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Original Article

Methylation status of PAX1 and SF-1: implications for diagnosis and prognosis in endometrial cancer

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Abstract: Endometrial carcinoma (EC) is a common malignancy of the female reproductive system, often diagnosed at advanced stages due to the lack of reliable early biomarkers. Gene methylation has emerged as a key epigenetic mechanism in cancer development, offering potential for early detection and prognostic evaluation. This study aimed to explore the methylation status of Paired Box Gene 1 (PAX1) and Steroidogenic Factor 1 (SF-1) as potential biomarkers for EC diagnosis and prognosis. A total of 110 EC patients and 75 non-EC patients, enrolled between January 2020 and January 2022, were retrospective analyzed using methylation-specific polymerase chain reaction (MSP) to assess the clinical utility of PAX1 and SF-1 methylation in diagnosis, prognosis, and recurrence surveillance. EC patients exhibited significantly higher PAX1 and SF-1 methylation levels compared to controls, with SF-1 methylation showing superior diagnostic efficacy (AUC = 0.735). PAX1 methylation was significantly associated with key clinicopathological features, including tumor differentiation grade ($P = 0.001$), FIGO staging ($P < 0.001$), and myometrial invasion depth ($P = 0.030$). It also showed a strong correlation with overall survival (OS) and cumulative incidence of recurrence (CIF) ($P < 0.001$). These results highlight the important role of PAX1 methylation in the diagnosis and prognostic evaluation of EC. Multivariate Cox regression analysis identified PAX1 methylation positivity as an independent risk factor for poor prognosis, whereas SF-1 methylation had limited prognostic impact. These findings highlight PAX1 methylation as a valuable biomarker for enhancing diagnostic accuracy and refining prognostic stratification in EC. In contrast, SF-1 methylation primarily contributes to diagnosis. Together, these results offer new insights into the development of personalized diagnostic and therapeutic strategies for EC.

Keywords: Endometrial carcinoma, PAX1, SF-1, methylation, biomarkers, diagnosis, prognosis, Cox regression analysis

Introduction

Endometrial carcinoma (EC) is a prevalent malignancy of the female reproductive system, with a rising incidence worldwide [1, 2]. According to GLOBOCAN 2022 data from the International Agency for Research on Cancer (IARC), breast cancer remains the most common cancer in women, followed by lung and cervical cancers [3]. EC often presents with symptoms such as abnormal vaginal bleeding and pelvic discomfort, frequently leading to diagnosis at more advanced stages [4]. Early detection and treatment are crucial for improving survival rates in EC. However, the lack of reliable early biomarkers and effective screening methods causes most patients being diagnosed at intermediate or advanced stages, thereby complicating treatment and prognosis [5]. Gene methylation, a key epigenetic regulatory mechanism, can result in gene silencing and is strongly associated to cancer development [6]. Assessing the methylation status of specific genes holds promise for identifying early diagnostic, therapeutic, and prognostic biomarkers [7]. Therefore, exploring methylation-based biomarkers in EC may significantly enhance diagnostic accuracy and prognostic evaluation.

PAX1 (Paired Box Gene 1) and SF-1 (Steroidogenic Factor 1) are critical regulators of embryonic development and tumorigenesis, gaining attention for their roles in epigenetic regulation [8, 9]. PAX1, a member of the PAX transcription factor family, regulates somite differ-

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entiation and organ formation, especially during the development of the spine, thymus, and reproductive system. Dysfunction of PAX1 may result in severe developmental malformations [10, 11]. In cancer, PAX1 hypermethylation often silences its expression, particularly in cervical cancer, where reduced expression correlates negatively with tumor stage, differentiation, and invasiveness [12]. PAX1 exerts tumor-suppressive effects by inhibiting cyclin proteins (e.g., Cyclin D1) and pro-apoptotic pathways (e.g., BAX/Caspase-3). Methylation-induced inactivation may promote tumor metastasis by disrupting the balance of epithelial-mesenchymal transition (EMT) [13]. Similarly, SF-1 (NR5A1), a nuclear receptor family member, is essential for gonadal and adrenal development and maintains hormonal homeostasis in adults by regulating steroidogenic enzymes (e.g., CYP17A1, CYP19A1) [14, 15]. Although PAX1 and SF-1 operate in distinct regulatory networks, their spatiotemporal co-expression and synergistic epigenetic inactivation during reproductive system development provide a strong rationale for their combined study in tumorigenesis.

This study aims to evaluate the methylation levels of PAX1 and SF-1 in EC patients and assess their potential as diagnostic and prognostic biomarkers. Although both genes are expressed in EC, their clinical significance remains underexplored. Thus, we investigate the methylation status of PAX1 and SF-1 and analyze their association with EC prognosis. To our knowledge, this is the first study to examine the methylation status of these genes and their prognostic implications in EC.

Methods and materials

Sample size calculation

According to Liu et al. [16], the mean ΔCp for SF-1 was 8.28 (SD = 4.07) in the cervical cancer malignant group and 17.42 (SD = 4.85) in the non-malignant group, yielding an effect size of 2.04 and a combined standard deviation of 4.47. Using the formula ($n = \frac{2(Z_{\alpha/2} + Z_{\beta})^2 \times \sigma^2}{d^2}$) for sample size calculation. In statistical hypothesis testing, the α represents the significance level, typically set at 0.05, which indicates the probability of making a Type I error (rejecting a true null hypothesis). The Zα/2 value corresponds to the critical value from the standard normal distribution for a two-tailed test at the significance level α, with a typical value of 1.96 for α = 0.05. Power refers to the probability of correctly rejecting the null hypothesis when it is false, and is typically set at 80%, implying a 20% chance of a Type II error (failing to reject a false null hypothesis). The Z_{β} value corresponds to the critical value for Type II error; for a power of 80% (β = 0.2), it is typically 0.84. A minimum of 76 patients per group was required. The actual sample size for clinical data collection was determined by practical constraints.

Sample collection

This retrospective study included 110 patients with EC who underwent comprehensive staging surgery from January 2020 to January 2022 at the 980 (Bethune International Peace) Hospital of PLA Joint Logistics Support Forces, comprising the EC group. Additionally, 75 patients who underwent surgery for other gynecological conditions (e.g., uterine fibroids, adenomyosis) and had pathologically confirmed normal endometrial tissue were included as the control group. All participants provided written informed consent. This study has been approved by the 980 (Bethune International Peace) Hospital of PLA Joint Logistics Support Forces Ethics Committee (**Figure 1**).

Inclusion and exclusion criteria

Inclusion criteria: Histopathologically confirmed primary endometrial carcinoma (all histological subtypes and differentiation grades) [17]; age between 18 and 80 years (reproductive and postmenopausal stages); no history of preoperative radiotherapy, chemotherapy, or targeted therapy to avoid confounding effects on gene expression; availability of complete clinical and pathological data.

