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Analysis of diagnostic and prognostic value of lncRNA MEG3 in cervical cancer

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Abstract. The present study aimed to explore the diagnostic and prognostic value of lncRNA maternally expressed 3 (MEG3) in cervical cancer. Eighty-four patients with cervical cancer from February 2013 to March 2014 were enrolled in the observation group (OG), and another 58 female subjects who underwent physical examination at Huangshi Central Hospital were enrolled as the control group (CG). The serum MEG3 expression of patients in the two groups was detected by RT-qPCR, and the ability of MEG3 to aid in the diagnosis of cervical cancer, lymph node metastasis and FIGO staging, as well as to predict mortality was evaluated by ROC curve. In addition, the patients in the OG were divided into high- and low-expression groups according to the median value of MEG3. Kaplan Meier was employed to analyze the survival status, and Cox regression to analyze the independent prognostic factors of cervical cancer patients. The results of the present study revealed that the serum MEG3 expression in the OG was significantly lower than that of the CG ($P<0.05$). The area under the curve (AUC) of MEG3 in diagnosing cervical cancer was 0.844, the AUC in predicting mortality was 0.858, while that in diagnosing lymph node transfer was 0.707, and that in diagnosing FIGO staging was 0.791. The 5-year survival rate of the high-expression group was higher than that of the low-expression group ($P=0.020$). Multivariate analysis indicated that MEG3 (HR, 0.173; 95 CI%, 0.028-0.919), lymph node metastasis (HR, 2.259; 95 CI%, 1.004-5.025) and FIGO staging (HR, 0.008; 95 CI%, 1.453-6.248) were independent prognostic factors for cervical cancer patients. Collectively, lncRNA MEG3 may be a diagnostic marker and prognostic indicator for cervical cancer, and has a certain diagnostic value for lymph node metastasis and FIGO staging. Lymph node metastasis, FIGO stage III and IV, and low MEG3 levels were revealed to be independent prognostic factors for cervical cancer patients.

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Introduction

Cervical cancer is the second most common female cancer in the world and the fourth most common cause of cancer-related deaths (1). Infection with human papillomavirus (HPV) is a prerequisite for the disease, and approximately 80% of women will be infected with cervical HPV by the age of 50, but not all infected individuals will develop the disease (2,3). Among them, 13% of cervical cancer patients are only diagnosed at an advanced stage. When cervical cancer progresses to metastatic cervical cancer, the 5-year survival rate is only 16.5%, with local cervical cancer accounting for 91.5% of the total cases, and the 5-year survival rate of patients past stage III is <40% (4). Therefore, early diagnosis and effective prognosis prediction of cervical cancer patients will markedly enhance the survival treatment of patients. However, to date, cervical cancer does not have an effective biomarker for diagnosis and prognosis, thus it is particularly important to identify a key non-invasive molecular marker.

Long non-coding RNAs (lncRNAs) are a class of RNA molecules with a transcription length >200 nt. Although they lack protein-coding features, they are able to widely regulate the expression of some genes and participate in cell proliferation, differentiation, apoptosis as well as other processes, thereby attracting the attention of several scientists in recent years (5,6). With the progress in lncRNA research, it has been revealed that lncRNAs are closely related to numerous cancer diseases (7,8). For example, Misawa *et al* (9) revealed that lncRNASOCS2-AS1 could inhibit apoptosis of prostate cancer cells through the androgen receptor target gene. In addition, Chen *et al* (10) demonstrated that the expression of lncRNA n336928 in bladder cancer tissues was significantly higher than that in adjacent tissues, and the total survival time of bladder cancer patients with low expression of lncRNA n336928 was also lower than that of patients with high expression. lncRNA maternally expressed 3 (MEG3), a member of the lncRNA family, is located in the imprinted region of DLK1-MEG3 on chromosome 14, which contains multiple imprinted genes that exerts inhibition in most cancers (11,12). Studies have revealed that MEG3 expression in endometrial cancer tissue is significantly lower than that in normal endometrial tissue, and

that overexpressed MEG3 inhibits proliferation, invasion and metastasis of endometrial cancer cells, promotes apoptosis. Moreover, the expression of MEG3 in ovarian cancer cells has also been revealed to be significantly lower than normal ovarian cells (13-15). However, the diagnostic value of lncRNA MEG3 in cervical cancer and its impact on prognosis remain poorly understood.

Therefore, by observing the serum lncRNA MEG3 expression of cancer patients, the present study aimed to elucidate its diagnostic and prognostic value in cervical cancer, thus providing direction and basis for clinical application.

