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Significance analysis of PAX8 expression in endometrial carcinoma

Shan Hu, MD[a], Hua Gan, MD[b], Fengmei Yang, MD[a,*]

Abstract

To analyze the expression and prognostic value of paired-box 8 (PAX8) expression in uterine corpus endometrial carcinoma (UCEC) by bioinformatics. The expression of PAX8 gene in UCEC was analyzed by R language and immunohistochemistry. The correlation between PAX8 expression and clinicopathological features was analyzed by R language. The prognostic factors was analyzed by univariate/multivariate regression. The survival curve of patients was analyzed by Kaplan–Meier Plotter (K–M Plotter). The diagnostic value of PAX8 in UCEC was analyzed by receiver operating characteristic curve, and the relationship between PAX8 expression and methylation was analyzed by Ualcan. The relationship between methylation and prognosis was analyzed by MethSurv database. The expression of PAX8 in cancer tissues was significantly higher than that in normal tissues. The expression of PAX8 was related to clinical stage, age, histological type, histologic grade, tumor invasion and disease-specific survival event. Univariate/multivariate regression analysis showed that clinical stage, tumor invasion, and PAX8 expression were the influence factors of overall survival (OS), while histologic grade and PAX8 expression were the influence factors of disease-specific survival, and patients with low expression had a longer OS. The area under the curve of receiver operating characteristic curve was 0.81 for PAX8 diagnosis of UCEC. PAX8 was hypomethylated in cancer tissue, and patients with hypermethylated PAX8 had a longer OS. The high expression of PAX8 induced by hypomethylation may play an important role in the occurrence and prognosis of UCEC.

Abbreviations: DSS = disease-specific survival, OS = overall survival, PAX8 = paired-box 8, PFS = progression-free survival, TCGA = The Cancer Genome Atlas, UCEC = uterine corpus endometrial carcinoma.

Keywords: bioinformatics, expression, methylation, PAX8, prognosis, UCEC

1. Introduction

Uterine corpus endometrial carcinoma (UCEC) is a major malignant tumor of the female reproductive system.[1] The current International Federation of Gynecology and Obstetrics grading scheme provides prognostic information that can be used to guide the extent of surgery and use of adjuvant chemotherapy or radiation therapy. However, UCEC has high malignancy and poor prognosis. Its incidence has been gradually increasing in recent years. Thus, indicators of early diagnosis and prognosis need to be identified.[2]

Paired-box 8 (PAX8), a member of the PAX protein family, plays an important role in embryo development, central nervous system, angiogenesis, immune regulation, and tumor metastasis.[3] It is also a restricted transcription factor that is involved in physiological activities such as cell proliferation and differentiation, and is closely related to the occurrence and development of a various types of tumor, such as lung cancer,[4] ovarian cancer,[5,6] and breast cancer.[7]

The transcription factor PAX8 is critical in urogenital system development. Comprehensive genomic screens indicate an additional oncogenic role for PAX8 in ovarian cancers.[8] Given its high expression in primary ovarian cancer, PAX8 has been considered as a target for ovarian cancer treatment.[9] The rs10175462 mutation of PAX8 is significantly associated with overall cervical disease.[10] However, data regarding PAX8 expression in UCEC are limited. Bioinformatics analysis has been used to determine the value of PAX8 expression a in UCEC prognosis, and results have provided a theoretical basis for early diagnosis.

2. Materials and Methods

2.1. Analysis of PAX8 expression

The expression of *PAX8* gene in UCEC unpaired samples (174 cancer tissues and 91 normal tissues) and paired samples (23 cancer tissues and 23 corresponding adjacent tissues) from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) and Genotype-TissueExpression (https://gtexportal.org/home/) database was analyzed using R language,[11] and the expression of the PAX8 protein in UCEC cancer tissues and normal tissues in Human Protein Atlas (https://www.proteinatlas.org/) online database was analyzed through immunohistochemistry. Antibody staining in the annotated cell types (glandular cells) in the current human tissue is reported as not detected, low, medium, or high. In addition, the score is based on the staining

SH and HG contributed equally to this work.

