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Original Article

Aberrant expression of MCM_2 and Ki-67 are associated with endometrial carcinoma and its survival rate

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Abstract: Objective: Endometrial carcinoma (EC) is one of the most common gynecologic malignancies. Minichromosome maintenance-2 (MCM_2) and Ki-67 are both expressed during whole cell cycle to regulate proliferation and they have been proposed as potential prognostic markers in various tumors. In present study, we aim to examine the relationship between endometrial carcinoma and aberrant expression of MCM_2 and Ki67. Methods: The expression of MCM_2 and Ki-67 in normal endometrium tissues, atypical hyperplasia of endometrium (EAH) tissues and endometrioid adenocarcinoma (EAC) tissues were detected by immunohistochemical staining and statistically analyzed subsequently. Results: The positive expression rate of MCM_2 and Ki67 both showed statistical difference among normal tissues, EAH tissues, and EAC tissues. For clinical pathological characteristics, the positive expression of MCM_2 was related to the histopathologic grade and depth of stromal invasion, while the positive expression of Ki-67 was related to the lymph node metastasis. There was a positive correlation between MCM_2 and Ki-67 protein in EA tissues. Moreover, the 3-years survival rate in MCM_2 high expression group was significantly lower than that in the low expression group. *Conclusions:* MCM_2 and Ki-67 expression are significantly higher in EC tissues and their expressions are positively correlated. Expression of MCM_2 affects the 3-year survival rate in patients with EAC, indicating MCM_2 can be regarded as a prognosticator in EAC diagnosis.

Keywords: Endometrial carcinoma, MCM_2, Ki-67

Introduction

Endometrial carcinoma (EC) is a common malignant tumor of the female reproductive system and is encountered by most gynecologists on clinic. Recently, the incidence of endometrial carcinoma is rising, even becomes the highest in gynecological tumors in some developed countries. Meanwhile, its 5-year survival rate is declining. Therefore, the study of endometrial carcinoma is getting more attention over these years. Estrogen-dependent endometrial adenocarcinoma (EAC) is the most common type of endometrial carcinoma, accounting for 80%-85% in entire endometrial carcinoma [1-4]. However, the incidence and development mechanism of endometrial carcinoma is still unclear.

Tumor is basically a disease with disorder of cell cycle and uncontrolled growth due to the combined effects of hereditary factors and environmental factors. Regulation of cell cycle is complicated and involves an extensive variety of genes and proteins, among which the minichromosome maintenance (MCM_{2-7}) proteins are essential DNA replication and cell cycle initiation factors [5, 6]. During the cell cycle, the MCM proteins form a hexametric complex, which is a pivotal component of the pre-replication complex. In addition, MCM proteins restrict DNA synthesis to only once per cell cycle, and also regulate DNA elongation. The MCM_2 is an important member of MCM protein family, which is responsible for stabilizing and regulating the pre-replication complex. The expression of MCM_2 is constitutively high in proliferating cells but low or undetectable in quiescent cells. Therefore, its time-course expression pattern can accurately indicate the cell proliferative activity [7-10]. MCM_2 has been studied in gastrointestinal cancer, renal cancer, oral squamous cell cancer, bladder cancer and

Table 1. Expression of MCM_2 in endometrium tissues

Tissue types	Case (n)	MCM_2				Positive Expression Rate (%)	*P* value
		-	+	++	+++		
Normal	20	17	3	0	0	15.0	0.013[a]
Atypical Hyperplasia (EAH)	20	11	7	2	0	45.0	0.021[b]
Endometrioid Adenocarcinoma (EAC)	50	13	9	10	18	74.0	< 0.001[c]
							< 0.001[d]

[a]Comparison between normal tissues and EAH tissues (Mann-Whitney U test); [b]Comparison between EAC tissues and EAH tissues (Mann-Whitney U test); [c]Comparison between normal tissues and EAC tissues (Mann-Whitney U test); [d]Comparison among three types of tissues (Kruskal-Wallis test).

