MEOX1 is a risk factor for unfavorable prognosis in human endometrioid endometrial adenocarcinoma

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Research

Keywords: Endometrioid endometrial adenocarcinoma, Immunohistochemistry, MEOX1, Prognosis

Posted Date: February 17th, 2020

DOI: <https://doi.org/10.21203/rs.2.23728/v1>

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Abstract

Background: Mesenchyme homeobox 1 (MEOX1) is a homeobox transcription factor. Although MEOX1 has an impact on cancer, no studies investigated its expression in human endometrioid endometrial adenocarcinoma (EEA). This study aims to check the expression level of MEOX1 and detect its role in unfavorable prognosis.

Methods: We used Western blot analysis by fresh frozen tissues and Immunohistochemical (IHC) method to detect the protein levels of MEOX1.

Results: MEOX1 expression at the protein levels in EEA was gradually higher than that in normal group and benign hyperplastic endometrium group (both $P < 0.001$). The high expression of MEOX1 was correlated with depth of myometrial invasion, lympho-vascular space involvement, FIGO stage, lymph node metastasis and histological grade by univariate survival analysis. MEOX1 high-expression status in EEA was an independent prognostic factor for recurrence-free survival (RFS) and overall survival (OS) by Multivariate survival analysis. Kaplan-Meier analysis demonstrated that MEOX1 expression was related to RFS and OS. Compared with the high expression MEOX1 group, the low expression MEOX1 group had longer RFS and OS (both $P < 0.001$).

Conclusion: MEOX1 may be a new potential biomarker for prognosis and recurrence in human EEA.

Background

Endometrial carcinoma is the most common cancer in female reproductive organs, and endometrioid endometrial adenocarcinoma (EEA) is the most common type [1]. Since endometrial carcinoma belongs to one of the gynecological cancers, more than 61 000 patients with endometrial cancers were diagnosed in 2017, while the estimated death number was 10920 [2]. Although EEA is common in developed countries and regions, the disease has a good prognosis [3]. EEA tumors present as low-stage, well-differentiated, that are confined to the uterus and are curable with surgery [4]. Approximately 20% of patients have a recurrence. Therefore, investigating therapeutic targets and reliable biomarker that can predict prognosis of EEA is needful.

MEOX1 is essential for the somite formation as a pivotal homeobox transcription factor [5]. It is located on human chromosome 17q21.31. According to some research, MEOX1 might promote the growth of carcinomas. Some research have demonstrated that in ovarian cancers MEOX1 mediates PBX1. MEOX1 is a cofactor of PBX1 and a pivotal target gene [6]. Besides, MEOX1 affected the progression of triple-negative breast cancer [7,8]. In addition, siRNA knockdown inhibits breast cancer cell frequency and the growth of tumor through down-regulation of MEOX1 [9]. However, the clinicopathological significance of MEOX1 in EEA remain unclear. Thus, in current paper, we aimed to explore MEOX1 protein over-expression in specimens of EEA. We also demonstrated that MEOX1 was associated with recurrence of EEA patients.

Materials And Methods

Patients and tissue specimens

We collected 201 cases of EEA patients, 50 cases of benign endometrial lesions (including atypical hyperplasia and complex hyperplasia) and 55 cases of normal endometrium from the Harbin Medical University Cancer Hospital between April 1, 2010 to June 30, 2012. Samples of EEA were obtained from patients undergoing hysterectomy, pelvic lymphadenectomy, para-aortic lymphadenectomy and bilateral salpingo-oophorectomy, while the benign and normal group were obtained from patients undergoing hysterectomy. All the patients had never been received radiation therapy or chemotherapy before surgery. 20 fresh EEA tissues, 20 normal endometrium specimens and 20 benign endometrial lesions were obtained for Western blot analysis. This study was approved by Harbin Medical University Medical Ethics Committee.

Patient Cohorts And Clinical Follow Up

Patients' mean age was 53.59 ± 9.61 years (26–79). The stages of tumor were assessed based on the International Federation of Gynecology and Obstetrics (FIGO) 2009 staging system. All EEA patients were followed regularly until study closing date (June 30, 2019) or until death. Median follow-up time was 65 (4 to 84) months.