Exclusion criteria: Presence of other gynecological malignancies (e.g., ovarian or cervical cancer) or a history of non-gynecological primary cancers; severe endocrine disorders (e.g., Cushing's syndrome, hyperthyroidism), autoimmune diseases, or chronic infections (e.g., HIV, HBV) that could influence gene expression or the immune microenvironment; prior pelvic radiotherapy, chemotherapy, or hormone therapy (e.g., tamoxifen, GnRH agonists); pregnancy or lactation.

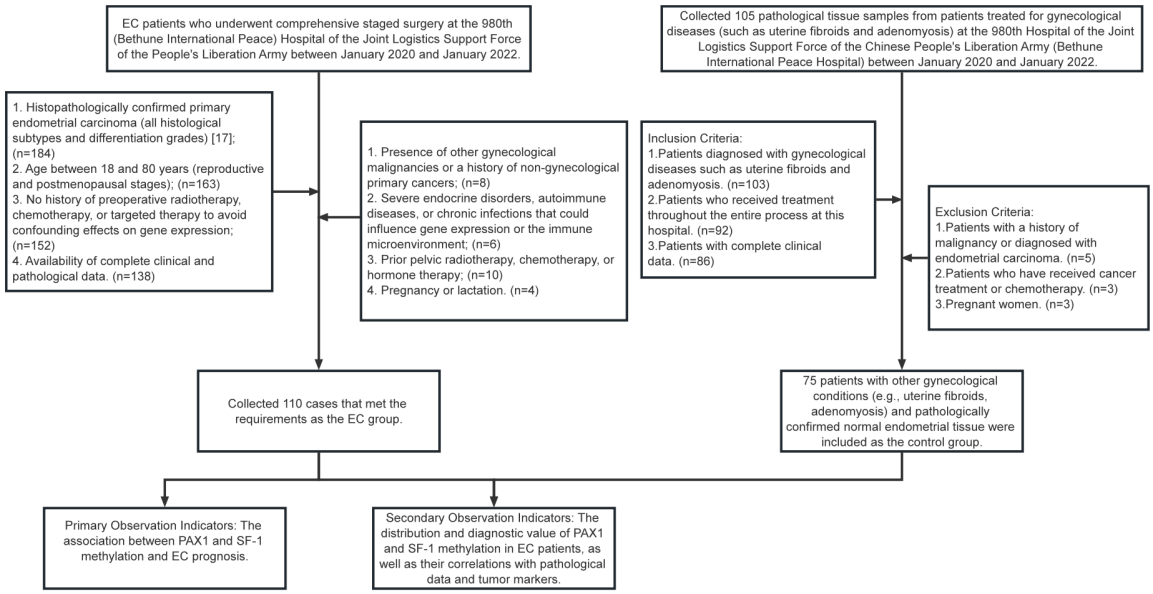

Figure 1. Sample inclusion flowchart.

Methylation detection

Tissue samples were obtained from cancerous and adjacent normal regions (≥ 3 cm from the tumor, pathologically confirmed as normal endometrial tissue), with 0.2 g of each sample used for DNA extraction. DNA was extracted using the Wizard SV Genomic DNA Purification Kit (Promega, USA), and purity was assessed with a Multiskan SkyHigh Full-Wavelength Enzyme Reader (Thermo Fisher, USA), selecting samples with an A260/A280 ratio of 1.8-2.0. To ensure complete bisulfite conversion, DNA was processed using the MethylCode™ Bisulfite Conversion Kit (Thermo Fisher, USA) for conversion, desulfurization, and purification. Methylation status of PAX1 and SF-1 was analyzed via methylation-specific PCR, with primers synthesized by Shanghai Jerei Co. (**Table 1**). The PCR reaction mixture consisted of 2 μL DNA template, 1 μL each of methylation- and non-methylation-specific primers, 25 μL Premix Ex Taq DNA polymerase, and 21 μL RNase-free ddH_2O. Thermal cycling was performed under the following conditions: initial denaturation at 95°C for 3 minutes; 30 cycles of 98°C for 2 seconds, 67°C for 15 seconds, and 72°C for 10 seconds; followed by a final extension at 72°C for 1 minute, and storage at 4°C. Amplification products (20 μL) were mixed with 6× DNA Loading Buffer and analyzed by agarose gel electrophoresis with a 10 μL DNA ladder using the E-Gel Imager Gel Imaging System (Thermo Fisher, USA). Methylation was deemed positive when the methylation-specific primer generated a prominent band, and the non-methylation primer either showed no amplification or produced a faint band. Conversely, methylation was considered negative if the methylation-specific primer was negative and the non-methylation primer was positive. Primer sequences are listed in **Table 1**. The methylation detection data used in this study were collected retrospectively from existing medical records.

Clinical data collection

Clinical data were retrieved from electronic medical records and outpatient follow-up records, encompassing demographic information, gynecological history, tumor characteristics, tumor markers, molecular biology data, and follow-up outcomes. Demographic variables included age, age at menarche, and body mass index (BMI). Gynecological history covered menopausal status, hypertension, diabetes, heart disease, smoking, and alcohol use. Tumor characteristics included differentiation grade (G1, G2, G3), FIGO stage (International Federation of Gynecology and Obstetrics staging system for EC), myometrial invasion depth, cervical stromal involvement, and lymph node metastasis. Tumor markers comprised cancer antigen (CA)-125, CA-199, and human epididy-

Table 1. Methylation primers

Primer name	Primer sequences	Primer size/bp
PAX1 M upstream primers	5'-TGTGATATTAGTCGGTAGTTTCGC-3'	152
PAX1 M downstream primers	3'-TAATCCCGAATATACTTAACCACGT-5'	
PAX1 U upstream primers	5'-GTGATATTAGTTGGTAGTTTTGTGT-3'	150
PAX1 U downstream primers	3'-AATCCCAAATATACTTAACCACATT-5'	
SF-1 M upstream primers	5'-TATTAAGGAAAAGGTATGATGTCGT-3'	266
SF-1 M downstream primers	3'-TAAAAAAATCACCAATAAACGC-5'	
SF-1 U upstream primers	5'-TGTATTAAGGAAAAGGTATGATGTTGT-3'	273
SF-1 U downstream primers	3'-CTACCTAAAAAAATCACCAATAAACAC-5'	

Note: PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1; M, Methylation; U, Unmethylation; bp, Base Pair.

mis protein 4 (HE4). Molecular data included PAX1 and SF-1 methylation status and expression levels. Follow-up data recorded overall survival (OS), progression-free survival (PFS), and recurrence status. All data were collected prior to treatment to ensure accuracy and completeness.