Table 2. The relationship between the expression of MCM_2 and the pathological characteristics in endometrium tissues

pathological characteristics	Case (n)	MCM_2		Positive Expression Rate (%)	X^2	*P* value
		Negative (-)	Positive (+ ~ +++)			
Age (y)					0.156	0.693
≤ 50	17	5	12	70.0		
> 50	33	8	25	35.0		
Surgical pathology Level					0.748	0.688
Stage I	19	6	13	68.4		
Stage II	15	4	11	73.3		
Stage III	16	3	13	81.2		
Histopathologic grade						
G_1	13	7	6	46.1	4.535	0.033[a]
G_2	25	5	20	80.0	0.812	0.356[b]
G_3		1	11	91.6	5.940	0.015[c]
Degree of infiltration					10.546	0.001
≤ no or 1/2	23	11	12	52.2		
> 1/2	27	2	25	92.6		
Lymphatic metastasis					2.225	0.135
No	34	11	23	67.6		
Yes	16	2	14	87.5		

[a]Comparison between grade G_1 and grade G_2; [b]Comparison between grade G_2 and grade G_3; [c]Comparison between grade G_1 and grade G_3.

pituitary adenoma and these studies indicate that aberrant expression of MCM_2 exists in a variety of cancers [5, 6, 11-16].

Ki-67 is another important cell proliferative biomarker, which is a nuclear protein closely associated with mitosis process. During mitosis, Ki-67 undergoes phosphorylation and de-phosphorylation modification at the breakdown and the reorganization of the nucleus, two key occasions of the cell cycle [17-19]. Although recognized as a cell proliferation associated protein, the specific functions of Ki-67 remain unclear. Since Ki-67 presents throughout the complete cell cycle except for G0 phase [19], it has been widely used for clinical diagnosis and study for the kinetics of cell proliferation in various tumors, including breast cancer, cervical cancer, prostate cancer, and brain cancer [20-22]. Several studies have been demonstrated that Ki-67 plays an important role in the tumor metastasis and invasion [20-24].

Although MCM_2 and Ki-67, as two cell proliferation makers, have been previously studied in various cancers, few studies are conducted to assess the potential prognostic effect of these two markers in patients with EC. In this study, we examined the expression of MCM_2 and Ki-67 in endometrial adenocarcinoma tissues

Table 3. Expression of Ki-67 in endometrium tissues

Tissue types	Case (n)	MCM_2				Positive Expression Rate (%)	*P* value
		-	+	++	+++		
Normal	20	14	5	1	0	30.0	0.027[a]
Atypical Hyperplasia (EAH)	20	7	10	3	0	65.0	0.350[b]
Endometrioid Adenocarcinoma (EAC)	50	12	9	14	15	76.0	< 0.001[c]
							< 0.017[d]

[a]Comparison between normal tissues and EAH tissues (Mann-Whitney U test); [b]Comparison between EAC tissues and EAH tissues (Mann-Whitney U test); [c]Comparison between normal tissues and EAC tissues; (Mann-Whitney U test); [d]Comparison among three types of tissues (Kruskal-Wallis test).

by immunohistochemical staining, and analyzed the relationship between these two markers and various clinical and pathological features in patients with EC.

Materials and methods

Tumor and patient data

90 Paraffin-embedded tissues from China Jiangxi maternal and child health hospital between 2006 and 2009 were investigated in this project. Among them, 20 cases were normal endometrium tissues, 20 cases were atypical hyperplasia of endometrium and 50 cases were endometrioid adenocarcinoma tissues. Comparisons of the clinical pathological features of the 50 endometrioid adenocarcinoma tissues are showed in **Tables 1**-**3**. None of the patients had received any hormonotherapy or chemoradiotherapy before and were followed-up for three years.