Materials and methods

Clinical data. Eighty-four patients with cervical cancer, with an average age of 51.0±6.3 years, were enrolled as the observation group (OG) from February 2013 to March 2014 at Huangshi Central Hospital. In addition, 58 female subjects with an average age of 50.7±5.8 years, who underwent physical examination at Huangshi Central Hospital, concurrently, were assigned into the control group (CG). The present study was approved by the Medical Ethics Committee of Huangshi Central Hospital, and all patients provided signed informed consent.

Inclusion and exclusion criteria. Patients pathologically diagnosed with cervical cancer that could be staged according to the International Federation of Gynecology and Obstetrics (FIGO) pathological staging (16), who were informed of the purpose of this study and signed the informed consent, and those willing to cooperate with the follow-up, with complete clinical data were included.

Patients complicated with other tumors or gynecological diseases, who had received chemoradiotherapy before this study or presented with congenital liver, kidney and heart function defects, or those with a life expectancy of <3 months were excluded.

Patient follow-up. The patients were followed-up for 5 years, with the diagnosis time as the starting point, and the death of a patient (due to cervical cancer and complications), loss of follow-up or the end of the follow-up time as the end point. A reexamination was carried out every 6 months. The follow-up was conducted by telephone, door-to-door and outpatient reexamination. In the first year of follow-up, the patients were followed-up every 3 months, and every 6 months for the following 4 years.

Quantitative real-time polymerase chain reaction (qPCR) detection. Fasting venous blood (5 ml) was collected from all the research participants, and the serum was collected by centrifugation (3,000 x g at 4°C for 10 min) in a pro-coagulation tube. Then the total RNA in serum was extracted with a TRIzol kit (Invitrogen; Thermo Fisher Scientific, Inc. cat. no. 15596018), whose purity, concentration and integrity was then detected by UV spectrophotometer and agarose gel electrophoresis. Subsequently, reverse transcription was conducted using TransScript® II Green Two-Step qRT-PCR SuperMix kit (cat. no. AQ301-01; Beijing TransGen Biotech Co., Ltd.) in strict accordance with the manufacturer's instructions. PCR amplification was then performed, and the PCR reaction system was as follows: cDNA, 1 μl; upstream and downstream primers, each 0.4 μl; 2X TransScript® Tip Green qPCR, SuperMix 10 μl; Passive Reference Dye (50X), 0.4 μl, and finally nuclease-free water was added for a total reaction volume of 20 μl. The PCR reaction conditions were as follows: Pre-denaturation at 94°C for 30 sec, denaturation at 94°C for 5 sec, annealing at 60°C for 30 sec, totaling 40 cycles. The primer sequences for MEG3 and GAPDH are presented in Table I. Three replicate wells were set for each sample and the experiment was performed in triplicate. GAPDH was used as the internal reference, and the data was analyzed by $2^{-\Delta\Delta Cq}$ in this experiment (17).

Observation indicators

Primary endpoints. MEG3 expression was compared between the OG and the CG. The 5-year survival of the patients was recorded, and multivariate Cox regression was used to analyze the risk factors of mortality according to the 5-year survival of the patients. Receiver operating characteristic (ROC) curve was applied to analyze the diagnostic and death-predicting value of MEG3 in cervical cancer.

Secondary endpoints. The MEG3 expression between patients who survived and those who succumbed to the disease, lymph node metastasis and non-metastasis, FIGO stage III and IV and I and II were compared. ROC was used to analyze the diagnostic value of MEG3 in lymph node metastasis and FIGO staging.

Statistical analysis. The collected data were statistically analyzed by SPSS 20.0 (Shanghai Cabit Information Technology Co., Ltd.), and the acquired images were plotted using GraphPad Prism 7 (Shenzhen Softhead Technology Co., Ltd., China). The counting data represented by percentage (%) were compared using Chi-square and expressed as χ^2. While the measurement data were expressed as the mean ± standard deviation (SD). All measurement data were in line with normal distribution, and the comparison between the two groups was compared by an independent sample t-test, represented by t. The ability of MEG3 in diagnosing cervical cancer, lymph node metastasis and FIGO staging, as well as in predicting mortality was assessed by ROC. The 5-year survival of patients was analyzed by Kaplan-Meier (K-M) survival analysis using log-rank test. Cox regression was employed to analyze the factors affecting the prognosis of patients. P<0.05 indicated a statistically significant difference between the two groups.