Western Bolt Analysis

A total of 20 fresh EEA samples, 20 normal endometrium specimens and 20 benign endometrial lesions were homogenized in RIPA buffer. The lysate was centrifuged at 16,000 g at 4 °C for 20 minutes. The background-corrected absorbance (BCA) method (Beyotime, China) was used to estimate the concentration. Equal amounts of extracts were electrophoresed on a SDS-polyacrylamide gel and then transferred onto POLYVINYLIDENE DIFLUORIDE membranes (Millipore, BILLERICA, MA, USA). The filters were blocked with 5% dry milk one hour, incubated with MEOX1 rabbit polyclonal antibody (dilution 1:1000; HPA045214, Sigma-Aldrich, USA) at 4 °C for 24 hours. THE BLOTS WERE THEN INCUBATED WITH horseradish peroxidase secondary antibody at 37 °C for 60 minutes. Enhanced chemiluminescence substrate (Millipore, BILLERICA, MA, USA) was performed. β -actin (WL0001, Wanleibio, China) was used as an internal control.

Immunohistochemistry And Staining Evaluation

The formalin-fixed tissues were routinely embedded in paraffin, cut into 4 μ m thick sections that were stained with HE. After immersing in buffer, the slides were autoclaved at 121 °C for 15 minutes. The sections were incubated with a rabbit polyclonal antibody MEOX1 (HPA045214, Sigma-Aldrich; dilution 1:100) at 4 °C for 24 hours. The slides were immersed in 0.05% diaminobenzidine tetrahydrochloride (DAB).

The expression of MEOX1 was evaluated by semiquantitative method [8]. The number of positive cancer cell was scored between 0 to 3 (0, 0%; 1, $\leq 25\%$; 2, 26%-50%; 3, $\geq 50\%$). The staining intensity was scored between 0 to 3 (0 = no reaction;1 = weak;2 = moderate;3 = strong). The overall score = intensity score + positive percentage score. Scores of 4–6 were considered as high expression.

Statistical Analysis

The test data were statistically analysed by SPSS 19. Kaplan-Meier analysis was applied to draw the survival curves. The χ^2 test was performed to assess the relation between clinicopathological variables and MEOX1 high-expression. The log-rank test was applied to evaluated the univariate analysis. Multivariate and univariate logistic regression were applied to evaluate the effect of MEOX1 high expression on cancer recurrence. $P < 0.05$ was statistically significant .

Results

MEOX1 expression was elevated in EEA

Western blot was applied to detect the levels of MEOX1 expression. MEOX1 was IDENTIFIED as A BAND OF 31 KDA (Fig. 1). MEOX1 is overexpressed in EEA tissues, which WAS FURTHER CONFIRMED BY IHC. THE POSITIVE STAINING CELLS WERE MAINLY LOCALIZED within the nucleus (Fig. 2). MEOX1 high expression was positive in 54.7% (110/201) of EEA. The proportion of MEOX1 high expression was 36.0% (18/50) in benign endometrial lesion group, while 25.5% (14/55) in normal endometrium group. The rate of MEOX1 high-expression was higher in EEA group, compared with normal endometrium group ($P = 0.001$) and benign endometrial lesion gropu ($P = 0.018$). But there was no significant difference between benign endometrial lesion group and normal endometrium group ($P = 0.391$) (Table 1).

Table 1
Expression status of MEOX1 in different endometrial tissues

	No.	MEOX1 expression status		P
		High %		
Normal endometrium	55	14	25.5	0.001 ^a 0.391 ^b 0.018 ^c
Benign endometrial lesion	50	18	36	
EEA	201	110	54.7	
^a EEA versus normal endometrium				
^b benign endometrial lesion versus normal endometrium				
^c EEA versus benign endometrial lesion				

MEOX1 high-expression is associated with progression and poor survival in EEA

The relation between MEOX1 and clinicopathological parameters in the 201 cases of EEA samples were displayed in Table 2. MEOX1 high-expression was significantly correlated with recurrence ($P < 0.001$), lymph node metastasis ($P < 0.001$), FIGO stage ($P = 0.002$), a deep myometrial invasion ($P = 0.037$), vascular or lymphatic invasion ($P = 0.010$) and histological grade ($P = 0.004$).