Follow-up

The follow-up cutoff date was January 2025. OS and PFS were assessed through telephone follow-ups and outpatient visits. In the first year, follow-ups occurred every three months; thereafter, they were conducted every six months. Telephone interviews with patients or their families documented survival and disease progression. For patients attending outpatient visits, clinical data were updated during the consultations. All follow-up data were rigorously reviewed for accuracy and completeness.

Observation indicators

Primary observation indicators: The association between PAX1 and SF-1 methylation and EC prognosis.

Secondary observation indicators: The distribution and diagnostic value of PAX1 and SF-1 methylation in EC patients, as well as their correlations with pathological data and tumor markers.

Statistical analysis

Data were analyzed using SPSS 26.0 software, and visualization generated with GraphPad Prism 10. The chi-square test was used for comparisons of categorical data, with results expressed as percentages. Survival analysis was performed using Kaplan-Meier survival curves, with Log-rank tests to compare survival differences between groups. For multiple event data, cumulative incidence function (CIF) analysis was used to estimate the occurrence rates of different events while accounting for competing risks. Receiver operating characteristic (ROC) analysis was performed using the pROC package (1.18.5) in R to assess the diagnostic performance of PAX1 and SF-1 methylation, with area under the curve (AUC) values reported. Cox proportional hazards regression analysis was used to examine the independent effects of multiple variables on survival time, with results presented as hazard ratios (HRs) and 95% confidence intervals (CIs). A *P*-value of < 0.05 was considered statistically significant.

Results

Comparison of baseline data between the control and EC groups

This study compared the baseline data between the EC group and the control group, and found no significant differences between the two groups in age, age at menarche, BMI, menopausal status, hypertension, diabetes, or history of heart disease, smoking, or alcohol use ($P > 0.05$, **Table 2**).

Methylation status of PAX1 and SF-1 and their diagnostic value in EC patients

Significant differences were observed in the methylation status of PAX1 ($\chi^2 = 4.581$, $P = 0.032$) and SF-1 ($\chi^2 = 40.313$, $P < 0.001$) between EC and non-EC patients (**Figure 2A** and **2B**). ROC curve analysis of a predictive model demonstrated that PAX1 and SF-1 methylation had diagnostic utility, with AUC values of 0.600

Table 2. Comparison of baseline data between EC patients and control subjects

Index	Total	EC group (n = 110)	control group (n = 75)	χ^2	*P*-value
Age (Year)					
≥ 60	95 (51.35%)	58 (52.73%)	37 (49.33%)	0.206	0.650
< 60	90 (48.65%)	52 (47.27%)	38 (50.67%)		
Menarche Age (Year)					
≥ 14	52 (28.11%)	30 (27.27%)	22 (29.33%)	0.094	0.760
< 14	133 (71.89%)	80 (72.73%)	53 (70.67%)		
BMI (kg/m^2)					
< 23	54 (29.19%)	31 (28.18%)	23 (30.67%)	0.708	0.702
23-25	83 (44.86%)	48 (43.64%)	35 (46.67%)		
> 25	48 (25.95%)	31 (28.18%)	17 (22.67%)		
Menopausal Status					
Yes	134 (72.43%)	78 (70.91%)	56 (74.67%)	0.315	0.574
No	51 (27.57%)	32 (29.09%)	19 (25.33%)		
Hypertension					
Yes	61 (32.97%)	37 (33.64%)	24 (32.00%)	0.054	0.816
No	124 (67.03%)	73 (66.36%)	51 (68.00%)		
Diabetes					
Yes	41 (22.16%)	26 (23.64%)	15 (20.00%)	0.342	0.559
No	144 (77.84%)	84 (76.36%)	60 (80.00%)		
Heart attack					
Yes	34 (18.38%)	21 (19.09%)	13 (17.33%)	0.092	0.762
No	151 (81.62%)	89 (80.91%)	62 (82.67%)		
Smoking History					
Yes	52 (28.11%)	32 (29.09%)	20 (26.67%)	0.130	0.719
No	133 (71.89%)	78 (70.91%)	55 (73.33%)		
Alcohol History					
Yes	11 (5.95%)	6 (5.45%)	5 (6.67%)	0.117	0.732
No	174 (94.05%)	104 (94.55%)	70 (93.33%)		

Note: BMI, Body Mass Index; EC, Endometrial carcinoma.

and 0.735, respectively (**Figure 2C**). Notably, SF-1 methylation exhibited superior diagnostic performance.

Correlation between PAX1 and SF-1 methylation and clinical data in EC patients

PAX1 methylation was significantly correlated with differentiation grade (P = 0.001), FIGO stage (P < 0.001), myometrial invasion depth (P = 0.030), but was not significantly correlated with cervical stromal involvement (P = 0.057). Specifically, 75.61% of patients with well-differentiated (G1) tumors exhibited PAX1 methylation, whereas PAX1 positivity was lower in poorly differentiated (G3) tumors. Higher PAX1 methylation rates were observed in FIGO stage I-II patients, suggesting a potential association with early-stage EC (**Table 3**).

For SF-1 methylation, significant associations were found with FIGO stage (P = 0.046), myometrial invasion depth (P = 0.025), and history of alcohol consumption (P = 0.011). SF-1 methylation was more prevalent in FIGO stage I-II patients (92.31%) and in those with superficial myometrial invasion (< 1/2). Additionally, patients with a history of alcohol consumption showed higher SF-1 methylation rates (**Table 4**).

Correlation between methylation of PAX1 and SF-1 and tumor markers

PAX1 methylation was significantly positively correlated with CA-125 (P = 0.020) but showed no significant association with HE4 or CA-199 (P > 0.05). In contrast, SF-1 methylation was significantly negatively correlated with CA-125

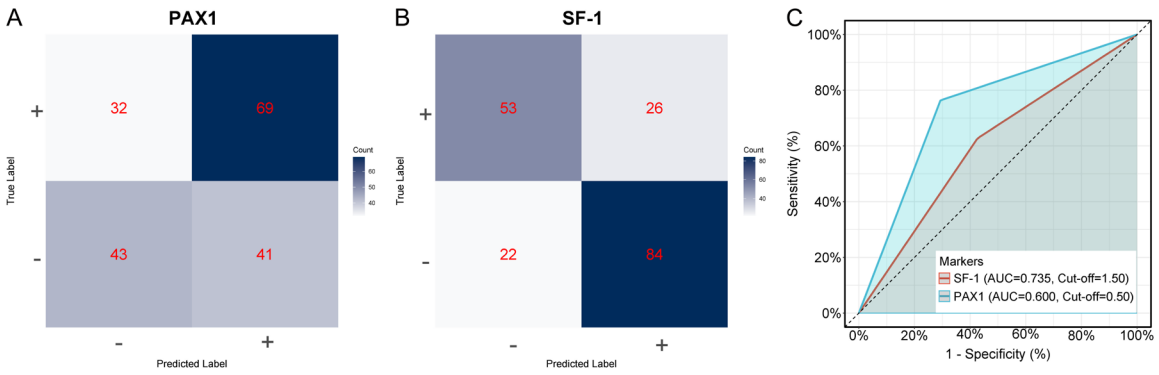

Figure 2. Methylation status of PAX1 and SF-1 and their diagnostic value in EC patients. A. Methylation prediction of PAX1 in EC (n = 110) and non-EC (n = 75) patients. B. Methylation prediction of SF-1 in EC (n = 110) and non-EC (n = 75) patients. C. ROC curve for the combined diagnostic performance of PAX1 and SF-1 methylation, comparing their diagnostic accuracy. Note: PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1; EC, Endometrial Carcinoma.