Results

Clinical data. No significant difference was observed in the clinical data of patients in the two groups in terms of age, BMI, smoking history, alcohol history, place of residence, and reproductive history (P>0.05; Table II).

Comparison of MEG3 expression in the two groups and its diagnostic value in cervical cancer. By observing the expression of MEG3, it was revealed that the expression of MEG3 in the OG (0.74±0.20) was significantly lower than that in the CG

Table I. Primer sequences.

Gene	Upstream primer	Downstream primer
MEG3	5'-TCGCTCTTCTCCATCGAACCG-3'	5'-GTAGGGCGACGACTTTGAGT-3'
GAPDH	5'-ATGGTGAAGGTCGGT-GTGA-3'	5'-CCATGTAGTTGAG-GTCAATGAG-3'

MEG3, maternally expressed 3.

Table II. Clinical data of patients.

Factors	OG (n=84)	CG (n=58)	t/χ^2-value	P-value
Age (years)	51.0±6.3	50.7±5.8	0.288	0.774
BMI (kg/m^2)	21.36±1.53	21.54±1.42	0.709	0.479
Smoking history				
Yes	15 (17.86)	11 (18.97)	0.028	0.867
No	69 (82.14)	47 (81.03)		
Drinking history				
Yes	13 (15.48)	7 (12.07)	0.329	0.566
No	71 (84.52)	51 (87.93)		
Residence				
Urban	67 (79.76)	47 (81.03)	0.035	0.851
Rural	17 (20.24)	11 (18.97)		
Reproductive history				
Yes	62 (73.81)	48 (82.76)	1.574	0.210
No	22 (26.19)	10 (17.24)		
Menopause				
Yes	48 (57.14)	35 (60.34)	0.145	0.704
No	36 (42.86)	23 (39.66)		
Tumor size				
≤2 cm	45 (53.57)			
>2 cm	39 (46.43)			
FIGO staging				
I+II	51 (60.71)			
III+IV	33 (39.29)			
Histological type				
Squamous cell carcinoma	57 (67.86)			
Adenocarcinoma	27 (32.14)			
Vaginal infiltration	28 (33.33)			
Parametrial involvement	24 (28.57)			
Lymph node metastasis	31 (36.90)			

OG, observation group; CG, control group.

(1.02±0.22). The AUC of MEG3 in the diagnosis of cervical cancer was 0.844, 95 CI%, 0.778-0.911. When the cut-off point was 0.850, the specificity was 75.00%, the sensitivity was 82.76%, and the Youden index was 57.76% (Fig. 1).

Survival and mortality prediction value of patients with high and low expression of MEG3. Over the 5-year follow-up, 31 patients did not survive while 53 survived in the OG, with a survival rate of 63.10%. All patients in the OG were divided into a high-expression group and a low-expression group according to the median value of MEG3 expression. It was determined that the survival of the high-expression group was significantly increased compared to the low-expression group, and MEG3 expression of the non-surviving patients was significantly lower than that of the surviving patients. The area under the ROC curve of MEG3 in predicting

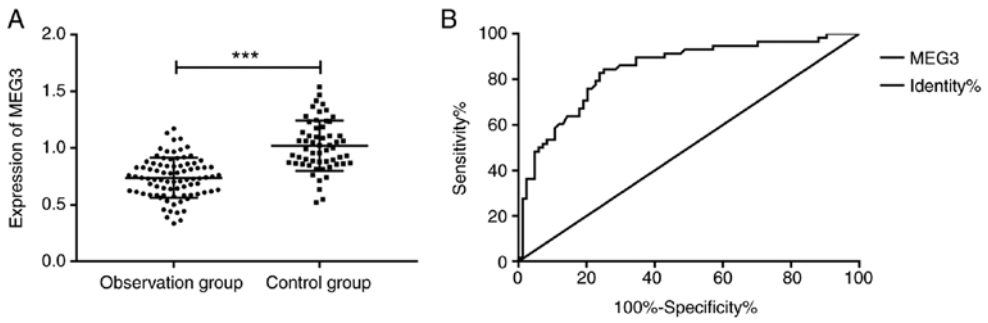

Figure 1. MEG3 is of diagnostic value in cervical cancer. (A) The MEG3 level of the OG was significantly lower than that of the CG. (B) ROC curve of MEG3 in the diagnosis of cervical cancer. ***P<0.001. MEG3, maternally expressed 3; OG, observation group; CG, control group.