Table 2. Association between MEOX1 elevated expression and clinicopathological factors.

Variables	No. of cases	MEOX1 expression status		P
		Low (%)	High (%)	
Age (years)				0.485
< 54	96	41 (42.7)	55 (57.3)	
≥ 54	105	50 (47.6)	55 (52.4)	
FIGO Stage				0.002
I	105	59 (56.2)	46 (43.8)	
II	53	21 (39.6)	32 (60.4)	
III-IV	43	11 (25.6)	32 (74.4)	
Histologic grade				0.004
G1	79	47 (59.5)	32 (40.5)	
G2	73	25 (34.2)	48 (65.8)	
G3	49	19 (38.8)	30 (61.2)	
Depth of MI				0.037
< 50%	103	54 (52.4)	49 (47.6)	
≥ 50%	98	37 (37.8)	61 (62.2)	
LVSI				0.010
No	143	73 (51.1)	70 (48.9)	
Yes	58	18 (31.0)	40 (69.0)	
Lymph node metastasis				<0.001
No	157	82 (52.2)	75 (47.8)	
Yes	44	9 (20.5)	35 (79.5)	
Recurrence				<0.001
No	152	81 (53.3)	71 (46.7)	
Yes	49	10 (20.4)	39 (79.6)	
BMI (kg/m ²)				0.495
< 25	114	54 (47.4)	60 (52.6)	
≥ 25	87	37 (42.5)	50 (57.5)	
ER				0.801
Negative	82	38 (46.3)	44 (53.7)	

Positive	119	53 (44.5)	66 (55.5)	0.912
PR				
Negative	87	39 (48.8)	48 (55.2)	
Positive	114	52 (45.6)	62 (54.4)	

FIGO: International Federation of Gynecology and Obstetrics; G1: Well; G2: Moderate; G3: Poor; EC: Endometrioid cancer; MI: Myometrial invasion; LVSI: Lympho-vascular space involvement; BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor.

Log-rank test and Kaplan–Meier analysis were applied to evaluate the survival time. Patients with MEOX1 high-expression were likely to have shorter RFS ($P < 0.001$, Fig. 3B) and shorter OS ($P < 0.001$, Fig. 3A). Univariate survival analysis showed that MEOX1 high-expression predicted an unfavorable RFS and OS ($P < 0.001$; Table 3). Lymph node metastasis ($P < 0.001$), vascular or lymphatic invasion ($P < 0.001$), deep myometrial invasion ($P = 0.005$), FIGO stage ($P < 0.001$) and histological grade ($P = 0.001$) were correlated with unfavorable clinical outcomes (Table 3).

Table 3. Univariate survival analysis of 201 patients with EEA

Variables	OS			RFS		
	Median	5-year (%)	P	Median	5-year (%)	P
Age (years)			0.279			0.206
< 54	65.0	84.5		63.0	79.9	
≥ 54	67.0	83.9		63.0	74.5	
FIGO Stage			<0.001			<0.001
I	70.0	92.7		68.0	90.0	
II	63.0	86.3		61.0	84.1	
III-IV	60.0	61.8		26.0	29.9	
Histologic grade			0.001			0.001
G1	68.0	97.4		67.0	92.3	
G2	63.0	78.4		61.0	73.9	
G3	63.0	70.2		35.0	54.1	
Depth of MI			0.005			0.004
< 50%	67.0	87.3		65.0	83.8	
≥ 50%	65.0	77.6		62.0	70.3	
LVSI			<0.001			<0.001
No	67.0	86.7		65.0	82.6	
Yes	63.0	71.6		43.5	55.8	
Lymph node metastasis			<0.001			<0.001
No	67.0	88.0		67.0	85.9	
Yes	56.0	69.4		34.0	40.2	
BMI (kg/m ²)			0.143			0.188
<25	67.0	83.9		65.0	80.3	
≥ 25	65.0	80.7		62.0	72.8	
ER			0.392			0.252
Negative	64.0	85.1		63.0	80.2	
Positive	67.0	80.7		65.0	75.0	
PR			0.295			0.468
Negative	65.0	78.3		63.0	74.6	