The datasets generated during and/or analyzed during the current study are publicly available.

The authors have no conflicts of interest.

[a] Department of Obstetrics and Gynecology, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, P.R. China, [b] Department of Orthopaedic, XiangZhou District Renmin Hospital, Xiangyang, Hubei, P.R. China.

** Correspondence: Fengmei Yang, Department of Obstetrics and Gynecology, Taihe Hospital, Hubei University of Medicine, Renmin Southern 32, Shiyan, Hubei 442000, P.R. China (e-mail: 29256175@qq.com).*

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intensity and fraction of the stained cells. Moreover, the clinical data of patients with UCEC were downloaded from the TCGA database to analyze the correlation between clinicopathological features and PAX8 expression. In accordance with the Declaration of Helsinki, all protocols were approved by the Ethics Committee of Taihe Hospital.

2.2. Correlation analysis of PAX8 expression and prognosis

The clinical data (clinical stage, age, tissue type, tissue grade, degree of invasion, etc.) of patients with UCEC and data of PAX8 expression were combined to construct a Cox model, A univariate/multivariate regression analysis was conducted to analyze the prognostic factors. The K–M plotter was used to analyze and plot the survival curve of PAX8 expression in UCEC patients.

2.3. Methylation analysis of PAX8 gene

The Ualcan online tool (http://ualcan.path.uab.edu/index.html) was used to analyze the correlation between the expression of PAX8 and methylation, and MethSurv online database (https://biit.cs.ut.ee/methsurv/) was used to analyze the correlation between methylation at different PAX8 sites and OS.

2.4. Mutation analysis of PAX8 gene

PAX8 gene mutation in UCEC patients was analyzed by cBioportal online analysis tool (http://www.cbioportal.org/).

2.5. Diagnostic value analysis of PAX8

The clinical data of patients with UCEC were downloaded from the TCGA database, the receiver operating characteristic curve was analyzed and plotted using R language, and the AUC was obtained.

2.6. Statistical analysis

Statistical calculations were performed using SPSS software version 19.0. All measurement data were expressed as mean ± standard deviation and analyzed by 2 Independent sample *t* test. All enumeration data were expressed as percentage and analyzed by Chi-square test. The survival curve was analyzed by Kaplan–Meier method and log-rank test was performed. Correlation was analyzed by Pearson correlation test, and $P < .05$ indicated statistically significant differences.

3. Result

3.1. Expression of PAX8 in cancer and normal tissues

First, the expression of PAX8 in cancer and normal tissues of patients with UCEC were analyzed using R language. As shown in Figure 1A and B, PAX8 expression was significantly higher in cancer tissues than in normal tissues in the unpaired samples and paired samples. Immunohistochemistry from the Human Protein Atlas database was used for validation (Fig. 1C and D).