(P = 0.015) and CA-199 (P = 0.001) but not with HE4 (P > 0.05) (**Figure 3**).

Survival and recurrence in EC patients with PAX1 and SF-1 methylation

The follow-up cutoff date was January 2025, with all patients followed for an average OS of 44.8 months and PFS of 38.5 months. During follow-up, 25 patients died, and 33 experienced disease progression. Kaplan-Meier survival analysis revealed that PAX1 methylation-positive patients had significantly shorter OS compared to methylation-negative patients (P = 0.002) (**Figure 4A**). In contrast, no significant difference in OS was observed between SF-1 methylation-positive and -negative patients (P = 0.090) (**Figure 4B**).

CIF analysis showed that PAX1 methylation-positive patients had a significantly higher recurrence rate than methylation-negative patients (P < 0.001) (**Figure 4C**). Similarly, SF-1 methylation-positive patients exhibited a higher recurrence rate compared to methylation-negative patients (P = 0.017) (**Figure 4D**). These findings indicate that PAX1 and SF-1 methylation are associated with survival and recurrence in EC, with PAX1 methylation potentially serving as a key prognostic marker.

Cox regression analysis for factors affecting OS in EC patients

Univariate Cox regression analysis identified several factors significantly associated with OS, including differentiation grade (G2: HR = 4.065, P = 0.009; G3: HR = 12.888, P < 0.001), FIGO stage (III+IV: HR = 12.212, P < 0.001), myometrial invasion depth (< 1/2: HR = 0.335, P = 0.013), cervical stromal involvement (HR = 0.198, P < 0.001), lymph node metastasis (HR = 0.055, P < 0.001), and PAX1 methylation positivity (HR = 0.186, P = 0.006). In contrast, SF-1 methylation was not significantly associated with OS (HR = 2.716, P = 0.104) (**Table 5**).

Multivariate analysis confirmed that FIGO stage (III-IV: HR = 5.037, P = 0.004), cervical stromal involvement (HR = 0.279, P = 0.011), and lymph node metastasis (HR = 0.194, P = 0.004) were independent prognostic factors for OS. In contrast, differentiation grade (G2: HR = 2.342, P = 0.126; G3: HR = 1.962, P = 0.317) and PAX1 methylation positivity were not statistically significant (HR = 0.566, P = 0.401) (**Table 6**).

Cox regression analysis for factors affecting PFS in EC patients

Univariate Cox regression analysis identified factors significantly associated with PFS, including differentiation grade (G2: HR = 5.691, P < 0.001; G3: HR = 13.607, P < 0.001), FIGO stage (III+IV: HR = 9.371, P < 0.001), myometrial invasion depth (< 1/2: HR = 0.478, P = 0.040), cervical stromal involvement (HR = 0.267, P = 0.001), lymph node metastasis (HR = 0.069, P < 0.001), PAX1 methylation positivity (HR = 0.129, P = 0.001), and SF-1 methylation positivity (HR = 3.844, P = 0.026) (**Table 7**).

Multivariate analysis confirmed that differentiation grade (G2: HR = 3.490, P = 0.010), FIGO

Table 3. Association between PAX1 methylation and clinicopathological characteristics in EC patients

Index	Total	PAX1		χ^2	*P*-value
		Positive (n = 69)	Negative (n = 41)		
Age (Year)					
≥ 60	58 (52.73%)	37 (53.62%)	21 (51.22%)	0.060	0.807
< 60	52 (47.27%)	32 (46.38%)	20 (48.78%)		
Menarche Age (Year)					
≥ 14	30 (27.27%)	20 (28.99%)	10 (24.39%)	0.274	0.601
< 14	80 (72.73%)	49 (71.01%)	31 (75.61%)		
BMI (kg/m^2)					
< 23	78 (70.91%)	47 (68.12%)	31 (75.61%)	0.700	0.403
23-25	32 (29.09%)	22 (31.88%)	10 (24.39%)		
> 25	31 (28.18%)	17 (24.64%)	14 (34.15%)		
Menopausal Status					
Yes	48 (43.64%)	32 (46.38%)	16 (39.02%)	1.186	0.553
No	31 (28.18%)	20 (28.99%)	11 (26.83%)		
Hypertension					
Yes	37 (33.64%)	22 (31.88%)	15 (36.59%)	0.255	0.614
No	73 (66.36%)	47 (68.12%)	26 (63.41%)		
Diabetes					
Yes	41 (37.27%)	27 (39.13%)	14 (34.15%)	0.273	0.601
No	69 (62.73%)	42 (60.87%)	27 (65.85%)		
Heart attack					
Yes	21 (19.09%)	12 (17.39%)	9 (21.95%)	0.346	0.556
No	89 (80.91%)	57 (82.61%)	32 (78.05%)		
Smoking History					
Yes	32 (29.09%)	23 (33.33%)	9 (21.95%)	1.615	0.204
No	78 (70.91%)	46 (66.67%)	32 (78.05%)		
Alcohol History					
Yes	6 (5.45%)	2 (2.90%)	4 (9.76%)	2.345	0.126
No	104 (94.55%)	67 (97.10%)	37 (90.24%)		
Differentiation					
G1	59 (53.64%)	28 (40.58%)	31 (75.61%)	13.049	0.001
G2	36 (32.73%)	28 (40.58%)	8 (19.51%)		
G3	15 (13.64%)	13 (18.84%)	2 (4.88%)		
FIGO Substage					
I+II	86 (78.18%)	47 (68.12%)	39 (95.12%)	10.996	< 0.001
III+IV	24 (21.82%)	22 (31.88%)	2 (4.88%)		
Myometrial Invasion Depth					
≥ 1/2	55 (50.00%)	40 (57.97%)	15 (36.59%)	4.705	0.030
< 1/2	55 (50.00%)	29 (42.03%)	26 (63.41%)		
Cervical Stromal Involvement					
Yes	14 (12.73%)	12 (17.39%)	2 (4.88%)	3.625	0.057
No	96 (87.27%)	57 (82.61%)	39 (95.12%)		
Lymph Node Metastasis					
Yes	6 (5.45%)	6 (8.70%)	0 (0.00%)	3.771	0.052
No	104 (94.55%)	63 (91.30%)	41 (100.00%)		

Note: PAX1, Paired Box Gene 1; BMI, Body Mass Index; FIGO, International Federation of Gynecology and Obstetrics; EC, Endometrial Carcinoma.

