that miR-1202 played a role as a tumor suppressor gene in cervical cancer, so its expression in cervical cancer was downregulated. In the study of Philp *et al* (22), it was found that miR-195 could act as a cancer suppressor gene in cervical cancer, so its expression in cervical cancer tissues was downregulated compared to normal tissues. The results are consistent with our findings, confirming the role of miR-1202 and miR-195 as cancer suppressor genes in cervical cancer. The diagnostic values of miR-1202 and miR-195 detection alone and their combined detection in early cervical cancer were evaluated. The results showed that the sensitivity, specificity and AUC value of miR-1202 diagnosis alone was 81.82%, 65.67% and 0.7225, respectively and in miR-195 diagnosis alone was 80.00%, 49.25% and 0.6544, respectively, and in the combined diagnosis of miR-1202 and miR-195 was 89.09%, 58.21% and 0.7544, respectively. The sensitivity and AUC value of the combined diagnosis were

significantly higher than those of miR-1202 and miR-195 diagnosis alone, showing that the value of the combined diagnosis of miR-1202 and miR-195 in early cervical cancer is higher than that of their diagnosis alone.

In conclusion, the expression of miR-1202 and miR-195 in cervical cancer tissues is significantly downregulated. The value of the combined detection is higher than that of the detection alone in early cervical cancer, which can be used as a preferred solution for the clinical diagnosis of early cervical cancer. However, there are few research studies on miR-1202 and miR-195 in cervical cancer, and the sample size is small. Therefore, more investigations are required to carry out extensive research.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XY conceived the study and drafted the manuscript. HY directed the project and was also involved in the conception and design of the study. ZY, LZ and HN acquired the data. LZ and YW analyzed the data and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Third Affiliated Hospital of Kunming Medical University and Yunnan Cancer Hospital (Kunming, China) and the participants and their family members signed an informed consent form.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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