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Original Article

A miRNA-205-based model for prediction of the recurrence of endometrioid endometrial cancer

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Abstract: Aim: To develop a miRNA-205 based model for prediction of the recurrence of endometrial cancer. Methods: The FIGO (International Federation of Gynecology and Obstetrics) stage, grading, myometrial infiltration, lymph node status and miRNA-205 expression levels were extracted from 90 endometrioid endometrial cancer patients, recurrence related risk factors were analyzed by Cox regression analysis. A risk model was then developed. Results: A total of 90 endometrial cancer patients were retrospectively included for the analysis. The FIGO stage and expression levels of miRNA 205 were independently associated with the recurrence-free survival of the patients. The FIGO stage and expression levels of miRNA 205 were used for a prognostic model of recurrence-free survival. The c-index of the model reached 0.764, and the output of the model (risk score) could stratify the patients into different groups on the risk of recurrence. Conclusion: A miRNA-205 based model could predict the risk of recurrence for endometrioid endometrial cancer, and the model could provide a risk stratification of patients by recurrence risk.

Keywords: Endometrioid endometrial cancer, miRNA-205, risk score, recurrence

Introduction

Endometrial carcinoma (EC) is a group of epithelial malignant tumors that occur in the endometrium. Adenocarcinoma derived from endometrial glands is the most common [1, 2]. It is one of the most common gynecologic malignancies and seriously threatens women's health. Among women worldwide, the number of patients with endometrial cancer is expected to increase by 63,230 in 2018, ranking fourth in the incidence of female malignancies. It is estimated that the number of new deaths from endometrial cancer is 11,350, and the mortality rate ranks sixth among malignant tumors and the second among female reproductive tract malignant tumors, and has an upward trend of 2% year by year [3-5].

How to effectively improve the prognosis of EC patients and reduce the recurrence rate and mortality rate has always been an important direction to explore and study. With the deepening of clinical research and the development of molecular biology, more factors have been found to be related to the recurrence of endometrial cancer. miRNAs play an important role in tumor formation and are mainly involved in the regulation of tumor cell proliferation, apoptosis, and adhesion. There are more than 700 kinds of miRNA that have been discovered, and many studies have shown that miRNA-205 is abnormally expressed in various tumor cells, suggesting that miRNA-205 may be a cancer-related gene. Previous studies have shown that miRNA-205 has a significant correlation with the prognosis of EC patients [6, 7]. However, such an important correlation is still not applied in clinical practice. The expression level of miRNA-205 might be an important indicator for the prognosis of the EC patients. Therefore, the present study aimed to develop a prognostic model based on the expression level of miRNA-205 for EC patients.

Table 1. Baseline characteristics of the included patients

Characteristic	Patients, N (percent)
FIGO stage	
IA	25 (27.8)
IB	24 (26.7)
II	13 (14.4)
IIIA	4 (4.4)
IIIB	5 (5.6)
IIIC	12 (13.3)
IVB	7 (7.8)
Grading	
G1	34 (37.8)
G2	42 (46.7)
G3	14 (15.6)
Myometrial infiltration ≥ 0.5	
Yes	54 (60.0)
No	36 (40.0)
Positive lymph nodes	
Yes	15 (16.7)
No	75 (83.3)

FIGO, International Federation of Gynecology and Obstetrics.

Methods

Patients

This is a retrospective study. The patients' data were collected from Dryad database, and a total of 90 EC patients were included for the analysis. All patients underwent laparoscopic or abdominal surgery between 2002 and 2014. There were data on the FIGO (International Federation of Gynecology and Obstetrics) stage, grade, myometrial infiltration and lymph nodes status in the database. The study was approved by the Clinical Research Ethics Committee of The First Affiliated Hospital of Jinan University.

MiRNA-205

MiRNA-205 expression levels were evaluated in formalin-fixed paraffin-embedded tissues. RNA was isolated with the use of miRNeasy FFPE Kit (Qiagen), according to the manufacturer's protocol. All samples were deparaffinized, digested with proteinase K (followed by heat treatment). Supernatant was treated with DNase, then mixed with buffer red blood cells. 100% ethanol was used to adjust binding conditions. Subsequently, the sample was transferred to RNeasy MinElute Spin Column and elution with RNase-free water was performed. Total mRNA concentrations were evaluated with spectrophotometry and the quality of samples was validated based on the ratio of absorptions at 260 nm vs 280 nm. The next step of the procedure was cDNA synthesis. Reverse transcription was carried out by means of TaqMan MicroRNA Reverse Transcription Kit and microRNA-specific primers, according to manufacturer's protocol. Specific reagents for miRNA-205 were employed in real time polymerase chain reaction. Applied Biosystems 7900HT Fast Real-Time PCR System machine was used for real time PCR evaluation, according to manufacturer's recommendations. Samples were run in triplicate.

