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Research Article

Expression of SOX4 in Endometrial Cancer and its Relationship with Clinical-pathological Characteristics in Endometrial Cancer Patients

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Abstract: ***Objective:*** To investigate the expression of SOX4 in endometrial carcinoma tissues and its relationship with clinicopathological features. ***Methods:*** The clinical and pathological data of 51 patients with pathologically diagnosed endometrial carcinoma who underwent panhysterectomy in the Wenzhou Central Hospital from March 2017 to March 2019 were retrospectively analyzed. There were 22 cases of type I endometrial carcinoma and 29cases of type Ⅱ endometrial carcinoma. The immunohistochemical expression of SOX4 was detected, and its relationship with clinicopathological parameters was analyzed. ***Results:*** Compared with the control group, SOX4 in endometrial cancer group increased significantly (P<0.05); High SOX4 expression were closely related to differentiation, clinical stage and lymph node metastasis of endometrial cancer (P<0.05). ***Conclusion:*** SOX4 is related to the occurrence and development of EC. SOX4 may be a clinical evaluation index of EC and a reference index for clinical pathological diagnosis, it is helpful for clinicians to better analyze the high risk factors of EC patients and provide new ideas for early diagnosis, prognosis evaluation and targeted treatment of EC.

Keywords: Endometrial cancer; SOX4; Immunohistochemistry

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Endometrial carcinoma (EC) is one of the three major malignant tumors occurring in the female reproductive system, accounting for 4%-7% of all female malignancies. In recent years, its incidence has significantly increased, with the age at onset becoming increasingly young[1]. The SOX (Sex-determining region Y-related high mobility group box) family is a group of genes related to the sex-determining region Y (SRY) gene. All members of this family have HMG (high mobility group) structural domain that can bind to DNA. Based on the HMG domain and other functional regions, the SOX family is further divided into 10 subgroups A–J, and SOX_4 is a transcription factor belonging to the C subgroup of the SOX family. The SOX_4 gene is located on the human chromosome 6p23 and can encode a 47 kD protein with 474 amino acids. The HMG domain at the N-terminus and the TAD domain at the C-terminus are the major functional regions of SOX_4[2]. The abnormal changes in the SOX gene are closely related to the occurrence of various tumors and other malignant biological behaviors. Previous studies have shown that SOX_4 genes are overexpressed in a variety of solid tumors and are related to the poor prognosis of patients[3-7]. In recent years, the role of SOX family in EC development has been getting more and more attentions. To our best knowledge, there are few studies on the relationship between SOX_4 gene and EC at home and abroad. Therefore, in this study, by detecting the

expression of SOX_4 protein in 51 EC patients using immunohistochemical methods, we analyzed the clinical-pathological parameters in EC and further explored the relationship between SOX_4 expression and the clinical pathology and prognosis of EC.

1 Materials and methods

1.1 General data of EC patients

Retrospective analysis of clinical and pathological data of 51 patients, who were diagnosed as having EC and underwent panhysterectomy in the Wenzhou Central Hospital from March 2017 to March 2019, was performed. All patients met following inclusion criteria: the first surgical treatment of each patient was performed in the Wenzhou Central Hospital; EC was diagnosed and confirmed in all patients in the same hospital; the results were consistent after double-blind studies; complete clinical and follow-up data were all available. Moreover, following exclusion criteria were strictly implemented: patients with metastatic EC were excluded; exclude uterus and ovaries Both parties cannot determine the primary tumor; patients with a history of other malignancies were excluded; patients who had received radiotherapy or hormone therapy before surgery were excluded. All patients were between 45 to 61 years old with a mean age of (51.37 ± 4.16) years; among these patients, 22 cases were type 1 EC(EC I) and 29 cases were type 2 EC(EC II). Of all patients, 5 cases were in the first pathological stage (Stage I), 8 cases were in the Stage II, 13 cases were in the Stage III and 25 cases were in the Stage IV. Of all patients, 8 cases had Grade I EC, 13 cases had Grade II EC, and 30 cases had Grade III EC. And 21 cases had lymph node metastasis, and 30 cases had no lymph node metastasis. Hematoxylin-eosin staining and immunohistochemical staining of tissue sections were assessed by two experienced pathologists, and the diagnosis is consistent with the pathological report.

1.2 Methods

All samples were fixed with 40 g/L formaldehyde solution, embedded in paraffin and sectioned. The primary antibody, Rabbit polyclonal SOX_4 antibody, was procured from Abcom, USA; the secondary antibody and diaminobenzidine (3-3'-diaminobenzidine, DAB) chromogenic reagent were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.; EnVision System was used in immunohistochemical analysis according to protocols provided by the manufacturer.