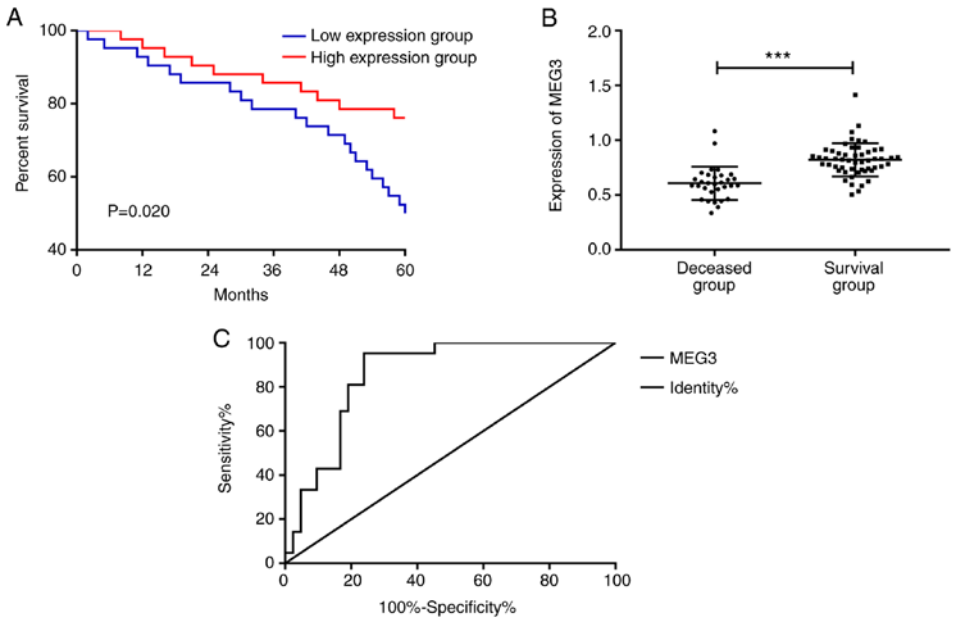

Figure 2. Low MEG3 expression has decreased survival and predictive value of mortality. (A) The survival time of the MEG3-low expression group was significantly decreased compared with the MEG3-high expression group. (B) MEG3 expression in the non-survival group was significantly lower than that in the survival group. (C) ROC curve of MEG3 for the prediction of cervical cancer mortality. ***P<0.001. MEG3, maternally expressed 3; ROC, receiver operating characteristic.

cervical cancer death was 0.858, 95 CI%, 0.773-0.943. When the cutoff point was 0.706, the specificity was 76.19%, the sensitivity was 92.86%, and the Youden index was 69.05% (Fig. 2).

Cox analysis. The clinical data of patients were collected and assigned, and then Cox regression and the Enter Regression method were used for analysis. It was determined that FIGO staging, vaginal infiltration, lymph node metastasis and MEG3 were prognostic factors for cervical cancer patients. Then, backward logistic regression (LR) was further selected for the multivariate analysis of the factors with differences, which revealed that MEG3 (HR, 0.173; 95 CI%, 0.028-0.919), lymph node metastasis (HR, 2.259; 95 CI%, 1.004-5.025), and FIGO staging (HR, 0.008; 95 CI%, 1.453-6.248) were independent prognostic factors for cervical cancer patients (Tables III and IV).

Diagnostic value of MEG3 for risk factors. By comparing the expression of MEG3 in patients with lymph node metastasis and non-metastasis and those with stage I and II or III and IV,

Table III. Factors and assignments of cervical cancer.

Factors	Assignments
Age	≥50 years old=1; <50 years old=0
BMI	≥21 kg/m^3=1; <21 kg/m^3=0
Reproductive history	Yes=1; no=0
Menopause	Yes=1; no=0
Tumor size	>2 cm=1; ≤2 cm=0
FIGO staging	III+IV=1; I+II=0
Histological type	Squamous cell carcinoma=1; adenocarcinoma=0
Vaginal infiltration	Yes=1; no=0
Parametrial involvement	Yes=1; no=0
Lymph node metastasis	With metastasis=1; without metastasis=0
MEG3	Raw data analysis for continuous variables.

MEG3, maternally expressed 3.

Table IV. Cox regression.