Table 4. Relationship between SF-1 methylation and clinical data in EC patients

Index	Total	SF-1		χ^2	*P*-value
		Positive (n = 26)	Negative (n = 84)		
Age (Year)					
≥ 60	58 (52.73%)	12 (46.15%)	46 (54.76%)	0.590	0.442
< 60	52 (47.27%)	14 (53.85%)	38 (45.24%)		
Menarche Age (Year)					
≥ 14	30 (27.27%)	8 (30.77%)	22 (26.19%)	0.210	0.647
< 14	80 (72.73%)	18 (69.23%)	62 (73.81%)		
BMI (kg/m^2)					
< 23	78 (70.91%)	17 (65.38%)	61 (72.62%)	0.504	0.478
23-25	32 (29.09%)	9 (34.62%)	23 (27.38%)		
> 25	31 (28.18%)	8 (30.77%)	23 (27.38%)		
Menopausal Status					
Yes	48 (43.64%)	11 (42.31%)	37 (44.05%)	0.114	0.945
No	31 (28.18%)	7 (26.92%)	24 (28.57%)		
Hypertension					
Yes	37 (33.64%)	11 (42.31%)	26 (30.95%)	1.147	0.284
No	73 (66.36%)	15 (57.69%)	58 (69.05%)		
Diabetes					
Yes	41 (37.27%)	8 (30.77%)	33 (39.29%)	0.616	0.433
No	69 (62.73%)	18 (69.23%)	51 (60.71%)		
Heart attack					
Yes	21 (19.09%)	4 (15.38%)	17 (20.24%)	0.303	0.582
No	89 (80.91%)	22 (84.62%)	67 (79.76%)		
Smoking History					
Yes	32 (29.09%)	8 (30.77%)	24 (28.57%)	0.046	0.829
No	78 (70.91%)	18 (69.23%)	60 (71.43%)		
Alcohol History					
Yes	6 (5.45%)	4 (15.38%)	2 (2.38%)	6.510	0.011
No	104 (94.55%)	22 (84.62%)	82 (97.62%)		
Differentiation					
G1	59 (53.64%)	16 (61.54%)	43 (51.19%)	1.318	0.517
G2	36 (32.73%)	8 (30.77%)	28 (33.33%)		
G3	15 (13.64%)	2 (7.69%)	13 (15.48%)		
FIGO Substage					
I+II	86 (78.18%)	24 (92.31%)	62 (73.81%)	3.983	0.046
III+IV	24 (21.82%)	2 (7.69%)	22 (26.19%)		
Myometrial Invasion Depth					
≥ 1/2	55 (50.00%)	8 (30.77%)	47 (55.95%)	5.037	0.025
< 1/2	55 (50.00%)	18 (69.23%)	37 (44.05%)		
Cervical Stromal Involvement					
Yes	14 (12.73%)	1 (3.85%)	13 (15.48%)	2.418	0.120
No	96 (87.27%)	25 (96.15%)	71 (84.52%)		
Lymph Node Metastasis					
Yes	6 (5.45%)	0 (0.00%)	6 (7.14%)	1.964	0.161
No	104 (94.55%)	26 (100.00%)	78 (92.86%)		

Note: PAX1, Paired Box Gene 1; BMI, Body Mass Index; FIGO, International Federation of Gynecology and Obstetrics; EC, Endometrial Carcinoma.

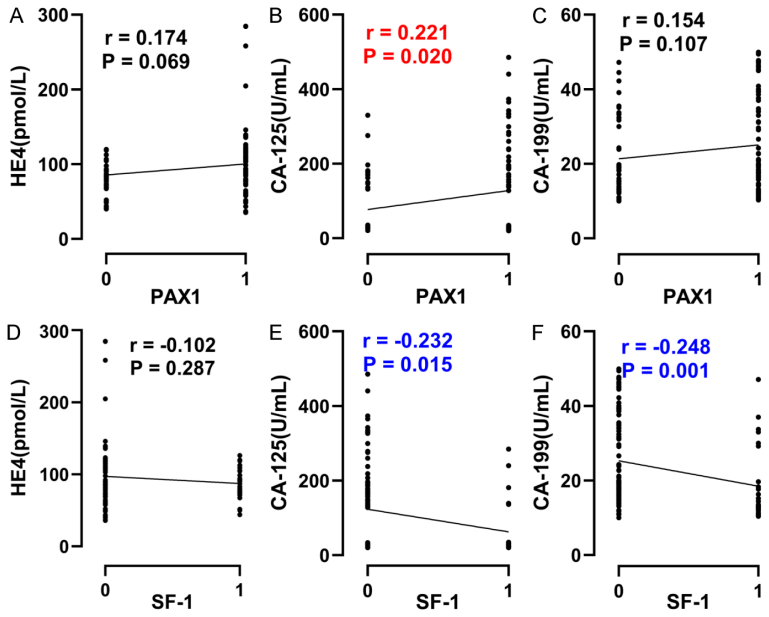

Figure 3. Correlation analysis between methylation of PAX1 and SF-1 and tumor markers. A. Correlation between PAX1 methylation and HE4 (n = 110). B. Correlation between PAX1 methylation and CA-125 (n = 110). C. Correlation between PAX1 methylation and CA-199 (n = 110). D. Correlation between SF-1 methylation and HE4 (n = 110). E. Correlation between SF-1 methylation and CA-125 (n = 110). F. Correlation between SF-1 methylation and CA-199 (n = 110). Note: PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1; HE4, Human Epididymis Protein 4; CA-125, Cancer Antigen 125; CA-199, Cancer Antigen 199.

stage (III+IV: HR = 4.219, P = 0.003), cervical stromal involvement (HR = 0.343, P = 0.022), lymph node metastasis (HR = 0.346, P = 0.056), and PAX1 methylation positivity (HR = 0.290, P = 0.049) were independent prognostic factors for PFS (**Table 8**).

Analysis of the correlation and interaction between PAX1 and SF-1

Although a negative correlation was observed between PAX1 and SF-1 methylation, Cox regression models showed that their interaction was not statistically significant for OS (P = 0.758) or PFS (P = 0.574). These results suggest that the interaction between PAX1 and SF-1 methylation had a limited impact on survival outcomes in this cohort (**Figure 5**; **Table 9**).

