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Expression of miR-128-3p, miR-193a-3p and miR-193a-5p in endometrial cancer tissues and their relationship with clinicopathological parameters

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ABSTRACT

In order to explore the expression of microRNA-128-3p (miR-128-3p), microRNA-193a-3p (miR-193a-3p) and microRNA-193a-5p (miR-193a-5p) in the endometrial carcinoma and their relationship with clinicopathological parameters, the post-operative clinical samples of 61 endometrial cancer patients who underwent surgical resection in our hospital from February 2019 to February 2022 were collected as cancer tissues. The post-operative clinical samples of 61 normal endometrium patients who underwent surgical resection due to non-tumor diseases in our hospital were collected as para-cancer tissues. miR-128-3p, miR-193a-3p and miR-193a-5p were measured by fluorescence quantitative polymerase, and the relationship between them and clinicopathological parameters and the correlation among them were analyzed. Results showed that miR-128-3p, miR-193a-3p and miR-193a-5p were lower in cancer tissues than in adjacent tissues (P<0.05). miR-128-3p, miR-193a-3p and miR-193a-5p were not related to the age and histopathological type of endometrial cancer patients (P>0.05). Still, they were related to FIGO stage, degree of differentiation, depth of myometrial invasion, lymph node metastasis and distant metastasis (P<0.05), and compared with FIGO stage I-II, medium and high differentiation, depth of myometrial invasion<1/2, no lymph node metastasis and no distant metastasis, FIGO stage III-IV, low differentiation miR-128-3p, miR-193a-3p and miR-193a-5p were lower in endometrial cancer patients with myometrial invasion depth≥1/2, lymph node metastasis and distant metastasis (P<0.05). miR-128-3p, miR-193a-3p and miR-193a-5p were the risk factors of endometrial carcinoma (P<0.05). miR-128-3p and miR-193a-3p were positively correlated (r = 0.423, P = 0.001); miR-128-3p and miR-193a-5p were positively correlated (r = 0.342, P = 0.007); miR-193a-3p and miR-193a-5p were positively correlated (r = 0.555, P = 0.001). miR-128-3p, miR-193a-3p and miR-193a-5p are low expressed in the cancer tissues of endometrial cancer patients and are related to the adverse clinicopathological parameters of patients. They are expected to become potential prognostic markers and therapeutic targets of the disease.

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Introduction

Endometrial cancer is an epithelial malignancy that starts in the endometrium and is mostly diagnosed in perimenopausal and postmenopausal women. Its pathogenesis has not yet been clinically known but is thought to be closely related to lifestyle (1). Endometrial cancer patients present no typical symptoms in the early stages, which makes it difficult to attract attention and is only detected incidentally during screening or gynecological examinations. But as the disease progresses, most patients report irregular vaginal bleeding, abnormal vaginal drainage, paroxysmal lower abdominal pain, and abdominal masses, severely besetting their daily life and work (2). While surgery is the main treatment for endometrial cancer with a good overall prognosis, the year-on-year increasing prevalence of endometrial cancer renders younger women more likely to develop this condition. Hence, it is important to find effective therapeutic targets for the treatment of this disease (3). Many previous research results have shown that the development of various malignant tumors is associated with the dysregulation of miRNA, which directly or indirectly regulates the expression of downstream target genes and thus participates in the progression of endometrial cancer (4,5). In this paper, the expression of miR-128-3p, miR-193a-3p and miR-193a-5p in patients with endometrial cancer was detected by fluorescence quantitative polymerase chain reaction (FQ-PCR) to investigate the correlation between the three miRNAs and clinicopathological parameters.

Materials and Methods

Subjects

Postoperative clinical samples were collected as cancer tissue from 61 patients with endometrial cancer treated by surgical resection at our hospital from February 2019 to February 2022, while 61 normal endometrium samples surgically resected due to non-neoplastic disease at our hospital were pooled as paracancerous tissue during the same period. Patients with endometrial cancer were aged 32-65 years with a mean age of (43.15±18.29) years, whereas patients with normal endometrium were aged 34-64 years with a mean age of (43.37±18.07) years. The data are comparable (P>0.05), and these samples were approved by the ethics committee.

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Inclusion criteria: confirmed diagnosis of endometrial cancer by pathological examination; patients in good general condition without serious respiratory, circulatory, or neurological complications; no other complicated tumors; no radiotherapy or targeted treatment of any kind prior to surgery; voluntary participation in this trial and signed informed consent.