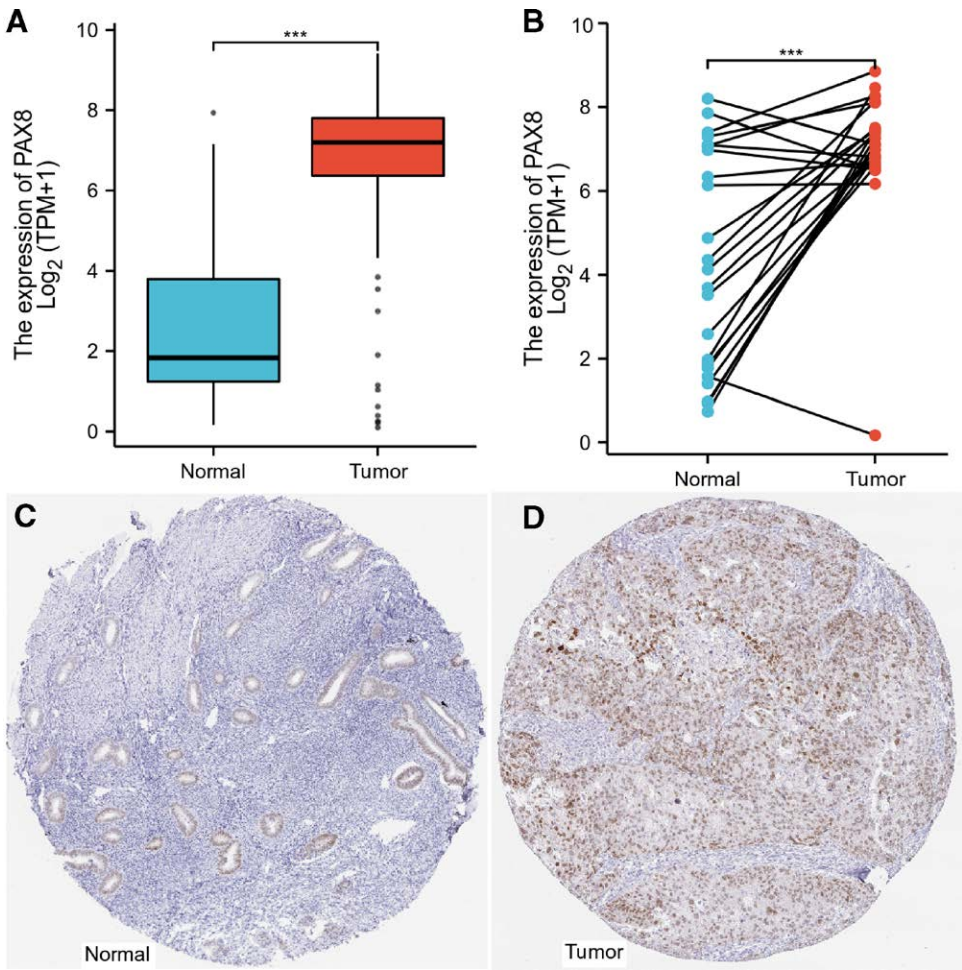

Figure 1. Expression of PAX8 in UCEC. (A) Unpaired sample, (B) paired sample, (C) normal tissue, and (D) tumor tissue. ***$P < .001$. PAX8 = paired-box 8, UCEC = uterine corpus endometrial carcinoma.

3.2. Relationship between PAX8 expression and clinicopathological features

The relationship between PAX8 expression and clinicopathological features was further analyzed by downloading clinical data of UCEC patients from TCGA database. Results of chi-square analysis showed PAX8 expression significantly influenced clinical stage, age, histological type, histologic grade, menopause status, and OS and disease-specific survival (DSS) (Table 1). Then, T test was used to analyze the correlation between case data and PAX8 expression. Results showed that PAX8 was higher in clinical stages (Stage III and Stage IV), age (>60 years), tissue Stage (mixed and serous), tissue grade (G3), depth of invasion (>50%), and DSS events (death cases) (Fig. 2).

3.3. Prognostic factors of UCEC

The prognostic factors of OS and progression-free survival (PFS) in patients with UCEC were analyzed using univariate/multivariate cox regression. Univariate regression analysis showed that clinical stage, age, histological type, histologic grade, tumor invasion, and PAX8 expression were the influencing factors of OS. Multivariate regression analysis showed that clinical stage, tumor invasion, and PAX8 expression were the factors affecting OS. Univariate regression analysis revealed that clinical stage, histological type, histologic grade, tumor invasion, and PAX8 expression were the influencing factors of PFS, whereas multivariate regression analysis showed that clinical stage, histologic grade, and PAX8 expression were the influencing factors of PFS (Fig. 3).

The patients were divided into high and low PAX8 expression groups, and the relationship between PAX8 expression and OS was further analyzed. Results showed that the patients in the low expression group had a significantly longer OS than those in the high expression group (Fig. 4A). Analysis of the relationship between PAX8 expression and subgroups of clinical data demonstrated that in patients with diabetes, those with low PAX8 expression had significantly longer OS than those with high PAX8 expression (Fig. 4B). Meanwhile, the difference between other clinical subgroups was not statistically significant.