Detection and scoring methods

The patient tissue samples were fixed in 10% formalin, embedded in paraffin and cut into 4 μm tissue sections. Immunohistochemistry staining was performed to determine the expression level of MCM_2 and Ki-67 by using Elivision™ plus Polyer HRP (Mouse/Rabbit) IHC Kit (KIT-9901, Fuzhou Maixin Biotech) according to the manufacturer's instructions. The primary antibodies (MCM_2: Mouse Monoclonal Antibody, MS1726P1, LabVision; Ki-67: RB-90-43, Fuzhou Maixin Biotech) were diluted at 1:200 in 2% bovine serum albumin and incubated with tissue sections for 1 hr at room temperature. All sections were counterstained with hematoxylin. Expression of MCM_2 and Ki-67 were scored by assessing the percentage of the nucleus with brown yellow or brown particles: "-" meant the percentage was less than 10%; "+" meant the percentage was between 10% and 50%; "++" meant greater than 50% but less than 75% and "+++" means the percentage is larger than 75%. The patient tissue samples marked with "-" or "+" were defined as low positive expression and "++" or "+++" were defined as high positive expression.

Statistics analysis

All statistical analyses were performed using SPSS 18.0 software. Non-graded enumeration data was assessed by Chi-square test or Fisher's exact test. Graded enumeration data was assessed by Mann-Whitney U-test or Kruskal-Wallis test. Kaplan-Meier survival analysis and Cox proportional hazards regression were used to identify the role of MCM_2 and Ki-67 in survival. *P* values less than 0.05 were considered as statistically significant.

Results

Expression of MCM_2 is associated with clinical pathological characteristics in EAC tissues

In this study, 20 cases of normal endometrium tissues, 20 cases of endometrial atypical hyperplasia (EAH) tissues and 50 cases of EAC tissues were examined. These tissues were grouped into 4 levels ("-", "+", "++", and "+++") according to the expression level of MCM_2, which was mainly located in nuclei (**Figure 1**). The tissue cases marked with "+" were defined as low positive expression and marked with "++" or "+++" were defined as high positive expression. Clinic information of the patietns are shown in Supplementary Table 1.

Of the 50 EAC tissue cases, 9 showed low expression of MCM_2 and 28 showed high positive expression of MCM_2, resulting a total positive rate of 74.0%. In EAH tissues, there were 7