	Univariate cox			Multivariate cox		
Factors	Exp(B)	95 CI%	Sig.	Exp(B)	95 CI%	Sig.
Age	1.072	0.896-1.226	0.473			
BMI	1.672	0.632-3.066	0.336			
Reproductive history	0.836	0.445-1.783	0.720			
Menopause	1.053	0.572-1.94	0.868			
Tumor size	1.274	0.937-1.725	0.173			
FIGO staging	3.739	1.780-7.458	<0.001	0.008	1.453-6.248	0.006
Histological type	0.937	2.745-10.247	0.963			
Vaginal infiltration	2.136	1.037-4.227	0.036	0.583	0.185-1.649	0.365
Parametrial involvement	0.146	0.061-0.351	0.400			
Lymph node metastasis	2.057	1.021-4.243	0.044	2.259	1.004-5.025	0.047
MEG3	0.137	0.038-0.573	0.003	0.173	0.028-0.919	0.042

MEG3, maternally expressed 3.

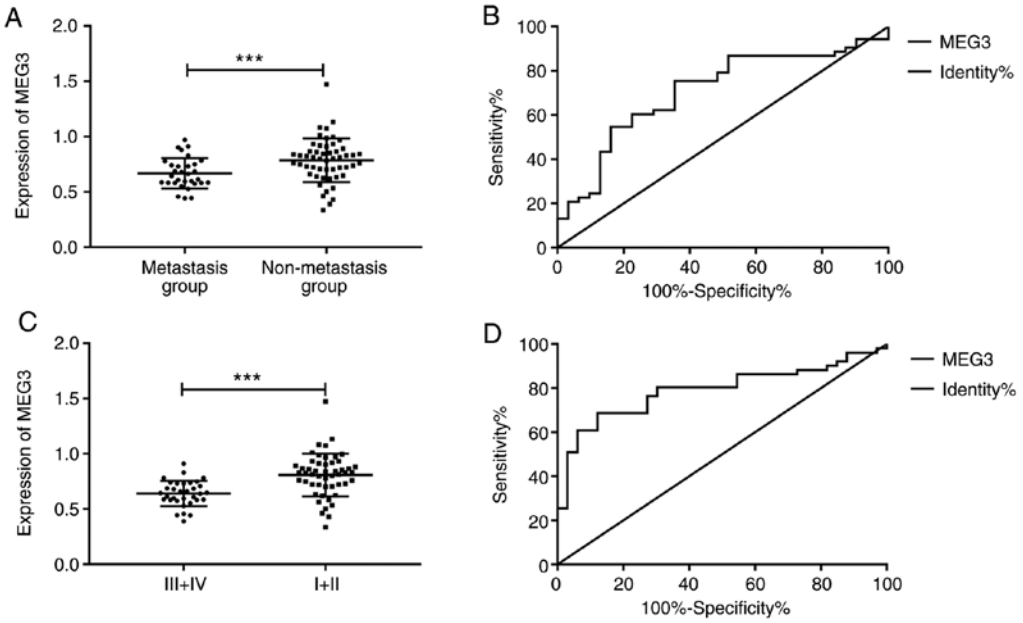

Figure 3. MEG3 is of diagnostic value for risk factors. (A) MEG3 expression in patients with lymph node metastasis was significantly lower than that in patients without metastasis. (B) ROC curve of MEG3 in diagnosing lymph node metastasis of cervical cancer patients. (C) Expression of MEG3 in patients with stage III and IV was significantly lower than that of patients in stage I and II. (D) ROC curve of MEG3 in staging of cervical cancer patients. ***P<0.001. MEG3, maternally expressed 3; ROC, receiver operating characteristic.

it was revealed that the expression of metastatic patients was significantly lower than that of non-metastatic patients. The area under the ROC curve for diagnosis of lymph node metastasis was 0.707, 95 CI%, 0.592-0.821. When the cut-off point was 0.750, the specificity was 77.42%, the sensitivity was 60.38%, and the Youden index was 37.80%. In addition, the expression of patients with stage III and IV was lower than that of patients with stage I and II. The area under the ROC curve for the diagnosis of FIGO staging was 0.791, 95 CI%, 0.694-0.889. When the cut-off point was 0.755, the specificity was 87.88%, the sensitivity was 66.67%, and the Youden index was 54.55%. (Fig. 3).

Discussion

The onset of cervical cancer is the contribution of different complex factors, resulting in the formation of invasive, chemo-resistant and radiation-tolerant tumor masses in HPV-infected cells, which are often caused by unstable mutations and disorders of the genome (18). At present, several lncRNAs have been demonstrated to play a relevant role in the development and progression of cervical cancer, and are expected to be new targets for the diagnosis, prognosis and targeted therapy of the disease (19). Although there are different treatment methods available customized to the different conditions of

cervical cancer patients, such as surgery, chemotherapy and radiotherapy (20), if the actual condition of patients can be accurately understood, more appropriate individualized treatment programs can be applied for treatment, thus improving the survival of patients (21,22).