3.4. Diagnostic value analysis

The above results were showed that PAX8 expression could affect the prognosis of patients with UCEC, and patients with low expression had better OS than those with high expression. An receiver operating characteristic curve was plotted to analyze the value of PAX8 in the early diagnosis of UCEC. Results showed that the sensitivity, specificity, positive predictive value, and negative predictive value of PAX8 in the diagnosis of UCEC were 77%, 86%, 26.5%, and 98.4%, respectively, and the AUC was 0.814 (Fig. 5A). In addition, the methylation level of PAX8 in normal tissues was higher than that in tumor tissues, and the difference was statistically significant (Fig. 5B). Analysis of the relationship between methylation at different sites of PAX8 and prognosis of patients revealed that the patients with hypermethylation at these sites also had better OS (Table 2).

3.5. Mutation analysis of PAX8 gene

The mutation of the *PAX8* gene in the database was also analyzed in this study. Results showed that 8 of the 529 samples were mutated, with a mutation frequency of 1.5%. No significant differences in DSS, OS, PFS, disease-free survival, and other prognostic indicators were found between the mutant and non-mutant group (P = .255, 0.455, 0.699, 0.831).

4. Discussion

Bioinformatics is the processing and analysis of a variety of omics data, which can conduct a comprehensive analysis of gene products. With the rapid development of computer technology and biotechnology, bioinformatics technology plays a very

Table 1

Relationship between gene expression and clinical data.

Characteristic	Low expression	High expression	*P*
Clinical stage, n (%)			.002**
Stage I	189 (34.2%)	153 (27.7%)	
Stage II	27 (4.9%)	24 (4.3%)	
Stage III	46 (8.3%)	84 (15.2%)	
Stage IV	14 (2.5%)	15 (2.7%)	
Age, n (%)			.002**
≤60	122 (22.2%)	84 (15.3%)	
>60	154 (28.1%)	189 (34.4%)	
Histological type, n (%)			<.001***
Endometrioid	242 (43.8%)	168 (30.4%)	
Mixed	9 (1.6%)	15 (2.7%)	
Serous	25 (4.5%)	93 (16.8%)	
Histologic grade, n (%)			<.001***
G1	66 (12.2%)	32 (5.9%)	
G2	73 (13.5%)	47 (8.7%)	
G3	134 (24.8%)	189 (34.9%)	
Tumor invasion (%), n (%)			.879
<50	132 (27.8%)	127 (26.8%)	
≥50	112 (23.6%)	103 (21.7%)	
Menopause status, n (%)			<.001***
Pre	28 (5.5%)	7 (1.4%)	
Peri	7 (1.4%)	10 (2%)	
Post	215 (42.5%)	239 (47.2%)	
Overall survival event, n (%)			.044*
Alive	238 (43.1%)	220 (39.9%)	
Dead	38 (6.9%)	56 (10.1%)	
Disease-specific survival event, n (%)			.007**
Alive	254 (46.2%)	233 (42.4%)	
Dead	21 (3.8%)	42 (7.6%)	