Statistical analysis

Constant variables are shown as mean ± standard deviation or median (range) as proper. The overall survival and recurrence-free survival were evaluated respectively. The overall survival was defined as the time from treatment to death, regardless of disease recurrence. The recurrence-free survival was defined as the time from treatment to disease recurrence. The univariate and multivariate survival analysis were used for the analysis of independent risk factors for the prognosis. Kaplan-Meier survival curves were used to analyze the relationship between the expression levels of miRNA-205 or other potential prognostic factors and patients' survival. Then we developed a predictive model for the recurrence-free survival of endometrioid endometrial cancer patients. The model performance was evaluated by the c-index. The ten-fold cross-validation was used for internal validation.

Results

A total of 90 endometrioid endometrial cancer patients were included in the analysis. The baseline characteristics of the patients are presented in **Table 1**. Several potential prognostic clinical factors were evaluated including the FIGO stage, grading, myometrial infiltration and also lymph node status. More than half of the patients were stage I, there were few patients with the advanced stage of cancer. The majority of the patients were grade 2, followed by then grade 1, and a few patients with grade 3

Table 2. Multivariate survival analysis of overall and recurrence-free survival

Characteristic	Hazard Ratio and 95% Confidence Intervals	
	Overall survival	Recurrence-free survival
FIGO stage		
Stage I-II	Reference	Reference
Stage III-IV	2.15 (0.88-5.23)	6.21 (1.76-21.88)
Grading		
G1	Reference	Reference
G2	1.27 (0.75-2.18)	2.52 (0.65-9.74)
G3	1.22 (0.55-2.71)	2.21 (0.46-10.76)
Myometrial infiltration ≥ 0.5		
Yes	Reference	Reference
No	0.75 (0.45-1.31)	4.57 (0.96-21.62)
Positive lymph nodes		
Yes	Reference	Reference
No	0.54 (0.44-4.97)	3.32 (0.86-12.91)
miRNA-205 expression levels		
High level	Reference	Reference
Low level	1.38 (0.16-12.01)	3.12 (1.77-4.59)

FIGO, International Federation of Gynecology and Obstetrics.

cancers. More than 60% of the patients had the myometrial infiltration ≥ 0.5. 83.3% of the patients were lymph node negative. The median follow-up duration of those patients were 46 months. 18 patients had the cancer recurrence by the follow-up. The baseline characteristics of patients with and without recurrence are listed in the Supplementary Table 1.

We performed a survival analysis to study clinical risk factors associated with overall and recurrence-free survival. As shown in Supplementary Figure 1, the grading of the cancer was not significantly associated with overall-survival, but significantly associated with recurrence-free survival. In Supplementary Figure 2, we observed that patients with FIGO stage I-II had a better overall survival than patients of stage III-IV, but the difference was not significant. There was a significantly better recurrence-free survival in stage I-II patients than stage III-IV patients.

Patients with myometrial infiltration ≥ 0.5 and positive lymph nodes had a significantly better recurrence-free survival (Supplementary Figures 3 and 4). Then we explored the prognostic value of the expression levels of miRNA 205. The X-tile software was used for the definition of the cutoff for the expression levels. With the defined cutoff, those patients were divided into high and low level groups. Patients with high levels of miRNA 205 had a significantly better recurrence-free survival than those patients with low levels (Supplementary Figure 5).