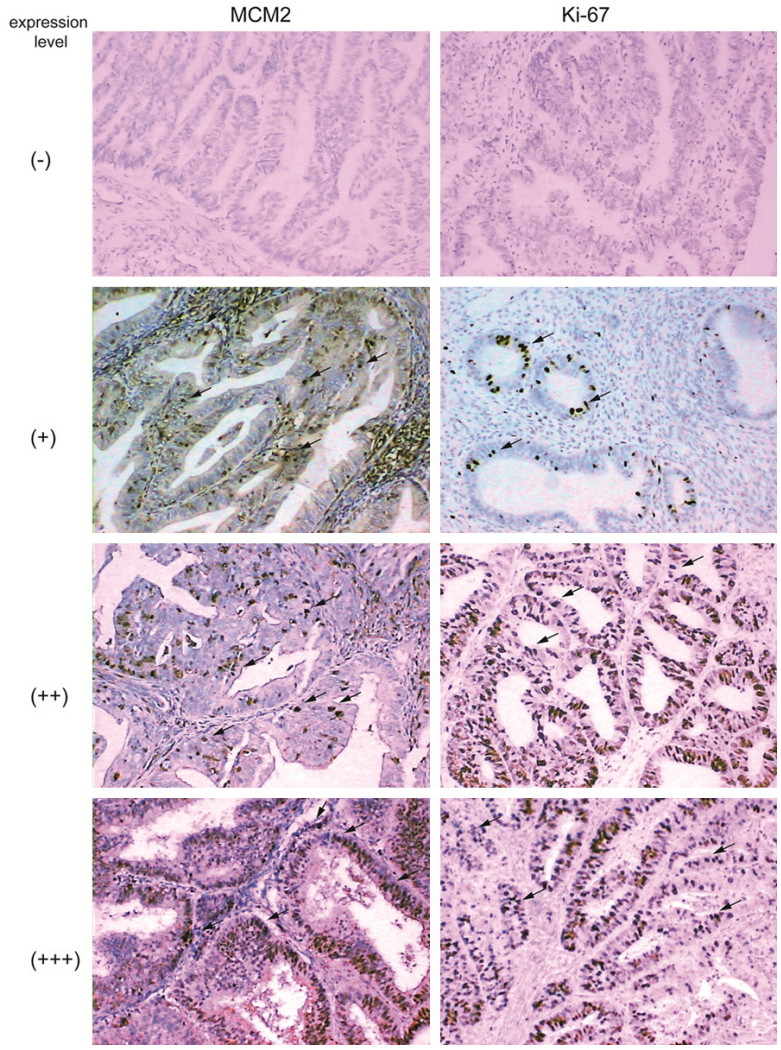

Figure 1. Expression pattern of proteins MCM$_2$ and Ki67 in EAC tissues. Black arrows indicate expression of MCM$_2$ or Ki67 located in the nucleus, 200×.

cases of low positive expression and 2 cases of high positive expression, resulting a total positive rate of 45.0%. But in the normal endometrium tissues, only 3 cases were positive expression, resulting a total positive rate of 15.0%. The difference of MCM$_2$ positive expression rate between each two groups and among all three groups were all statistically significant ($P < 0.05$) (**Table 1**).

The pathological characteristics analysis results in EAC tissues were showed in **Table 2**. Histopathologic grade analysis of 50 cases of EAC tissues indicated that the positive expression rate of MCM$_2$ were 46.1% in well-differentiated EAC, 80% in intermediate differentiated EAC, and 91.6% in poorly differentiated EAC, showing significant difference among them ($P < 0.05$). The positive expression rates between superficial myometrial invasion group (52.2%) and deep myometrial invasion group (92.6%) also showed significant difference ($P < 0.05$). Although the positive expression rate gradually increased in the clinical stage I, II, and III, there was no significant difference between these groups ($P > 0.05$). In addition, the MCM expression had no significant difference between lymphatic metastasis grade and different age stage.

Expression of Ki-67 is associated with clinical pathological characteristics in EAC tissues

Ki-67 was mainly located in the nucleus of tumor cells, as shown in **Figure 1**. Three types of tissues were also grouped into 4 levels ("-", "+", "++", and "+++") according to the expression level of Ki67. The positive expression rate of Ki-67 was 76.0% in 50 cases of EAC tissues, from which there were 29 cases with Ki67 high positive expression and 9 cases with low positive expression. In EAH tissues, 13 cases of high expression and 3 cases of low expression were found, resulting a total positive expression rate of 65.0%. However, in normal endometrium, there were only 5 cases with Ki 67 low expression and 1 case with high expression, resulting a total positive expression rate of 30%. The difference of Ki67 positive expression in these three tissue types were statistically significant ($P < 0.05$) (**Table 3**).

The pathological characteristics analysis in EAC tissues were showed in **Table 4**. For the histopathologic grade, the positive expression rate of Ki-67 showed statistically significant between G_1 (46.1%) and G_2 (84.0%), and G_1 and

Table 4. The relationship between the expression of Ki67 and the pathological characteristics in endometrium tissues

Pathological characteristics	Case (n)	Ki67		Positive Expression Rate (%)	X^2	*P* value
		Negative (-)	Positive (+ ~ +++)			
Age (y)						
≤ 50	17	4	13	76.5		
> 50	33	8	25	76.6	0.003	0.955
Surgical pathology Level						
Stage I	19	7	12	63.2		
Stage II	15	3	12	80.0		
Stage III	16	2	14	87.5	3.010	0.222
Histopathologic grade						
G_1	13	7	6	46.1	5.956	0.015[a]
G_2	25	4	21	84.0	0.408	0.523[b]
G_3	12	1	11	91.6	5.940	0.015[c]
Degree of infiltration						
≤ no or 1/2	23	9	14	60.9		
> 1/2	27	3	24	88.8	5.346	0.021
Lymphatic metastasis						
No	34	11	23	67.6		
Yes	16	1	15	93.8	4.064	0.044

[a]Comparison among grade G_1 and G_2; [b]Comparison between grade G_2 and grade G_3; [c]Comparison between grade G_1 and grade G_3.