Discussion

EC ranks among the most common malignancies of the female reproductive system, with its incidence steadily increasing in recent years. Early diagnosis is pivotal for enhancing patient survival rates [18, 19]. However, the lack of robust screening tools and biomarkers frequently results in advanced-stage diagnoses, which complicates treatment and prognostic assessment [20]. Consequently, identifying novel biomarkers, particularly epigenetic markers, is of vital clinical importance for early detection and prognosis evaluation.

This study establishes a strong correlation between elevated PAX1 gene methylation and adverse clinical features, including poor differentiation, advanced FIGO stage, and deep myometrial invasion in EC patients. Moreover, patients with positive PAX1 methylation demonstrate significantly worse OS and higher recurrence rates compared to those with negative methylation. These results are consistent with Liu et al. [21], who demonstrated that methylation-mediated silencing of PAX1 expression promotes malignant transformation by impairing its tumor-suppressive function. Mechanistically, PAX1 methylation-induced silencing may propel tumor progression through diverse pathways. For example, Zhang et al. [22] revealed that PAX1 suppresses cervical cancer cell proliferation and migration by inhibiting the WNT/TIMELESS pathway, and CRISPR-mediated demethylation restores its expression, thereby enhancing chemotherapy sensitivity. Likewise, Su et al. [23] reported that PAX1 activates DUSP phosphatases (e.g., DUSP1/5/6) to inhibit the EGF/MAPK signaling pathway, while its methylation-driven inactivation triggers aberrant kinase signaling, accelerating cell cycle progression. Cross-cancer studies further underscore the universal significance of PAX1 epigenetic regulation. Huang et al. [24] observed PAX1 methylation in 100% of esophageal squamous cell carcinoma cases, with a strong association with tumor invasiveness, highlighting its potential as a pan-cancer biomarker. Clinically, PAX1 methylation testing exhibits remarkable diag-

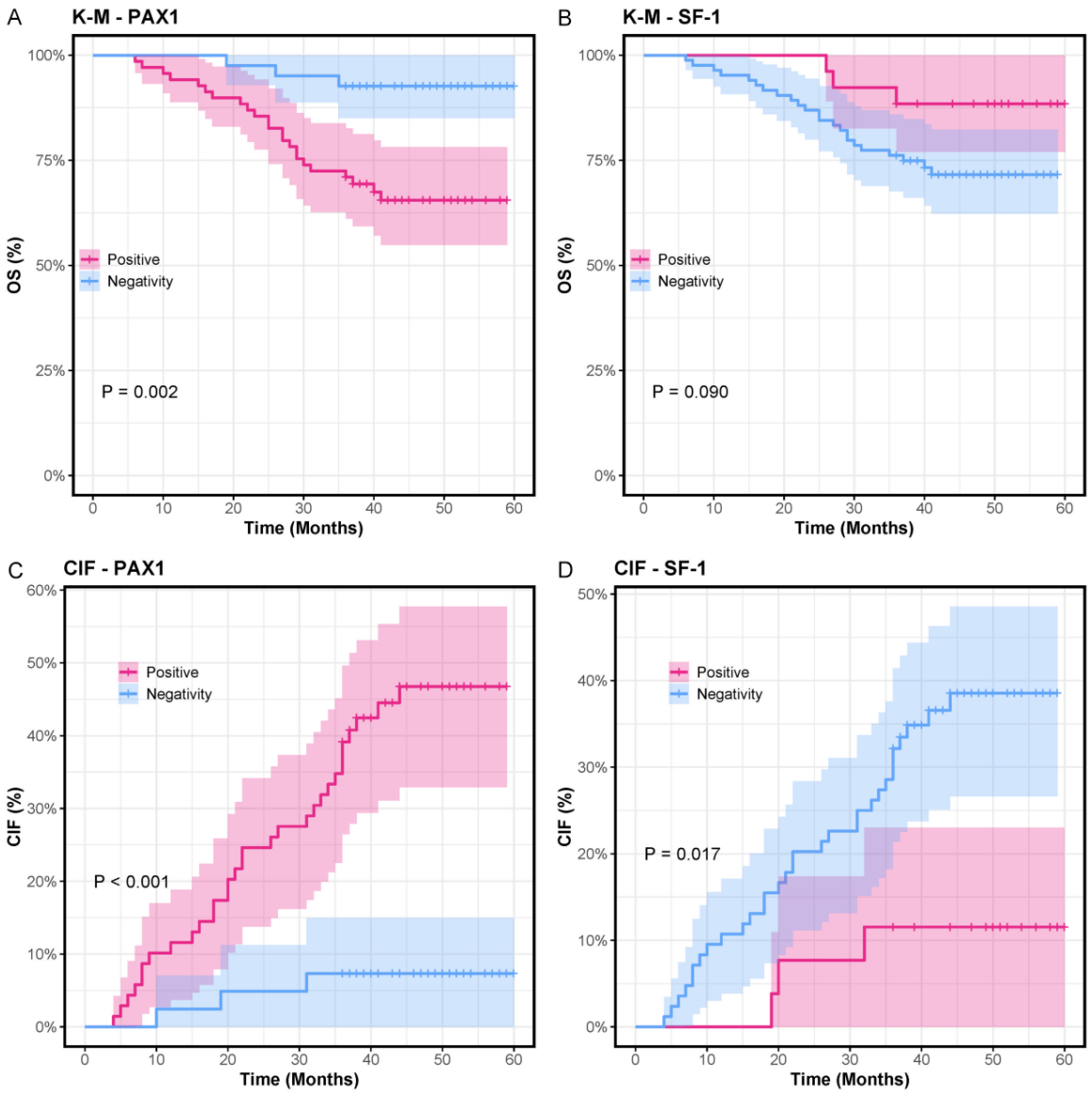

Figure 4. Role of PAX1 and SF-1 in EC patient survival and recurrence. A. Kaplan-Meier curve of OS stratified by PAX1 methylation status. B. Kaplan-Meier curve of OS stratified by SF-1 methylation status. C. CIF curve for recurrence according to PAX1 methylation status. D. CIF curve for recurrence according to SF-1 methylation status. Note: PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1; OS, Overall Survival; CIF, Cumulative Incidence Function.

nostic accuracy. Li et al. [25] reported a specificity of 95.36% for detecting high-grade cervical lesions, significantly outperforming traditional cytology (24.8%). Furthermore, He et al. [26] demonstrated that combining PAX1 and SEPT9 testing yielded an AUC of 0.86, providing a novel, non-invasive screening approach for EC.