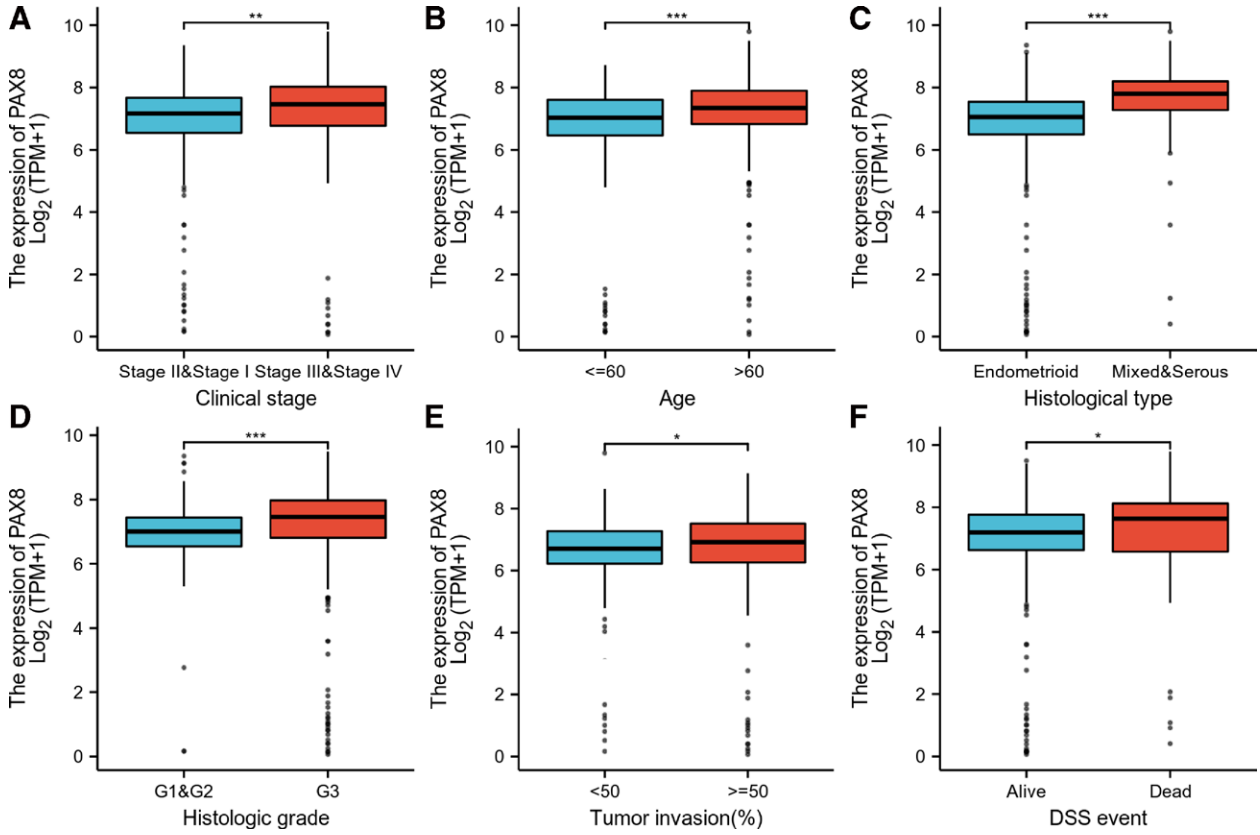

Figure 2. The relationship between PAX8 expression and clinicopathological features. *$P < .05$, **$P < .01$, ***$P < .001$. (A) Clinical stage, (B) age, (C) histological type, (D) histologic grade, (E) tumor invasion, and (F) disease-specific survival event. PAX8 = paired-box 8.