We performed multivariate analysis to find the independent risk factors for prognosis. The results are presented in the **Table 2**. The FIGO stage and expression level of miRNA 205 were independently associated with the recurrence-free survival of the patients. Therefore, the FIGO stage and expression level of miRNA 205 were used as a prognostic model for recurrence-free survival. The formula of the risk model is presented below:

risk score = 2.54 × *FIGO stage* + 0.93 × miRNA 205 expression levels

The c-index of the model reached 0.764. Then the output of the model (risk score) was used for the evaluation of the recurrence-free survival of the patients. As shown in the **Figure 1**, patients of high risk had a significantly worse recurrence-free survival than patients of low risk. As presented in **Figure 2**, the areas under the receiver operating characteristic curve for the prediction of the 3-year and 5-year recurrence were 0.78 (95% confidence interval [CI] 0.66-0.90) and 0.79

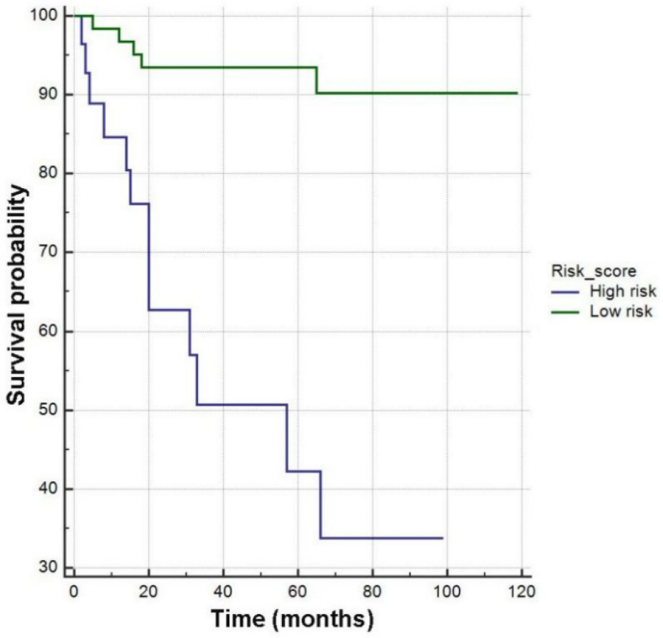

Figure 1. Kaplan-Meier curve of the risk score for recurrence-free survival.

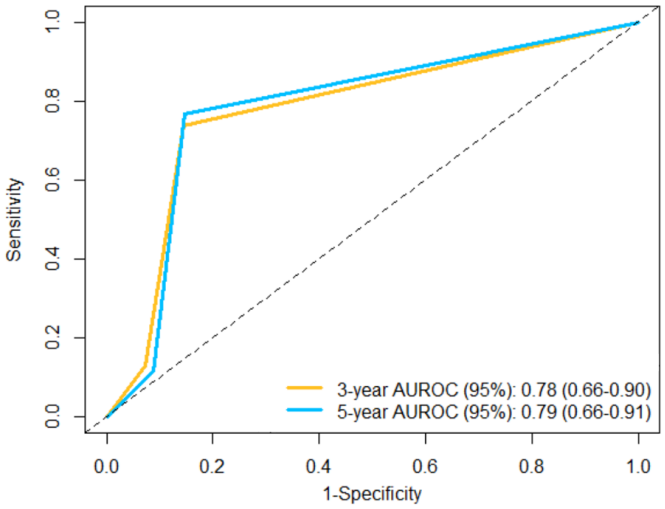

Figure 2. The receiver operating characteristic curve for prediction of the 3-year and 5-year recurrence. AUROC, area under the receiver operating characteristic curve.

(95% CI 0.66-0.91), respectively. We plotted the nomogram for the prediction of the recurrence in the **Figure 3**.

Discussion

In the present study, we explored the prognostic value of miRNA 205 in endometrial carcinoma (EC) patients. We found that the expression level of miRNA-205 was significantly associated with the recurrence of the EC. We developed a risk score based on the FIGO stage and expression level of miRNA 205 for the prediction of the recurrence. The predictive performance of the risk score was acceptable with the c-index reaching 0.79.

Our study was the first one that combined the expression of miRNA 205 into the clinical prediction model for recurrence. MiRNA 205 is located in the second intron of locus LOC642587 on chromosome 1 of human gene [8]. MiR-205 is highly conserved among different germlines [9]. A large number of studies have confirmed that the expression of miRNA 205 is related to the occurrence of various tumors [10]. The biologic role of miRNA 205 is different in different tumors.

There always was high expression of miRNA 205 in EC tissues and cell lines. Hiroki et al. found that miRNA 205 was upregulated by 267.8% in the expression profile [11]. Wu et al. analyzed the expression profiles of miRNAs in EC and paracancerous tissues, among which miRNA 205 was up-regulated by 18.9 times [12]. Karaayvaz et al. analyzed the miRNA expression levels of 48 pairs of EC and their normal endometrial samples by real-time qRT-PCR and found that miRNA 205 was significantly up-regulated [13].