Exclusion criteria: those with serious postoperative complications requiring resuscitation; those with serious chronic diseases; those with other complicated tumors postoperatively; those with recurrent endometrial cancer; those who voluntarily requested to withdraw from the trial.

Indicators

FQ-PCR was used to determine miR-128-3p, miR-193a-3p, and miR-193a-5p. RNA extraction: fresh tissues were stored with nitrogen solution and ground to extract total RNA using Trizol reagent. Then, the RNA was reverse transcribed to cDNA under the following conditions: 25°C, 50°C, 85°C (10 min, 45 min, and 5 min). cDNA was amplified. Primers: miR-128-3p; F: 5'-CGCGTCACAGTGAACCGGT-3', R: 5'-AGTGCAGGGTCCGAGGTATT-3', miR-193a-3p, F: 5'-TATGGCAGAAGGAGACCCG-3', R: 5'-CCATTCCTCACTGAACCCG-3' miR-193a-5p, F: 5'-AGTGTGGCATTAAGGGGCAAT-3', R: 5'-GCTCCTCTCGCTTCTGTTCTT-3'. Using U6 as the internal reference, F: 5'-GGTTAGAAGTCATACG-3', R: 5'-TGTCATGAATGATCC-3'. Reaction system: 10 μL BeyoFastTMSYBR Green qPCR Mix (2×), 2μL cDNA, 1μL upstream and downstream primers, and 6 μL H2O. Amplification conditions: 95°C pre-denaturation and denaturation both for 5 min, 95°C annealing and 72°C extension for 15s and 30s, respectively, 40 cycles. The relative expression of miR-128-3p, miR-193a-3p, and miR-193a-5p was calculated using 2-ΔΔCt.

Statistical analysis

SPSS 26.0 was used for data analysis. The Kolmogorov-Smirnov test was applied to test whether the data conformed to a normal distribution. Measurement data conforming to normal distribution were described by mean ± standard deviation ( )and compared between multiple groups using the F-test and between two groups using the independent sample t-test, while the data not conforming to normal distribution were expressed by M(Qn) after natural logarithm transformation using a non-parametric test. Count data expressed as a rate (%) were tested using c2 test. Correlation between miR-128-3p, miR-193a-3p, and miR-193a-5p was analyzed through Pearson's correlation coefficient, and P<0.05 was considered statistically different.

Results

miR-128-3p, miR-193a-3p, miR-193a-5p expression in endometrial cancer tissues

The miR-128-3p, miR-193a-3p and miR-193a-5p were significantly lower in cancer tissues than in paraneoplastic tissues, indicating statistical differences (P<0.05) (Table 1).

Association of miR-128-3p, miR-193a-3p, and miR-193a-5p with clinicopathological parameters in patients with endometrial cancer

First, miR-128-3p, miR-193a-3p, and miR-193a-5p were not associated with age and histopathological type in patients with endometrial cancer (P>0.05) but were associated with FIGO stage, differentiation, depth of myeloid infiltration, lymph node metastasis, and distant metastasis (P<0.05). Further, miR-128-3p, miR-193a-3p, and miR-193a-5p were lower in patients with FIGO stage III-IV cancer, low differentiation, myeloid infiltration depth ≥1/2, lymph node metastasis, and distant metastasis endometrial cancer than in those with FIGO stage I-II cancer, moderate to high differentiation, myeloid infiltration depth <1/2, no lymph node metastasis, and no distant metastasis endometrial cancer (P<0.05) (Table 2).

Multiple logistic regression of factors behind the occurrence of endometrial cancer

Endometrial cancer was the dependent variable (not occurring = 0, occurring = 1), whilst miR-128-3p, miR-193a-3p, and miR-193a-5p were the independent variables, whose elevated values were set to 1 and normal values to 0. They were included in a dichotomous multifactorial logistic regression model analysis, which showed that miR-128-3p, miR 193a-3p and miR-193a-5p were statistically different as risk factors that affected the occurrence of endometrial cancer (P<0.05) (Table 3).

Correlation between miR-128-3p, miR-193a-3p, and miR-193a-5p

The correlation analysis showed that miR-128-3p and miR-193a-3p were positively correlated (r=0.423, P=0.001); miR-128-3p and miR-193a-5p were positively

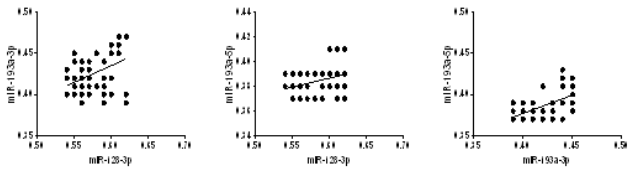

Figure 1. Correlation between miR-128-3p, miR-193a-3p, and miR-193a-5p.