In the present study, the expression of MEG3 was first compared between two groups (OG and CG). It was revealed that the OG presented markedly lower MEG3 expression than the CG, which was in line with a previous study (23). Moreover, in a study by Chen and Qu (24), MEG3 inhibited the survival and migration of cervical cancer cells mainly by inhibiting Rac1. Therefore, we hypothesized that MEG3 may be a diagnostic indicator for cervical cancer patients. With the advantage of a ROC curve, it was observed that the AUC of MEG3 in diagnosing cervical cancer was 0.844, and the corresponding specificity and sensitivity were 75.00 and 82.76% when the cut-off point was set as 0.850, which also indicated that MEG3 may be a diagnostic marker for cervical cancer. Then, the patients were followed-up for 5 years and it was revealed that the survival rate was 63.10%. The survival curve demonstrated that the survival rate of patients with low MEG3 expression was significantly lower than that of patients with high MEG3 expression. In addition, by comparing the expression of MEG3 between the surviving and non-surviving patients, it was revealed that the expression of MEG3 in the non-surviving patients was significantly lower than that in surviving patients. Furthermore, ROC curve revealed that the AUC of MEG3 in predicting cervical cancer death was 0.858, and when the cut-off point was 0.706, the specificity was 76.19% and the sensitivity was 92.86%, which further indicated that MEG3 may be used as a predictor of mortality of cervical cancer patients. Xiu *et al* (25) determined that the inhibition mechanism of MEG3 in ovarian cancer was realized by regulating ATG3 activity and inducing autophagy, and they also reported that the AUC was 0.763 by ROC curve detection, which revealed that MEG3 not only had diagnostic value in ovarian cancer, but also had diagnostic value in cervical cancer, while MEG3 may be more suitable for the diagnosis of the latter.

Furthermore, by exploring the independent factors affecting the prognosis of cervical cancer patients, we collected the clinical data of the patients for multivariate Cox regression analysis, and it was determined that lymph node metastasis, FIGO stage III and IV, and a low MEG3 level were independent prognostic factors for cervical cancer patients. Numerous studies have reported that patients with high FIGO staging and lymph node metastasis present poor prognosis (26,27). In addition, since lymph node metastasis and high FIGO staging are characteristics of high-risk patients, early diagnosis of lymph node metastasis and high FIGO stage will also be helpful for the treatment. Therefore, we compared the MEG3 level of these patients and utilized ROC curve to assess the diagnostic value of MEG3 in cervical cancer patients with lymph node metastasis and FIGO stage III+IV. It revealed that the MEG3 level of patients with metastasis was significantly lower than that of patients without metastasis. The area under the ROC curve was 0.707, with a specificity of 77.42% and a sensitivity of 60.38% when the cut-off point was 0.750. Moreover, the MEG3 level of patients with FIGO stage III and IV was significantly lower than that of patients with stage I and II. Therefore, the detection of MEG3 levels may be conducive to identify high-risk patients before treatment, in order to conduct more effective treatment. However, there are some limitations in the present study. First, the treatment methods were not restricted, which may irrevocably lead to inconsistencies in the treatment of patients, and the impact of these treatments is still unclear. Secondly, we have not conducted any cell-based experiments, and the specific regulatory mechanism of MEG3 as a tumor suppressor gene in cervical cancer remains a subject of investigation. Zhang *et al* (28) revealed that MEG3 inhibited the growth of tumors in cervical cancer by regulating miR-21-5p. Hence, our aim is to conduct corresponding basic experiments on MEG3 in the future to study corresponding target genes and affected signaling pathways, to verify whether it can be used as a target therapy. Finally, an in-depth investigation has not yet been performed on patients with recurrence, however, this will be carried out with the relevant research in a follow-up study.

In summary, lncRNA MEG3, which possesses diagnostic value for lymph node metastasis and FIGO staging, may serve as a diagnostic marker and prognostic indicator for cervical cancer. Furthermore, lymph node metastasis, FIGO stage III and IV, and a low MEG3 level were revealed to be independent prognostic factors for cervical cancer patients.

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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

SW wrote the manuscript, and analyzed and interpreted the general data of patients. HZ performed PCR and was responsible for observation indicator analysis. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Huangshi Central Hospital. Patients who participated in this research, provided signed informed consent and had complete clinical data. Signed written informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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