Table 5. Correlation analysis of the expression of MCM_2 and Ki-67 in endometrium tissues

Ki-67 \ MCM_2	Negative	Positive	Total	Spearman rank correlation
Negative	7	6	13	r = 0.414, P = 0.003
Positive	5	32	37	
Total	12	38	50	

Table 6. Relationship between the expression of MCM_2 and Ki-67 and three-year survival rate in endometrial adenocarcinoma patients with EAC

Gene	Expression Level	Case	Three-year survival rate	*P* value
MCM_2	Low[a]	22	95.5	
	High	28	67.9	0.010
Ki-67	Low	21	90.5	
	High	29	72.4	0.104

[a]Tissues above marked with "-" or "+" were defined as low expression level and "++" or "+++" were defined as high expression level.

G_3 (91.6%), respectively. For the degree of tumor infiltration, the positive expression rates were 60.9% and 88.8% in superficial myometrial invasion group and deep myometrial invasion group, showing significant difference (P < 0.05). In addition, Lymphatic metastasis, but not clinical stage and age, was associated with the positive expression rate.

MCM_2 expression was positively correlated with Ki-67 expression in patients with EAC tissues

As shown in **Table 5**, of 50 EAC tissue cases, 37 showed positive expression of MCM_2, whereas 32 showed positive expression of Ki-67. There were 7 cases expressing MCM_2 alone and 6 cases expressing Ki-67 alone. Spearman rank correlation analysis showed that MCM_2 expression was positively correlated with Ki-67 expression in endometrial adenocarcinoma tissues (r = 0.414, P = 0.003).

Relationship between the expression of MCM_2 and Ki-67 and three-year survival rate

Fifty cases of patients with EAC were followed up by telephone and outpatient reexamination. The duration of overall follow-up was from 6 to 60 months, with a median follow-up time of 36 months. Follow-up rate was 100%. 10 people died due to tumor recurrence during follow-up

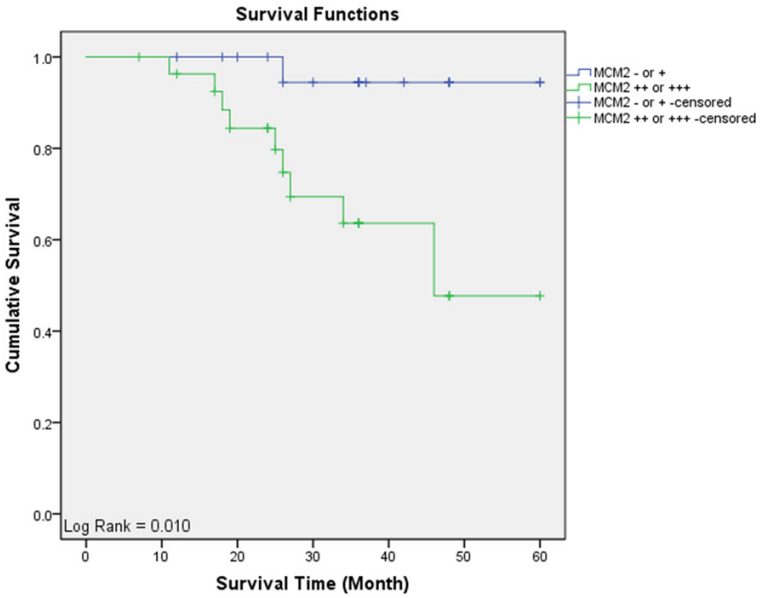

Figure 2. Kaplan-Meier survival curves for MCM_2 in patients with EAC.