1.3 Observed index

SOX_4 protein in endometrial cancer was examined; the relationship between SOX_4 expression and clinical-pathological parameters in EC tissues was analyzed.

1.4 Result Assessment

That SOX_4 was positive for nuclear staining, and normal endometrial tissue next to the cancer was used as an internal control.

1.5 Statistical analysis

The data were subject to statistical analysis using SPSS software (Version 20.0). Comparison between groups was performed using the χ^2 test. P value <0.05 was considered to be statistically significant.

2 Results

2.1 SOX_4 expression in EC tissue

In the EC tissue of 51 patients, SOX_4 was mainly expressed in the nucleus of tumor cells. Brown-yellow particles appeared in the positive cases, as shown in Figure 1, and no significant SOX_4 expression was observed in non-tumor endometrial tissue adjacent to the tumor (Figure 2). The positive rate of SOX_4 expression in EC tissues was 76.5% (39/51), while that in non-tumor endometrial tissues was 6.78%(4/59). Therefore, the expression of SOX_4 in endometrial cancer tissues was significantly higher than that in non-tumor tissues adjacent to the cancer (P<0.05) (Table 1).

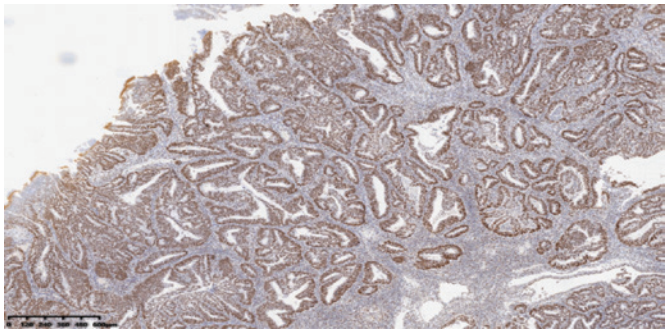

Figure 1. SOX_4 expression in endometrial cancer (Envision method × 200)

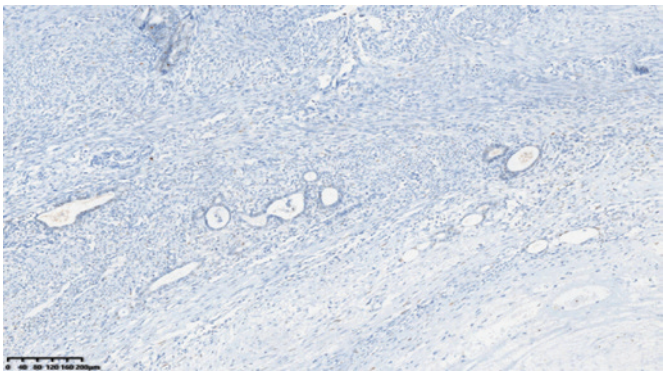

Figure 2. SOX_4 expression in the control group (Envision method × 200)

2.2 Relationship between SOX_4 expression in EC tissues and clinical-pathological parameters

Among the 51 EC patients, 39 cases were positive for SOX_4 with an expression rate of 76.4% .The differences of SOX_4 expression in the patients with different differentiation grading, in the patients at different FIGO staging , and between the patients with lymph node metastasis and those without lymph node metastasis, were statistically significant (P<0.05); However, there was no significant differences in the expressions of SOX_4 protein in terms of patients' age and histopathological classification (P>0.05), as shown in Table 2.

Table 1. SOX_4 expression in endometrial tissue

Group		SOX_4 Expression rate (%)	χ^2	*P* value
endometrial cancer group	51	76.4% (39/51)	55.799	0.00
control group	59	6.78% (4/59)		

Table 2. EC Relationship between SOX_4 expression in EC tissues and clinical-pathological parameters

Clinical-pathological characteristics	*n*	SOX_4 expression state		χ^2, *P*
		+(*n*=39)	-(*n*=12)	
Age				
<50	20	16	4	
⩾ 50	31	23	8	
Differentiation Grading				
Ⅰ	8	3	5	
Ⅱ	10	10	3	
Ⅲ	30	26	4	
FIGO Staging				
Ⅰ	5	1	4	
Ⅱ	8	6	2	
Ⅲ	13	10	3	
Ⅳ	23	22	3	
Histopathological type				
I type	22	15	7	
IItype	29	24	5	
Lymphatic metastasis				
Yes	20	19	1	
No	31	20	11	