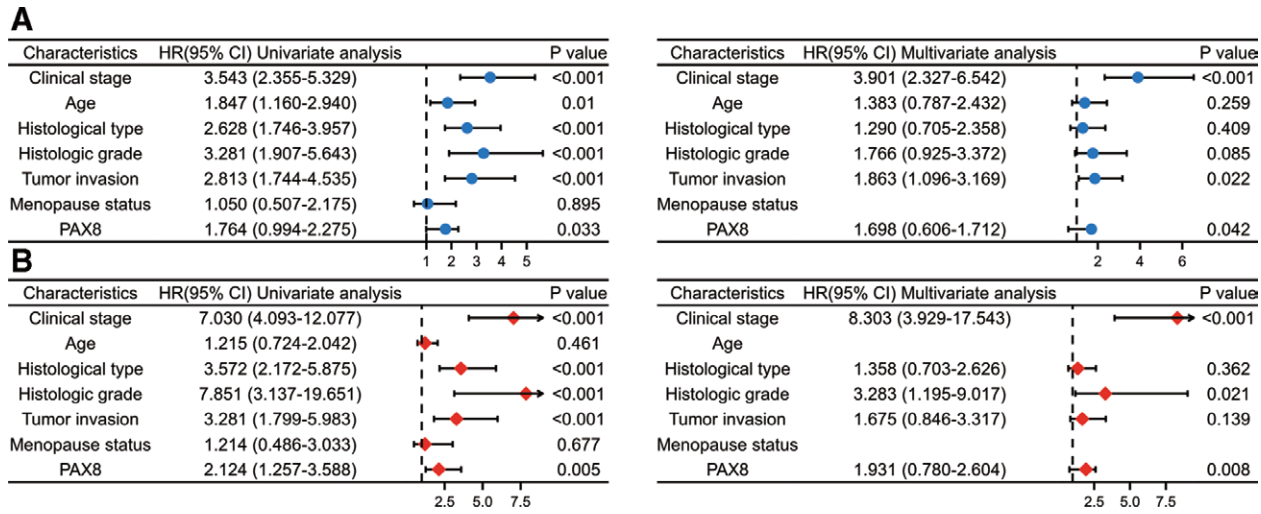

Figure 3. Univariate/multivariate regression analysis of prognostic factors. (A) Overall survival and (B) disease-specific survival.

important role in understanding and exploring the occurrence of diseases. The incidence of endometrial cancer, a common malignancy in the female reproductive system has gradually increased in recent years. The lack of specific early diagnostic indicators results in poor prognosis. The *PAX* gene is transcription factors and a key regulator of normal tissue formation and cell differentiation.[9] The *PAX8* gene is abnormally expressed in various solid tumors. In the present study, the significance of PAX8 gene expression in endometrial cancer was analyzed using bioinformatics.

We first analyzed the expression of PAX8 in endometrial cancer, and found that PAX8 was highly expressed in cancer tissues, especially in clinical patients with stage III and stage IV cancer, age (>60 years old), histological type (mixed and serous), histologic grade (G3), and tumor invasion (>50%). In addition, regression analysis showed that clinical stage, tissue invasion and PAX8 expression were the prognostic factors of patients with endometrial carcinoma, and patients with low PAX8 expression had better prognosis than those with high PAX8 expression. PAX8 + cells are the cellular origin of serous