We found an association between the expression level of miRNA 205 and the prognosis of the EC patients. The mechanism on that has been explored in several studies. The epithelial-mesenchymal transition (EMT) is related to EC muscle infiltration, and its process is closely related to miRNAs, which is an important factor for the prognosis of EC. The activation of serine-threonine protein kinase (AKT) has become a central feature of EMT, which is the first step in tumor metastasis in many cancer models. Jin et al. applied anti-miRNA 205 biological agents to EC cells HEC-50B and HEC-1-A by measuring EMT changes, and the results showed that miRNA 205 participates in the proliferation, invasion, and migration of EC by targeting and regulating the AKT pathway [14].

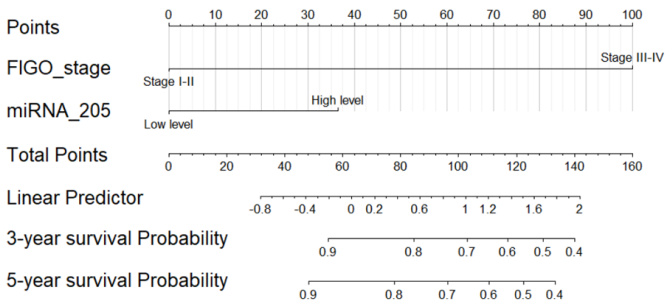

Figure 3. miRNA 205 based nomogram for prediction of recurrence. FIGO (International Federation of Gynecology and Obstetrics).

Torres et al. used miRNA 205 inhibitors to transfect EC cells (HEC-1-B, RL-95, KLE, Ishikawa), and found that locked nucleic acid inhibitors of miRNA 205 could reduce in vitro and in vivo EC cells' proliferation, and systemic delivery of their inhibitors is feasible without significant toxicity [15]. This confirmed that miRNA 205 can affect the invasion and metastasis of tumor cells. Su et al. found that downregulating miRNA 205 can inhibit the proliferation, growth, invasion, and metastasis of cancer cells [16]. Wilczynski et al. examined 100 formalin-fixed paraffin-embedded tissues by RT-PCR and observed higher levels of miRNA 205 expression in less than 50% of tumors with myometrial invasion and non-advanced cancer [17]. Therefore, the above studies confirm that miRNA 205 regulates the expression of related gene proteins and signaling pathways in EC, forms a regulatory network, and affects the aggressiveness and prognosis of tumor cells.

The miRNA 205-based risk score could be used in clinical practice. Our risk score could stratify the patients into high and low risk groups. For the patients of high risk, those patients should be treated actively for their high risk of recurrence. Follow-up strategy should be managed differentlyfor patients of high versus low risk. The nomogram is a visualization of the risk score which can be used in clinical practice more easily.

There were several limitations to the present study. First, the sample size was small, this would lead to selection bias. Second, the clinical model in the present study could not be reported as the TRIPOD checklist, due to that we lack of important external validations. Third, the inclusion and exclusion criteria of the study was not described with detail in the database that might lead to bias. Lastly, the mechanism of miRNA-205 on the prognosis of the EC patients was not explored.

The present study developed a miRNA-205-based model for prediction of the recurrence of endometrioid endometrial cancer. This model could be promoted in clinical practice and be helpful for the treatment for the endometrioid endometrial cancer.

Disclosure of conflict of interest

None.