Positive	66.0	85.8	63.5	75.8
MEOX1 expression status			<0.001	<0.001
Low	67.0	94.4	67.0	93.2
High	62.5	71.8	40.0	58.5

FIGO: International Federation of Gynecology and Obstetrics; G1: Well; G2: Moderate; G3: Poor; EC: Endometrioid cancer; MI: Myometrial invasion; LVSI: Lympho-vascular space involvement; BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor; OS: Overall survival; RFS: Recurrence-free survival.

Multivariate survival analysis demonstrated that MEOX1 high-expression together with vascular or lymphatic invasion ($P = 0.001$), FIGO stage ($P < 0.001$) and deep myometrial invasion ($P = 0.039$) were independently factors for RFS (Table 4). Multivariate survival analysis also suggested that MEOX1 high-expression together with FIGO stage ($P < 0.001$) and vascular or lymphatic invasion ($P = 0.013$) were independently factors for OS (Table 4).

Table 4. Multivariate survival analysis of 201 patients with EEA

Variables	OS			RFS		
	HR	95%CI	P	HR	95%CI	P
FIGO Stage						
I	1.000 (reference)			1.000 (reference)		
II	3.501	1.610-7.613	0.002	2.841	1.320-6.116	0.008
III-IV	6.452	3.244-12.835	<0.001	11.586	5.569-24.103	<0.001
Depth of MI						
< 50%	1.000 (reference)			1.000 (reference)		
≥ 50%			0.093			0.039
LVSI	1.633	0.921-2.895		1.808	1.030-3.172	
No						
Yes	1.000 (reference)		0.013	1.000 (reference)		0.011
MEOX1 expression status	2.022	1.161-3.521		2.057	1.184-3.576	
Low						
High	1.000 (reference)		0.007	1.000 (reference)		0.001
	2.613	1.297-5.261		3.377	1.669-6.831	

FIGO: International Federation of Gynecology and Obstetrics; MI: Myometrial invasion; LVSI: Lympho-vascular space involvement; OS: Overall survival; RFS: Recurrence-free survival; HR: Hazard ratio; CI: Confidence interval.

MEOX1 High-expression Independently Predicts Recurrence In EEA

The recurrence in EEA was related to FIGO stage ($P < 0.001$) and positive lymph node metastasis ($P = 0.014$) (Table 5). Multivariate logistic regression analysis showed that lymph node metastasis, FIGO stage and MEOX1 high-expression were independently related to EEA recurrence (Table 5).

Table 5. Multivariate regression analysis of the association between recurrence and MEOX1 elevated expression in EEA

Variables	B	S.E.	P	OR	95%CI
FIGO Stage					
I					
II	0.595	0.504	0.238	1.813	0.675-4.864
III-IV	2.369	0.505	< 0.001	10.683	3.971-28.737
Lymph node metastasis					
No					
Yes	1.105	0.451	0.014	3.020	1.248-7.312
MEOX1 expression status					
Low					
High	1.089	0.455	0.017	2.972	1.219-7.244

FIGO: International Federation of Gynecology and Obstetrics; B and S.E.: the parameter estimator of association coefficient and its standard error; OR: odds ratio; CI: confidence interval.

Discussion

Although the prognosis remains good for patients with who are diagnosed with early stage EEA, some patients still die of tumor recurrence [10 – 16]. It is necessary to find other effective MOLECULAR targets for prognosis analysis.

MEOX1 is radical for somite formation. As all know,mutations of MEOX1 may cause Klippel-Feil Syndrome [17]. Previous studies showed that MEOX1 may be involved in the development of some carcinoma. MEOX1 was over expressed in several cancers than normal tissues, such as prostate cancer, ovarian cancer, non-small-cell lung cancer and breast cancer [7,8,18,19]. Also, MEOX1 is related to signaling pathway. One study showed that HER2 signaling regulated MEOX1 in HER2 + breast cancer [9].Another report demonstrated that MEOX may control Bapx1 expression [20].