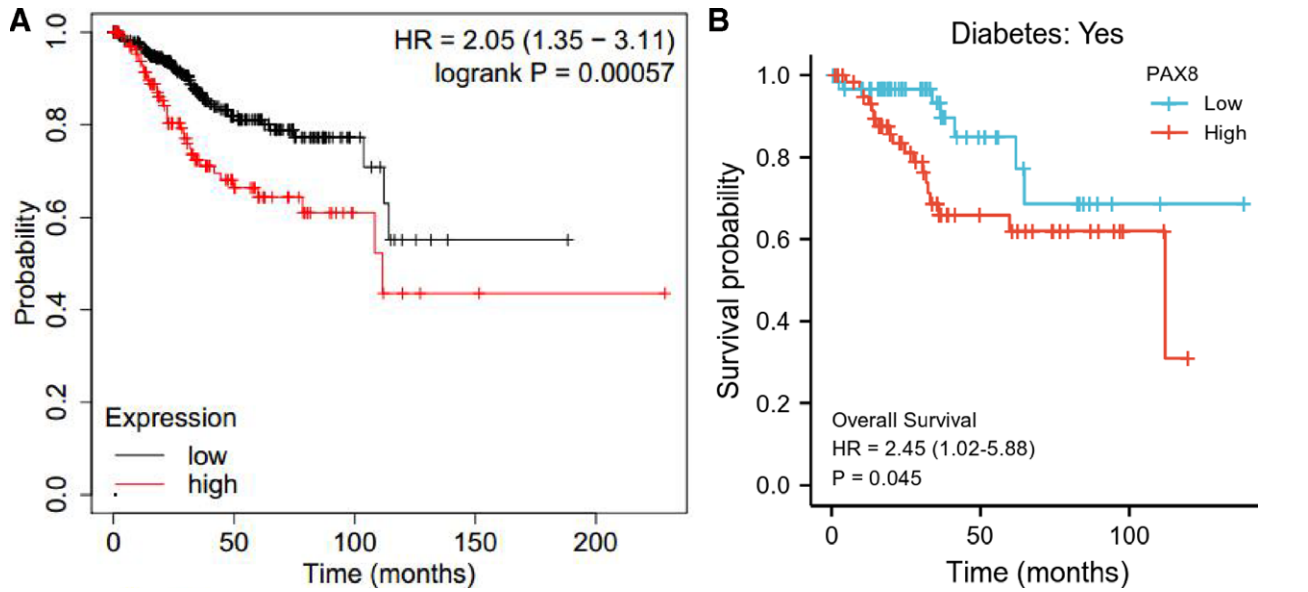

Figure 4. Survival curve analysis. (A) All patients and (B) diabetic patients.

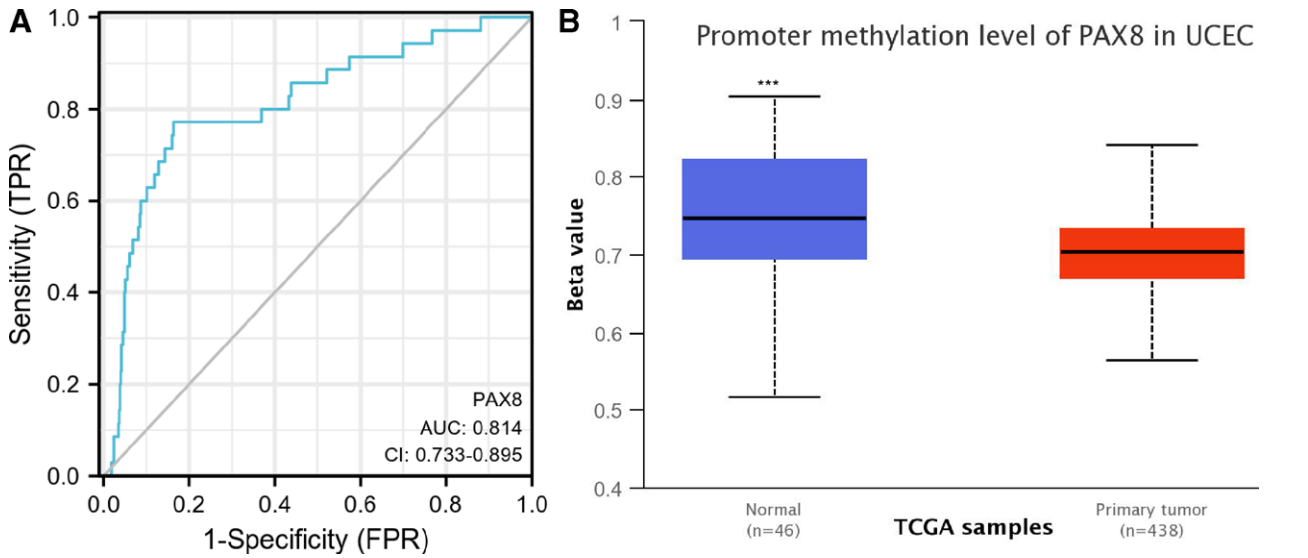

Figure 5. The value of PAX8 expression in the diagnosis of UCEC and relationship with methylation. (A) ROC and (B) relationship between PAX8 expression and methylation. PAX8 = paired-box 8, ROC = receiver operating characteristic curve, UCEC = uterine corpus endometrial carcinoma.

endometrial carcinoma in mice, and the P53 and RB pathways play a key role in the pathogenesis of serous endometrial carcinoma.[12] PAX8 positively regulates mutated p53, and missense p53 mutations have an oncogenic gain of function effect.[13] The pathogenesis of PAX8 in endometrial cancer patients in this region warrants further study.