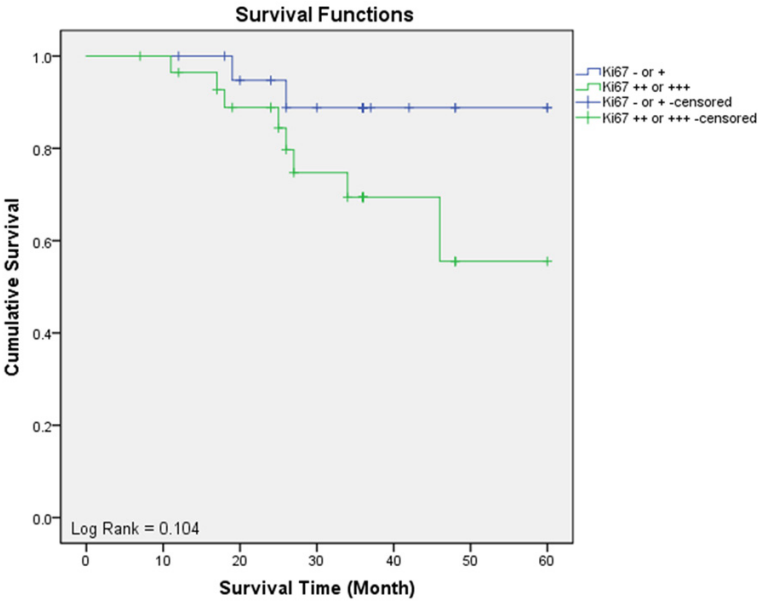

Figure 3. Kaplan-Meier survival curves for Ki-67 in patients with EAC.

study and 3-year survival rate of followed patients was 80.0%. Kaplan-Meier survival analysis showed that the 3-year survival rate of the high MCM_2 expression group in was 67.9%, which was significantly lower than the rate of the low MCM_2 expression group (95.5%, $P < 0.05$), as shown in **Table 6** and **Figure 2**. There was no statistically significant difference in 3-year survival rates between the low Ki-67 expression group (90.5%) and the high expression group (72.4%) (**Table 6** and **Figure 3**). When univariate and multivariate Cox proportional hazard regression model were applied, only MCM_2 expression was an independent prognostic factor for OS in all patients with EAC (**Tables 7**, **8**).

Discussion

Plenty of studies have been demonstrated that upregulated expression of MCM_2 is closely related to abnormal cell proliferation and tumor invasion, progression and poor prognosis in a variety of tumors. Dudderidge et al showed that MCM_2 expression increases dramatically with increasing histopathologic grade in renal cell carcinoma tissues [16, 25]. Gonzalez et al showed that the labeling index (LI) of MCM_2 was associated with the size of tumor and mitotic index in 221 cases of invasive breast carcinoma, indicating MCM_2 expression can reflect the degree of tumor invasion [25, 26]. Yang et al found that combined expression of MCM2 and gelsolin indicated serious poor prognosis [11]. The prognostic value of MCM_2 was also reported in bladder cancer and oligodendroglioma [15, 27]. Although there are few studies about MCM_2 in gynecological tumor, it has been applied for the diagnosis for cervical cancer. Mukherjee et al applied immunohistochemical method detecting MCM_2 to inspection of cytological smears from cervical exfoliated cells and showed it had better sensitivity to rule out false negative [28]. In present study, we showed that, consistent with previous studies in other tumors, the increased expression of MCM_2 was relevant to elevated histological

Table 7. Univariate analysis of the prognostic factors in patients with EAC

Factors	OS		
	OR (95% CI)	P	β
Age	0.986 (0.928~1.048)	0.656	-0.014
Surgical pathology Level	0.862 (0.408~1.822)	0.697	-0.149
Histopathologic grade	0.882 (0.365~2.135)	0.781	-0.125
Degree of infiltration	3.589 (0.754~17.083)	0.108	1.278
Lymphatic metastasis	1.925 (0.553~6.697)	0.303	0.655
MCM_2 expression	9.629 (1.177~73.653)	.0034	2.231
Ki67 expression	3.341 (0.709~15.742)	0.127	1.206

Table 8. Cox proportional hazards model analysis of the prognostic factors in patients with EAC