Table 1. Expression of miR-128-3p, miR-193a-3p, and miR-193a-5p in cancerous and paraneoplastic tissues ($\bar{x} \pm s$).

Group	Cases	miR-128-3p	miR-193a-3p	miR-193a-5p
Paracancerous tissue	61	5.97±0.63	8.16±0.84	10.69±1.05
Cancerous tissue	61	0.58±0.04	0.42±0.03	0.37±0.02
t		66.690	71.920	76.750
P		0.001	0.001	0.001

Table 2. Association of miR-128-3p, miR-193a-3p, and miR-193a-5p with clinicopathological parameters in patients with endometrial cancer ($\bar{x} \pm s$).

Clinicopathological parameters		miR-128-3p	miR-193a-3p	miR-193a-5p
Age	<50 years old (n=45)	4.23±0.46	7.63±0.69	8.94±0.93
	≥50 years old (n=16)	4.29±0.41	7.56±0.78	9.02±0.84
t		0.460	0.337	0.303
P		0.647	0.737	0.763
FIGO staging	I-II (n=52)	4.18±0.44	5.24±0.53	8.37±0.85
	III-IV (n=9)	5.63±0.59	7.36±0.78	10.25±1.06
t		9.325	8..362	5.908
P		0.001	0.001	0.001
Differentiation	Medium to high differentiation (n=48)	4.28±0.46	5.09±0.54	8.67±0.89
	Low differentiation (n=13)	5.13±0.54	7.24±0.76	10.28±1.05
t		5.695	11.630	5.568
P		0.001	0.001	0.001
Depth of muscle infiltration	< 1/2 (n=46)	3.92±0.41	5.24±0.56	8.31±0.86
	≥1/2 (n=15)	5.36±0.57	7.58±0.79	10.36±1.05
t		10.690	12.650	7.588
P		0.001	0.001	0.001
Lymph node metastasis	No (n=43)	4.09±0.43	5.83±0.61	8.79±0.88
	Yes (n=19)	5.36±0.57	7.95±0.82	10.06±1.04
t		9.678	11.320	4.952
P		0.001	0.001	0.001
Distant metastasis	No (n=47)	3.89±0.42	5.31±0.56	6.43±0.68
	Yes (n=14)	5.32±0.56	7.64±0.79	9.58±0.97
t		10.330	12.380	13.730
P		0.001	0.001	0.001
Histopathological type	Endometrioid (n=45)	4.26±0.35	5.36±0.44	8.52±0.96
	Non-endometrioid (n=16)	4.13±0.42	5.28±0.54	8.67±0.89
t		1.210	0.588	0.547
P		0.231	0.559	0.587

Table 3. Multiple logistic regression of factors behind the occurrence of endometrial cancer.

Indicators	*Beta*	*SE*	*Wald*	*P*	*OR*	95%*CI*
miR-128-3p	1.68	0.73	6.492	0.015	1.64	0.985-2.473
miR-193a-3p	1.27	0.64	6.813	0.009	1.83	0.864-2.135
miR-193a-5p	1.43	0.58	7.159	0.003	2.16	1.237-2.849

correlated (r=0.342, P= 0.007); miR-193a-3p, miR-193a-5p were positively correlated (r=0.555, P=0.001) (Figure 1).

Discussion

Endometrial cancer treatment often only requires surgery, but sometimes adjuvant therapies such as radiotherapy and chemotherapy may be needed, all of which are only effective for patients with early-stage endometrial cancer and little effective for those with advanced and recurrent disease (6). Some studies have shown (7) that the molecular mechanism of endometrial cancer changes during the development of endometrial cancer, and it is of great clinical value to study the molecular mechanism and find new therapeutic strategies to improve the prognosis. miRNAs, a non-coding RNA that consists of a combination of 22 nucleotides, are expressed in both liquid and solid tumors and are easily extracted from tissues and blood. It acts as a gene regulator by binding mRNAs of untranslated regions and target genes, thus playing an oncogenic or oncogenic role in the development of tumors (8). Therefore, this paper aimed to analyze the expression of miR-128-3p, miR-193a-3p and miR-193a-5p in cancer tissues of endometrial cancer patients to provide a new direction for the diagnosis, treatment and prognosis of clinical endometrial cancer.