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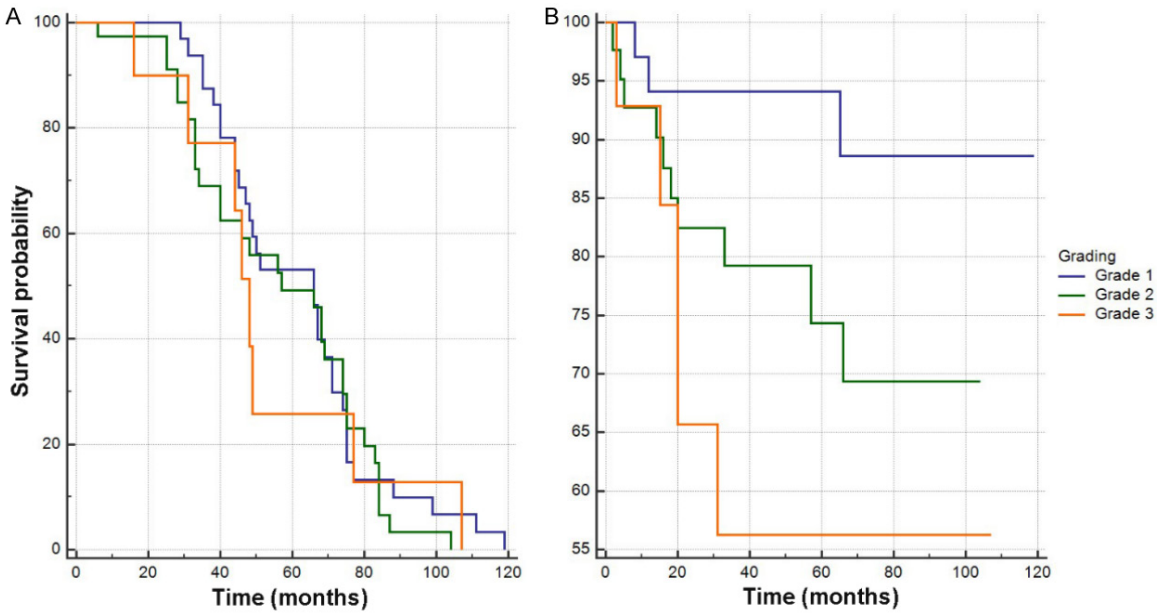
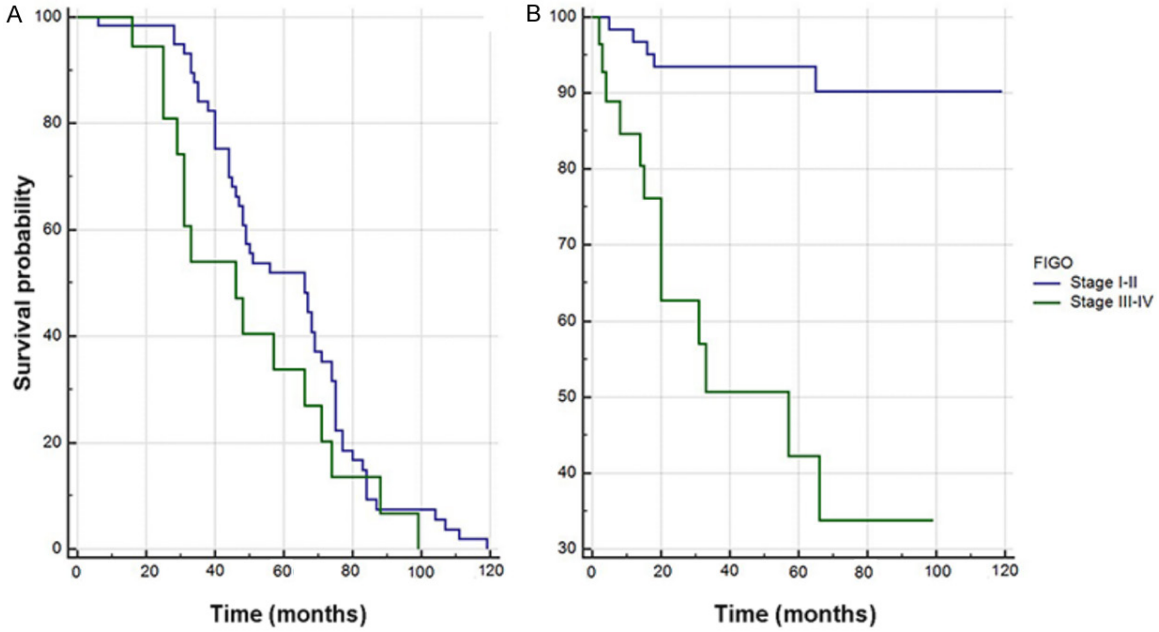
Supplementary Table 1. Baseline characteristics ofrecurrence and no recurrence patients