High expression of MACC1-AS1 can upregulate PAX8 expression in hepatocellular carcinoma cells, and MACC1-AS1 overexpression can promote hepatocellular carcinoma cell proliferation, epithelial-mesenchymal transition and invasion by regulating PAX8.[14] PAX8 expression is upregulated in primary stomach cancer tissue and could promote the proliferation of gastric cancer cells. Patients with high PAX8 expression have a poor prognosis.[15] PAX8 is widely expressed in high-grade serous ovarian cancer. In addition, silencing of PAX8, as a histological marker of high-grade serous ovarian cancer, could reduce the proliferation

Table 2

Relationship between methylation at different sites of PAX8 and prognosis.

Name	Cancer	HR	95% CI	*P* value
cg04345118	UCEC	0.36	(0.223–0.583)	3.25E−05
cg03197992	UCEC	0.397	(0.249–0.633)	.000103982
cg25758828	UCEC	0.435	(0.271–0.699)	.000581778
cg23564664	UCEC	0.466	(0.292–0.743)	.001326591
cg25243455	UCEC	0.534	(0.328–0.869)	.011576856
cg07594247	UCEC	0.569	(0.353–0.916)	.02031674
cg11763394	UCEC	1.745	(1.067–2.853)	.026425668
cg05106191	UCEC	0.582	(0.36–0.94)	.027071626
cg17445212	UCEC	0.596	(0.367–0.968)	.036618125

PAX = paired-box 8, UCEC = uterine corpus endometrial carcinoma.

and increase the apoptosis of human high-grade serous ovarian cancer cell lines.[16] PAX8 mediated multiple tumorigenic mechanisms, including proliferation, migration, angiogenesis, and apoptosis, in high-grade serous ovarian cancer. Reducing PAX8 levels or disrupting PAX8 transcriptional activity could inhibit these precancerous effects.[5,17] These results suggest that PAX8 promotes the development and progression of tumors. Analysis of the relationship between PAX8 expression and prognosis in clinical subgroups showed that the diabetic patients with low PAX8 expression had better prognosis than those in high PAX8 expression. Diabetes is a common risk factor for endometrial cancer, and type 2 diabetes is a poor prognostic factor for estrogen-dependent endometrial cancer.[18] The relationship between PAX8 and diabetes needs further study in patients with endometrial cancer.

Genetic and epigenetic changes play important roles in tumor development. In gastrointestinal stromal tumor, PAX8 expression is down-regulated, and PAX8 overexpression or PAX8 methylation suppression using DNA methyltransferase inhibitor 5-Aza-dC inhibits the proliferation, migration, and invasion of gastrointestinal stromal tumor cells while promoting their apoptosis.[19] In this study, *PAX8* gene mutation in endometrial carcinoma was analyzed. Results showed that the mutation rate was low and the mutation had almost no effect on the prognosis of the tumor. The methylation level in normal tissues was significantly higher than that in cancer tissues. Combined expression analysis showed that the high PAX8 expression caused by hypomethylation might play an important role in the development of endometrial cancer. PAX8 alone as a diagnostic indicator of endometrial cancer also had high specificity. Methylation changes were earlier than genetic changes and were reversible process. Patients with low PAX8 expression and hypermethylation at the CpG island site had a good prognosis. This study may serve as a theoretical basis for the use of PAX8 methylation as an indicator of early diagnosis and prognosis.

In conclusion, the high expression of PAX8 induced by hypomethylation may play an important role in the occurrence, development, and prognosis of endometrial cancer, and PAX8 has certain value in the early diagnosis of endometrial cancer.

Author contributions

Data curation: Hua Gan.
Formal analysis: Shan Hu.
Investigation: Fengmei Yang.
Methodology: Fengmei Yang.
Project administration: Fengmei Yang.
Resources: Hua Gan.
Software: Hua Gan.
Writing – original draft: Shan Hu.
Writing – review & editing: Fengmei Yang.

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