3 Discussion

EC is one of the most common malignant tumors in gynecology and its morbidity and mortality have increased year by year. In 2018, 63,230 new cases of EC are expected in the United States, which would cause 11,350 cases of death[8]. The incidence of EC in China is also increasing yearly. Abnormal changes of oncogenes or tumor suppressor genes are one of the important causes that trigger tumor progression. EC staging is closely related to clinical treatment and prognosis and the 5-year relative survival rate for patients in the early EC stage or undergoing metastasis is only between 10% and 30%[9]. Although imaging tests, such as transabdominal ultrasound, transvaginal ultrasound, CT and MRI, are sensitive in detecting structural changes, changes in form and structure often lag behind tumor-related dysfunction. Therefore, an important way to improve the diagnosis and prognosis of EC is to find molecular markers[10,11] that are closely related to the biological behavior of

tumors and have high sensitivity and specificity.

Since the SRY gene was discovered in mammals by Sinclair et al. in 1990, the SOX gene family was gradually discovered and recognized[12]. The sox gene family is super-conservative in evolution and its encoded products all share an HMG-box motif. The SOX gene family is involved in sex determination, cartilage tissue, and plays a vital role in the development of blood system, nervous system, embryo and lens. It is an important developmental gene family and many developmental genes are related to malignant tumors, such as members of PAX and HOX family. The SOX_4 gene is a member of SoxC subfamily and is mainly expressed in heart, central nervous system, and thymus during embryonic development. In addition, SOX_4 is also expressed in stem cellsto stabilize them. Therefore, mutations, deletions or overexpression of the SOX_4 gene not only cause congenital diseases, but also are closely related to tumor development. Some studies have shown that the overexpression of SOX_4 gene in a variety of solid tumors is related to the poor prognosis of cancer patients. It is discovered during research on carcinogenic genes that SOX_4 gene is one of the most sensitive and common site for insertion mutation caused by retroviruses, which can lead to development of myeloid leukemia and B-cell lymphoma in mice. In addition, silencing SOX_4 expression can lead to the apoptosis of tumor cells in the prostate cancer, submandibular adenocarcinoma, liver cancer and breast cancer. In a quantitative analysis study, it is revealed that SOX_4 gene is among the 64 up-regulated genes in human tumors; therefore, some scholars have listed it as one of the biomarkers for malignant tumors.[13] Moreover, it was reported that overexpression of SOX_4[14] is observed in primary gallbladder cancer and an analysis of 136 cases of primary gallbladder cancer by immunohistochemical staining revealed a positive SOX_4 expression rate of 75% (102/136). Andersen CL et al[15] reported that colorectal cancer patients with higher expression rate of SOX_4 also had a higher recurrence rate. The above-mentioned studies suggest that SOX_4 can serve as an oncogene and be involved in tumor invasion, metastasis and recurrence.

This study examined and compared the SOX_4 expression in EC tissue and non-tumor endometrial tissue. The results show that the expression rate of SOX_4 in EC tissues is significantly higher than that in non-cancer endometrial tissue. This is basically consistent with the results reported in the other studies[16,17], indicating that SOX_4 can play a role in cancer development. By analyzing the relationship between SOX_4 expression and clinical-pathological characteristics of EC, we found that high SOX_4 expression are closely related to the differentiation grade, FIGO stage and lymph node metastasis; a poorer differentiation grade and more advanced stage both indicate a poorer prognosis of the patient. Further analysis showed that SOX_4 expression in patients with Stage Ⅲ and IV EC is significantly higher than that in patients with Stage I and II EC, suggesting that SOX_4 expression increases with the advancement of clinical stage and resulting into a poor prognosis of patients. Meanwhile, high SOX_4 expression is also associated with lymph node metastasis in EC, suggesting that it may increase the risk of cancer lymph node metastasis, and play a role in the lymph node metastasis of EC; however, the specific mechanism still needs to be further explored.

4 Conclusion

SOX_4 is closely related to EC; moreover, SOX_4 expression in EC is associated with the differentiation grade, clinical stage and lymph node metastasis of EC, but is unrelated to the age and histologic type of patients. This may imply that SOX_4 is related to the occurrence and development of EC. SOX_4 may also serve as an indicator for clinical assessment of EC and a biomarker for clinical-pathological diagnosis, and help to better analyze high-risk factors in EC patients, thus providing a new insight into the early diagnosis, prognosis and targeted treatment of EC. Due to the diversity of SOX genes and the complexity of the carcinogenic mechanisms of EC, the exact role and mechanisms of SOX_4 gene in EC occurrence and development need to be further studied.

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