Conversely, SF-1 displays markedly reduced methylation in EC, with lower methylation levels strongly associated with FIGO stage, myometrial invasion depth, and recurrence risk. This finding is corroborated by Huang et al. [25], who noted a significantly lower SF-1 promoter methylation rate in EC tissue (8.2%) compared to adjacent normal tissue (40.9%). Treatment with the demethylating agent 5-Aza-CdR upregulated SF-1 expression, promoting tumor cell proliferation. Mechanistically, SF-1 drives EC progression by regulating estrogen synthesis enzymes and inflammatory pathways [27]. Environmental epigenetic factors also influence SF-1 regulation. Chen et al. [28] found

Table 5. Univariate Cox regression analysis of factors associated with OS

Index	Beta	Std Err	*P* Value	HR	Lower	Upper
Age (Year)						
≥ 60						
< 60	0.271	0.393	0.492	1.311	0.606	2.834
Menarche Age (Year)						
≥ 14						
< 14	-0.138	0.425	0.746	0.871	0.379	2.004
BMI (kg/m^2)						
< 23						
23-25	0.464	0.532	0.383	1.591	0.560	4.517
> 25	0.666	0.558	0.232	1.947	0.652	5.813
Menopausal Status						
Yes						
No	0.450	0.403	0.265	1.568	0.711	3.456
Hypertension						
Yes						
No	-0.020	0.412	0.960	0.980	0.437	2.198
Diabetes						
Yes						
No	0.141	0.412	0.732	1.152	0.513	2.585
Heart attack						
Yes						
No	-0.082	0.498	0.870	0.922	0.347	2.445
Smoking History						
Yes						
No	0.636	0.498	0.201	1.889	0.712	5.010
Alcohol History						
Yes						
No	-1.133	0.616	0.066	0.322	0.096	1.077
Differentiation						
G1						
G2	1.403	0.540	0.009	4.065	1.412	11.706
G3	2.556	0.551	< 0.001	12.888	4.381	37.916
FIGO Substage						
I+II						
III+IV	2.502	0.417	< 0.001	12.212	5.393	27.656
Myometrial Invasion Depth						
≥ 1/2						
< 1/2	-1.093	0.442	0.013	0.335	0.141	0.797
Cervical Stromal Involvement						
Yes						
No	-1.621	0.415	< 0.001	0.198	0.088	0.446
Lymph Node Metastasis						
Yes						
No	-2.909	0.481	< 0.001	0.055	0.021	0.140
PAX1						
Positive						
Negative	-1.683	0.614	0.006	0.186	0.056	0.620

SF-1						
Positive						
Negative	0.999	0.614	0.104	2.716	0.815	9.052

Note: OS, Overall Survival; BMI, Body Mass Index; FIGO, International Federation of Gynecology and Obstetrics; PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1.

Table 6. Multivariate Cox regression analysis of factors associated with OS

Index	Beta	Std Err	*P* Value	HR	Lower	Upper
Differentiation						
G1						
G2	0.851	0.556	0.126	2.342	0.787	6.967
G3	0.674	0.674	0.317	1.962	0.524	7.353
FIGO Substage						
I-II						
III-IV	1.617	0.563	0.004	5.037	1.671	15.183
Myometrial Invasion Depth						
≥ 1/2						
< 1/2	0.123	0.534	0.817	1.131	0.397	3.221
Cervical Stromal Involvement						
Yes						
No	-1.275	0.504	0.011	0.279	0.104	0.751
Lymph Node Metastasis						
Yes						
No	-1.639	0.574	0.004	0.194	0.063	0.599
PAX1						
Positive						
Negative	-0.570	0.678	0.401	0.566	0.150	2.135

Note: FIGO, International Federation of Gynecology and Obstetrics; PAX1, Paired Box Gene 1.

that copper exposure disrupted steroid metabolism in ovarian granulosa cells by reducing SF-1 promoter methylation, suggesting that toxic exposures may increase EC risk through epigenetic reprogramming. Notably, SF-1 methylation patterns are disease-specific. Xue et al. [29] reported SF-1 upregulation in endometriosis due to elevated intronic methylation, which contrasts with low promoter methylation in EC, highlighting the complexity of methylation site-specific gene regulation. Additionally, this study identified a significant negative correlation between SF-1 methylation and CA-125/CA-199 levels, suggesting that SF-1 may modulate EC's biological behavior by modulating the tumor microenvironment or metabolic pathways [30]. Intriguingly, a potential association between alcohol consumption history and SF-1 methylation was observed, indicating that environmental factors may influence EC occurrence or progression via epigenetic mechanisms. Alcohol may alter SF-1 promoter methylation by modulating DNA methyltransferase activity or inducing oxidative stress [31]. Furthermore, alcohol may disrupt estrogen metabolism, indirectly affecting SF-1 expression in EC. Future studies should collect detailed alcohol exposure data and validate alcohol's direct impact on SF-1 methylation through in vitro or in vivo experiments to clarify its role in EC pathogenesis.

The methylation profiles of PAX1 and SF-1 offer complementary clinical utility in EC. High PAX1 methylation serves as an independent predictor of poor prognosis, while low SF-1 methylation is associated with hormone-driven tumor growth. Combined testing of both markers could enhance diagnostic sensitivity and prognostic stratification accuracy. Gao et al. [32] developed a PAX1/SOX1 methylation model (AUC = 0.946), which significantly reduced unnecessary invasive procedures (e.g., cervical

Table 7. Univariate Cox regression analysis of factors associated with PFS

Index	Beta	Std Err	*P* Value	HR	Lower	Upper
Age (Year)						
≥ 60						
< 60	-0.017	0.344	0.960	0.983	0.501	1.928
Menarche Age (Year)						
≥ 14						
< 14	-0.090	0.377	0.810	0.914	0.437	1.911
BMI (kg/m^2)						
< 23						
23-25	0.433	0.453	0.340	1.541	0.634	3.747
> 25	0.561	0.484	0.246	1.753	0.679	4.524
Menopausal Status						
Yes						
No	0.340	0.359	0.343	1.405	0.695	2.840
Hypertension						
Yes						
No	0.242	0.376	0.519	1.274	0.609	2.665
Diabetes						
Yes						
No	0.250	0.367	0.495	1.284	0.626	2.635
Heart attack						
Yes						
No	-0.158	0.424	0.710	0.854	0.372	1.962
Smoking History						
Yes						
No	0.764	0.450	0.089	2.148	0.889	5.189
Alcohol History						
Yes						
No	-0.851	0.606	0.160	0.427	0.130	1.401
Differentiation						
G1						
G2	1.739	0.476	0.000	5.691	2.241	14.453
G3	2.611	0.512	0.000	13.607	4.983	37.152
FIGO Substage						
I+II						
III+IV	2.238	0.352	0.000	9.371	4.703	18.674
Myometrial Invasion Depth						
≥ 1/2						
< 1/2	-0.737	0.359	0.040	0.478	0.237	0.967
Cervical Stromal Involvement						
Yes						
No	-1.320	0.391	0.001	0.267	0.124	0.575
Lymph Node Metastasis						
Yes						
No	-2.672	0.472	0.000	0.069	0.027	0.174
PAX1						
Positive						
Negative	-2.051	0.605	0.001	0.129	0.039	0.421

SF-1						
Positive						
Negative	1.346	0.605	0.026	3.844	1.173	12.590

Note: BMI, Body Mass Index; FIGO, International Federation of Gynecology and Obstetrics; PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1.