Factors	OS		
	OR (95% CI)	P	β
Age	0.953 (0.886~1.026)	0.202	-0.048
Surgical pathology Level	0.633 (0.232~1.727)	0.372	-0.457
Histopathologic grade	0.505 (0.137~1.864)	0.305	-0.684
Degree of infiltration	1.836 (0.276~12.229)	0.530	0.608
Lymphatic metastasis	0.451 (.095~2.137)	0.316	-0.795
MCM_2 expression	429.629 (6.428~2871.233)	.005	6.063
Ki67 expression	0.037 (0.002~0.731)	.030	-3.304

grade in EAC tissues. Moreover, the positive expression of MCM_2 was remarkable increased in endometrial adenocarcinoma with deep myometrial invasion, which coincide with the study in bladder transitional cell carcinoma [29], suggesting that MCM_2 expression was involved with tumor invasion and development. Notably, our study did not show the significant difference between the groups with lymphatic metastasis and without lymphatic metastasis. This may be due to the insufficient case size.

Recently, as a novel cell proliferation antigen, Ki-67 has been widely used for investigating the biologic behavior of tumor and determining the dangers of tumor, although its detailed molecular mechanism in tumors is still unclear. Several studies have shown that elevated Ki-67 expression is associated with higher aggressiveness and invasiveness in several cancers [27, 30, 31]. The prognostic value of Ki-67 has been observed in cancers of breast, cervix, prostate and soft tissue [32]. A number of studies confirm that in endometrial adenocarcinoma, Ki-67 expression increased along with elevated surgical- pathologic stage and elevated histopathologic grade [33, 34]. Our study showed that aberrant expression of Ki-67 was associated with incidence of endometrial adenocarcinoma, which coincide with the reported in the literature. In addition, we showed that the group with lymphatic metastasis was significant higher than the group without lymphatic metastasis, which is in accordance with the study from Kimura et al. These results indicated that Ki-67 can be recognized as an important marker for reflecting the invasion ability and differentiation state of EAC.

MCM_2 and Ki67 are both belong to the antigens of cell proliferation. However, a few studies explored the co-expression of MCM_2 and Ki-67 in tumors and the conclusions are inconsistent [13, 14, 16, 35, 36]. The study from Jurić I et al showed that MCM_2 was a more optimal tumor biological marker than other two important cell proliferation markers, PACA and Ki-67 on account of its more sensitive ability to distinguish dysplasia atypical hyperplasia tissues and tumors tissues [35], Huang HY et al reported that MCM_2 could reflect the prognostic efficacy more accurately despite combined expression of MCM_2 and Ki-67 also can both indicate the dangers of tumor. This can be due to higher sensitivity of MCM_2 as an independent prognosticator. But Ki-67 was not expressed in early G_1 phase and was readily affected by metabolites [14]. Dudderidge et al found combined detection of expression of MCM_2 and Ki-67 can facilitate the clinical diagnosis in the kidney cancer. but the detailed mechanical association between MCM_2 and Ki-67 is still unclear [16]. However, Tokuyasu et al showed that high expression of MCM_2, but not Ki67, can be regarded as a good prognosticator in stage III gastric carcinoma [36].

In this study, we found that the expression of MCM_2 and Ki-67 both had positive correlations with EAC tissues ($r = 0.367$, $P = 0.012$). This was consistent with previous several studies to indicate that these both factors can be used for

diagnosing tumors. However, given our results of three-year survival rate, MCM_2 can be regarded as a better prognosticator in endometrial adenocarcinoma.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Clinic information of patients involved in this study