We firstly evaluated MEOX1 expression levels in EEA. In our study we detected the status of MEOX1. Our study demonstrated that MEOX1 was significantly elevated in cancer group at protein levels by western blot analysis and IHC staining. MEOX1 high expression was positive in 54.7% of EEA. The proportion of MEOX1 high expression was 36.0% in benign endometrial lesion group, while 25.5% in normal endometrium group. THE HIGH EXPRESSION RATE OF MEOX1 WAS SIGNIFICANTLY HIGHER IN EEA group, compared with normal endometrium group and benign endometrial lesion group.MEOX1 may play an oncogenic role in EEA .

Our study use clinical EEA tissues to demonstrate the association between the expression of MEOX1 and clinicopathological factors. MEOX1 high-expression was significantly related to recurrence,positive lymph

node metastasis, high histological grade, FIGO stage, a deep myometrial invasion and positive vascular or lymphatic invasion. This conclusion is consistent with some other studies.

MEOX1 may reflect the malignancy of the cancer. We used Kaplan–Meier analysis to draw the survival time. Patients with high-expression were likely to be with shorter OS and RFS. Univariate survival analysis demonstrated that MEOX1 high-expression was related with an unfavorable RFS and OS. Multivariate survival analysis showed that MEOX1 high-expression together with FIGO stage and positive vascular/lymphatic invasion for both OS and RFS.

As is known to all, some poorly differentiated EEA patients may develop pelvic lymph node metastasis [21]. Our results showed that MEOX1 high-expression was related to positive lymph node metastasis. FIGO stage, MEOX1 high-expression and lymph node metastasis independently correlated with recurrence. Unfortunately, there was no sensitive marker that could predict lymph node metastasis. We will look for more other factors that could predict lymph node metastasis in the future.

In conclusion, MEOX1 is overexpressed in EEA patients. MEOX1 high-expression is closely related to unfavorable prognosis and recurrence. MEOX1 may be a new potential biomarker for prognosis and recurrence in human EEA.

Abbreviations

MEOX1: Mesenchyme homeobox 1; EEA: endometrioid endometrial adenocarcinoma; IHC: Immunohistochemical; RFS: recurrence-free survival; OS: overall survival; FIGO: International Federation of Gynecology and Obstetrics.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of the Harbin Medical University Cancer Hospital.

Consent for publication

Consent was obtained to publish the paper.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by grants the National Natural *Science Foundation* of China (No.81772274), the Key Projects of Haiyan Foundation of Harbin Medical University Cancer Hospital (*JJZD2017-04*), Nn10 program of Harbin Medical University Cancer Hospital, the Youth Elite Training Foundation of the Affiliated Tumor Hospital of Harbin Medical University (JY2016-03), the Key Projects of Haiyan Foundation of Harbin Medical University Cancer Hospital (JJZD2019-02) and Outstanding Youth Programme of Harbin Medical University Cancer Hospital (JCQN2019-06).

Authors' contributions

XWC conceived and designed the study. YDL and DK collected samples and processed data. TBL provided technical support. TBL analyzed data. YDL drafted the manuscript. XWC revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

Figure 1

Western blot analysis of MEOX1 in 20 normal endometrium specimens, 20 fresh EEA tissues and 20 benign endometrial lesions. The expression of MEOX1 protein was significantly increased in EEA. The data are presented as SD, * $P < 0.01$

Figure 2

Representative examples of MEOX1 expression in endometrial tissue by immunohistochemistry. A and B: low expression of MEOX1 in normal endometrium .C and D : low expression of MEOX1 in benign endometrial lesion.E and F:high expression of MEOX1 in EEA.

Figure 3

Kaplan-Meier curves for survival of prognosis in 201 patients with EEA according to the categories of low and high expression of MEOX1 (analyzed with log-rank test). A, Overall survival. B, Recurrence-free

survival.