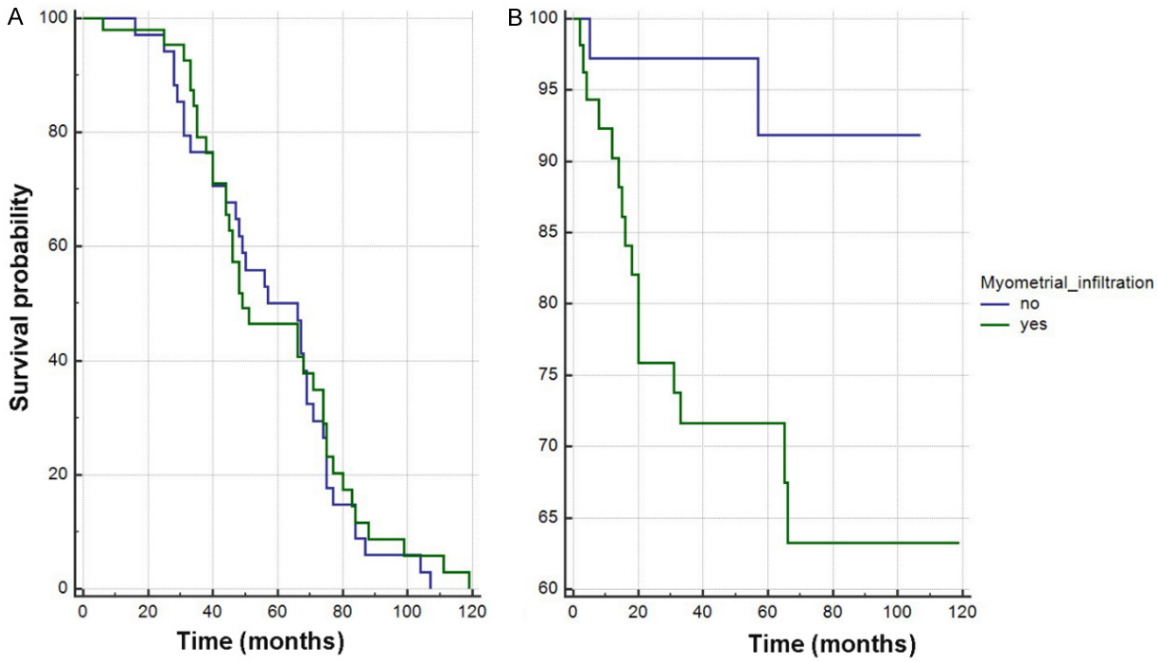
Characteristic	No Recurrence	Recurrence	*P* value
FIGO stage			0.003
IA	24 (33.3)	1 (5.6)	
IB	21 (29.2)	3 (16.7)	
II	12 (16.7)	1 (5.6)	
IIIA	3 (4.2)	1 (5.6)	
IIIB	2 (2.8)	3 (16.7)	
IIIC	6 (8.3)	6 (33.3)	
IVB	4 (5.6)	3 (16.7)	
Grading			0.074
G1	31 (43.1)	3 (16.7)	
G2	32 (44.4)	10 (55.6)	
G3	9 (12.5)	5 (27.8)	
Myometrial infiltration ≥ 0.5			0.005
Yes	34 (47.2)	2 (11.1)	
No	38 (52.8)	16 (88.9)	
Positive lymph nodes			0.005
Yes	64 (88.9)	11 (61.1)	
No	8 (11.1)	7 (38.9)	



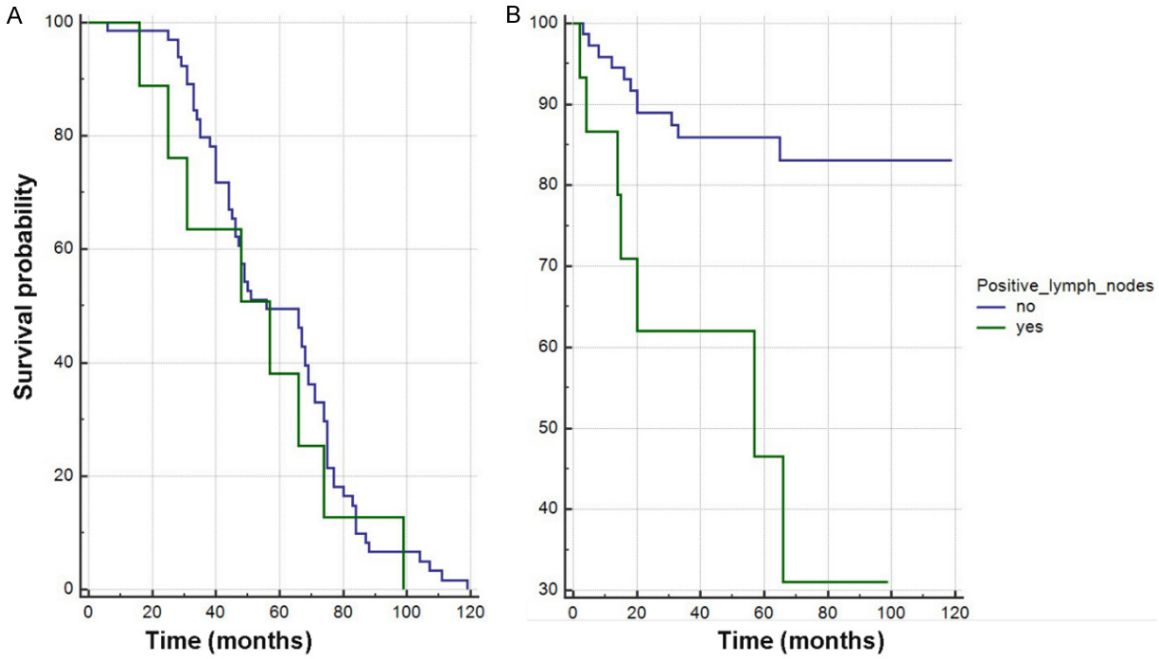
Supplementary Figure 1. Kaplan-Meier curve based on grading of patients. A, B. Represent overall and recurrence-free survival.