Case	MCM_2	Ki67	Survival Status	Survival Time (month)	Age	SP Level	His Grade	Infiltration	Metastasis
1	+	+	Death	26	48	Stage III	G_1	> 1/2	Yes
2	-	-	Survial	12	43	Stage I	G_1	≤ 1/2 or NO	No
3	+	+	Survial	18	39	Stage III	G_1	≤ 1/2 or NO	No
4	-	-	Survial	20	48	Stage I	G_1	≤ 1/2 or NO	No
5	-	-	Survial	24	47	Stage I	G_1	> 1/2	No
6	-	+	Survial	26	65	Stage I	G_3	≤ 1/2 or NO	No
7	+	-	Survial	30	58	Stage III	G_1	> 1/2	No
8	-	+++	Survial	36	75	Stage III	G_2	≤ 1/2 or NO	No
9	+	-	Survial	36	55	Stage I	G_3	≤ 1/2 or NO	No
10	+	-	Survial	36	52	Stage I	G_2	> 1/2	No
11	-	+	Survial	36	54	Stage I	G_1	≤ 1/2 or NO	No
12	+	-	Survial	36	67	Stage III	G_2	≤ 1/2 or NO	No
13	-	+	Survial	36	48	Stage II	G_2	≤ 1/2 or NO	No
14	+	-	Survial	36	67	Stage I	G_2	≤ 1/2 or NO	No
15	-	-	Survial	36	39	Stage II	G_1	≤ 1/2 or NO	No
16	-	-	Survial	37	55	Stage II	G_1	≤ 1/2 or NO	No
17	-	-	Survial	42	65	Stage I	G_2	≤ 1/2 or NO	Yes
18	+	+++	Survial	48	45	Stage I	G_2	> 1/2	No
19	-	-	Survial	48	63	Stage II	G_1	≤ 1/2 or NO	No
20	+	+	Survial	48	70	Stage I	G_2	> 1/2	No
21	-	+	Survial	60	72	Stage III	G_2	≤ 1/2 or NO	No
22	+	+	Survial	60	70	Stage II	G_2	> 1/2	No
23	4	+++	Death	11	48	Stage I	G_3	> 1/2	No
24	4	+++	Death	17	48	Stage II	G_2	> 1/2	No
25	4	4	Death	18	63	Stage II	G_2	≤ 1/2 or NO	No
26	-	+++	Death	19	66	Stage III	G_2	> 1/2	Yes
27	+++	+++	Death	25	70	Stage I	G_2	> 1/2	No
28	4	+++	Death	26	38	Stage II	G_2	> 1/2	Yes
29	+++	+++	Death	27	64	Stage II	G_2	> 1/2	No
30	4	4	Death	34	69	Stage II	G_1	≤ 1/2 or NO	Yes
31	+++	+++	Death	46	72	Stage I	G_2	> 1/2	Yes
32	4	4	Survial	7	78	Stage I	G_2	> 1/2	No
33	+++	+++	Survial	12	44	Stage III	G_2	> 1/2	No
34	+++	4	Survial	17	65	Stage I	G_3	≤ 1/2 or NO	No
35	+++	4	Survial	19	42	Stage II	G_3	> 1/2	Yes
36	+++	4	Survial	24	73	Stage II	G_2	> 1/2	No
37	4	4	Survial	24	68	Stage II	G_2	≤ 1/2 or NO	Yes
38	+++	+++	Survial	25	48	Stage I	G_2	> 1/2	No
39	4	4	Survial	26	52	Stage III	G_2	> 1/2	Yes
40	+++	4	Survial	27	58	Stage III	G_3	≤ 1/2 or NO	Yes
41	4	4	Survial	34	55	Stage I	G_1	> 1/2	Yes
42	+++	4	Survial	36	78	Stage I	G_1	≤ 1/2 or NO	No
43	4	+++	Survial	36	49	Stage II	G_2	≤ 1/2 or NO	Yes
44	4	+++	Survial	36	58	Stage III	G_2	> 1/2	No
45	4	4	Survial	36	49	Stage III	G_3	> 1/2	Yes
46	+++	4	Survial	36	57	Stage III	G_3	> 1/2	Yes
47	+++	+++	Survial	36	65	Stage III	G_1	≤ 1/2 or NO	Yes
48	+++	4	Survial	48	58	Stage II	G_3	> 1/2	No
49	4	4	Survial	48	63	Stage III	G_3	> 1/2	No
50	+++	4	Survial	60	73	Stage III	G_3	> 1/2	Yes