miR-128-3p is a newly discovered miRNA that is expressed specifically in a variety of malignancies (9). Zhang et al. (10) found that miR-128-3p was significantly low expressed in ovarian cancer, and by upregulating its expression level, SLC39A7 expression could be downregulated to promote apoptosis in ovarian cancer cells under endoplasmic reticulum stress and then stall disease progression. It has been shown (11,12) that miR-128-3p is aberrantly low in cervical cancer, and over-expressed miR-128-3p can exert its biological effect by targeting the

upregulation of BMI1 and promoting apoptosis in cervical cancer cells. Also, miR-128-3p serves as an oncogene in endometrial cancer, and up-regulated miR-128-3p can inhibit the proliferation, migration and invasion of endometrial cancer cells. In addition, its low expression was associated with differentiation, FIGO stage, vascular invasion and lymph node metastasis (13). In this study, the expression of miR-128-3p in endometrial cancer tissues was significantly lower than that in normal endometrial tissues as determined by FQ-PCR, and its low expression was closely related to FIGO stage, differentiation degree, depth of myofibrotic infiltration, lymph node metastasis, and distant metastasis. It is also a risk factor for the development of endometrial cancer and may be a biomarker for the prognostic assessment of endometrial cancer patients.

mir-193a-3p, located on human chromosome 17Q1.2, is a tumor inhibitor and parietal cell transforming factor. Clinical studies have confirmed that mir-193a-3p plays an important role in regulating the occurrence and development of a variety of diseases. For example, miR-193a-3p was aberrantly expressed in esophageal cancer tissues, correlated with lymph node metastasis and TNM stage. This indicates that mir-193a-3p may be involved in the malignant progression of esophageal cancer and further in the progression of esophageal cancer by targeting the ING1 gene (14,15). Previous clinical studies have suggested (16) that mir-193a-3p is characterized as a tumor suppressor gene in breast cancer to down-regulate the expression of cell cyclin-related proteins and inhibit the transformation of breast epithelial cells by regulating tyrosine kinase, thus inhibiting the occurrence and development of breast cancer. Associated with TNM staging and lymph node metastasis in breast cancer patients, it is a potential marker for breast cancer screening and prognosis. In this study, miR-193a-3p was found to be significantly less expressed in endometrial cancer tissues than in normal endometrial tissues by FQ-PCR, and its low expression was closely related to FIGO stage, differentiation, depth of muscle infiltration, lymph node metastasis, and distant metastasis. It was also a risk factor for endometrial cancer development and may be a biomarker for the prognostic assessment of patients with endometrial cancer. Its expression was abnormal in many malignant tumors such as bladder cancer, non-small cell lung cancer and esophageal squamous cell carcinoma. miR-193a-5p is highly expressed in ovarian epithelial cancer and correlated with lymph node metastasis, as confirmed by a clinical study (17). Meanwhile, patients with high miR-193a-5p expression have a poor prognosis and postoperative survival, which can be used as an indicator for the prognosis of ovarian epithelial cancer patients. miR-193a-5p was shown by animal experiments to inhibit the growth of endometrial cancer cells in nude mice, and its expression had an oncogenic effect in endometrial cancer patients as it correlated with their lymph node metastasis, FIGO stage and poor prognosis (18). This study was partially consistent with the above findings, where data analysis showed that miR-193a-5p expression was significantly lower in endometrial cancer tissues than in paracancerous tissues and closely correlated with FIGO stage, differentiation, myeloid infiltration depth, lymph node metastasis, and distant metastasis. However, the specific oncogenic effect of miR-193a-5p remains to be studied in depth.

In conclusion, miR-128-3p, miR-193a-3p and miR-193a-5p were significantly low expressed in cancer tissues of patients with endometrial cancer and were involved in the process of cancer development and metastasis. They are expected to be important markers for the early clinical diagnosis of endometrial cancer, providing a basis for improving clinical outcomes and assessing prognosis. However, there are still shortcomings in this study, including small sample size, single study subjects, and geographical restrictions on the source of cases. Therefore, increasing the sample size, including multicenter study subjects and expanding the range of case sources are needed in future studies to confirm the accuracy of this study.

Acknowledgments
Not applicable.

Interest conflict
The authors declare that they have no conflict of interest.

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