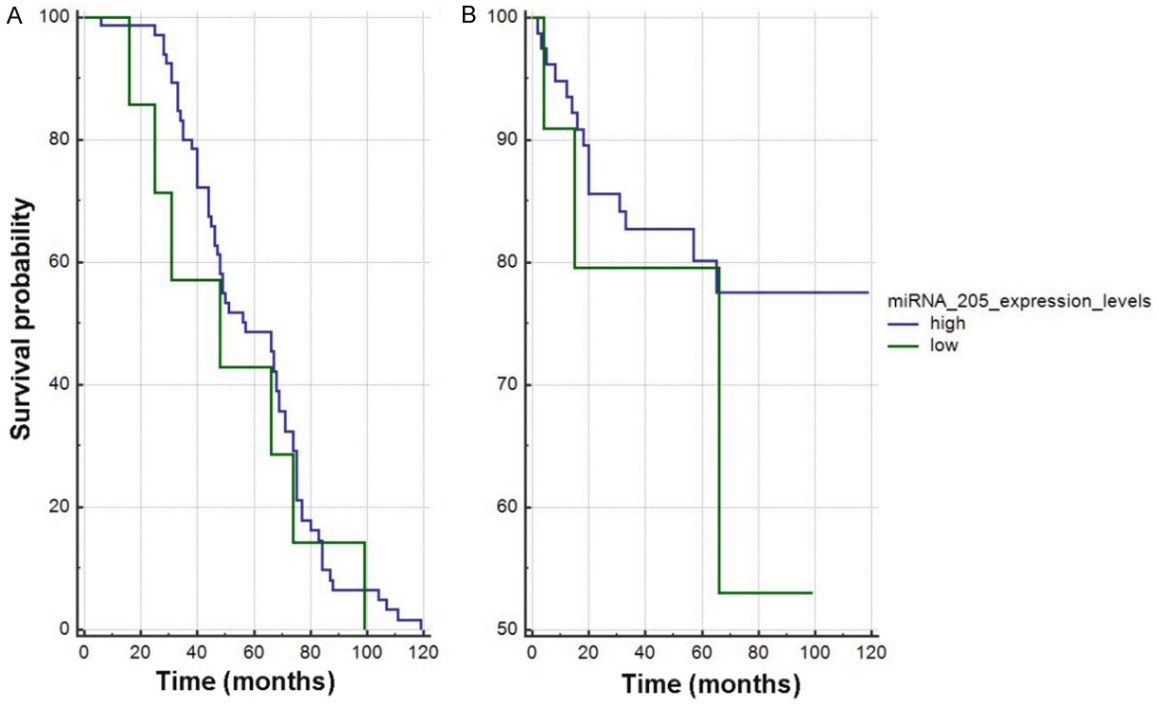
Supplementary Figure 2. Kaplan-Meier curve based on FIGO stage. A, B. Represent overall and recurrence-free survival.



Supplementary Figure 3. Kaplan-Meier curve based on myometrial infiltration status. A, B. Represent overall and recurrence-free survival.



Supplementary Figure 4. Kaplan-Meier curve based on lymph node status. A, B. Represent overall and recurrence-free survival.



Supplementary Figure 5. Kaplan-Meier curve of miRNA-205 expression levels. A, B. Represent overall and recurrence-free survival.