Table 8. Multifactor Cox regression analysis of factors associated with PFS

Index	Beta	Std Err	*P* Value	HR	Lower	Upper
Differentiation						
G1						
G2	1.250	0.487	0.010	3.490	1.345	9.060
G3	1.165	0.637	0.067	3.206	0.921	11.167
FIGO Substage						
I+II						
III+IV	1.440	0.481	0.003	4.219	1.642	10.841
Myometrial Invasion Depth						
≥ 1/2						
< 1/2	0.574	0.455	0.207	1.776	0.728	4.330
Cervical Stromal Involvement						
Yes						
No	-1.070	0.466	0.022	0.343	0.137	0.856
Lymph Node Metastasis						
Yes						
No	-1.061	0.556	0.056	0.346	0.116	1.028
PAX1						
Positive						
Negative	-1.236	0.631	0.049	0.290	0.084	1.000
SF-1						
Positive						
Negative	1.071	0.652	0.100	2.920	0.813	10.483

Note: FIGO, International Federation of Gynecology and Obstetrics; PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1.

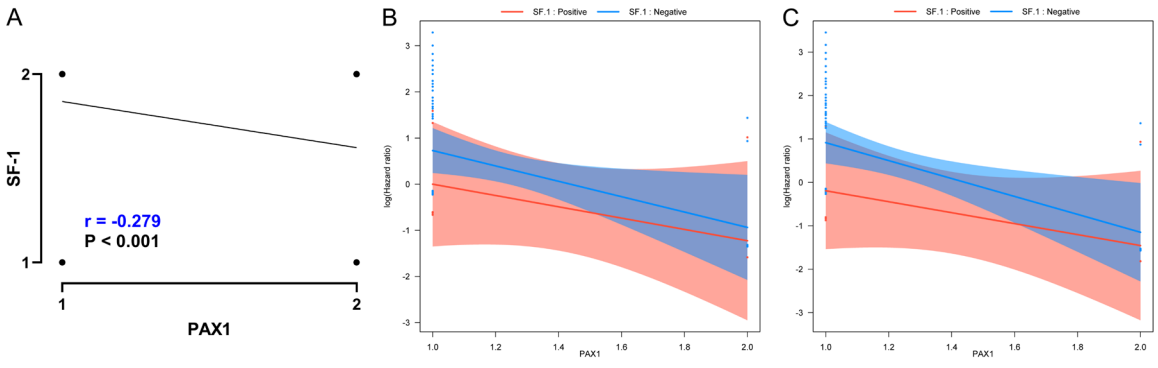

Figure 5. Correlation of PAX1 and SF-1 and interaction. A. Correlation of PAX1 and SF-1 (1 positive, 2 negative). B. PAX1 and SF-1 in OS. C. Interaction of PAX1 and SF-1 in PFS. Note: PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1; OS, Overall Survival; PFS, Progression-Free Survival.

scraping) in HPV-positive patients, providing a blueprint for precision EC management. Therapeutically, dynamic monitoring of PAX1 methylation shows predictive potential. Li et al. [33]

Table 9. Interaction analysis of PAX1 with SF-1 in OS with PFS

Index	OS					PFS				
	Coef	Exp	Se	Z-Value	*P*-Value	Coef	Exp	Se	Z-Value	*P*-Value
PAX1:SF.1	-0.4407	0.6436	1.4312	-0.308	0.758	-0.8017	0.4486	1.4265	-0.562	0.574

Note: PAX1, Paired Box Gene 1; SF-1, Steroidogenic Factor 1.

reported that patients with low PAX1 methylation responded poorly to concurrent chemoradiotherapy, whereas increased methylation during treatment were associated with complete remission (AUC = 0.84), suggesting its utility as a marker for therapeutic efficacy. Cross-cancer studies further validate the broad applicability of epigenetic markers. Aberrant PAX1 methylation in esophageal squamous cell carcinoma [24] and SF-1 methylation in ovarian cancer [34] support their potential clinical relevance across malignancies. Future research should investigate interplay between PAX1/SF-1 and other epigenetic factors and harness CRISPR-based epigenetic editing to develop targeted demethylation therapies, advancing personalized diagnosis and treatment for EC.

Survival analysis in this study elucidated the distinct prognostic impacts of PAX1 and SF-1 methylation in EC. Univariate Cox regression revealed that PAX1 methylation-positive patients had significantly reduced progression-free survival (HR = 0.129, P = 0.001), while multivariate analysis confirmed PAX1 methylation as an independent prognostic factor (HR = 0.290, P = 0.049). These findings indicate that high PAX1 methylation reliably predicts shorter PFS, likely due to the transcriptional silencing of its tumor-suppressive activity. In contrast, while SF-1 methylation was associated with increased PFS risk in univariate analysis (HR = 3.844, P = 0.026), it did not retain independent prognostic significance in the multivariate model (P = 0.100). This indicates that SF-1 methylation's prognostic impact may be modulated by other clinical or molecular factors, reflecting a more complex regulatory mechanism compared to PAX1. Further research is warranted to clarify the precise role of SF-1 methylation in EC pathogenesis and progression.

Despite providing preliminary evidence for the clinical utility of PAX1 and SF-1 methylation in EC, this study has several limitations. First, the small sample size and single-center design may limit the generalizability of the findings. Therefore, multi-center, large-scale studies are essential to validate reliability of these markers. Second, the study relied on methylation-specific PCR and qRT-PCR, which, although standard techniques, may be influenced by sample handling and experimental conditions, potentially affecting sensitivity and specificity. Future studies should consider using high-throughput sequencing methods for more precise and comprehensive methylation profiling. Third, the study did not evaluate PAX1 and SF-1 mRNA or protein expression levels, leaving the relationship between methylation and gene expression unclear. This warrants further investigation. Finally, while PAX1 and SF-1 methylation correlated with clinical features, their exact roles in tumorigenesis remain incompletely understood. Functional studies, such as gene knockout and overexpression experiments, are needed to deepen mechanistic insights and strengthen the theoretical foundation for their clinical applications.

Conclusion

The methylation statuses of PAX1 and SF-1 are intricately linked to the onset, progression, and prognosis of endometrial carcinoma, with PAX1 methylation emerging as a particularly promising biomarker for enhancing EC diagnosis and prognostic assessment.

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Disclosure of conflict of interest

None.

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