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Association of CYP7B1 expression with the prognosis of endometrial cancer: a retrospective study

Xiao-Fang Lu[1†], Tao Huang[2†], Chang Chen[3], Jing Zhang[1], Xu-Yong Fu[1], Bo Cheng[4], Ya-Yan Zhou[5], Jia Lei[2*] and Da-Lin Lu[1*]

Abstract

Background Endometrial cancer (EC) tissues express CYP7B1, but its association with prognosis needs to be investigated.

Methods Immunohistochemistry and image analysis software were used to assess CYP7B1 protein expression in paraffin-embedded endometrial tumor sections. Associations between CYP7B1 and clinical factors were tested with the Wilcoxon rank-sum test. Kaplan-Meier curves were employed to describe survival, and differences were assessed using the log-rank test. Cox regression analysis was used to assess the association between CYP7B1 expression and the prognosis of patients with EC.

Results A total of 307 patients were enrolled with an average age of 52.6 ± 8.0 years at diagnosis. During the period of follow-up, 46 patients (15.0%) died, and 29 (9.4%) suffered recurrence. The expression of CYP7B1 protein is significantly higher in the cytoplasm than in the nucleus ($P < 0.001$). Patients aged < 55 years ($P = 0.040$), ER-positive patients ($P = 0.028$) and PR-positive patients ($P < 0.001$) report higher levels of CYP7B1 protein. Both univariate (HR = 0.41, 95% CI: 0.18–0.90, $P = 0.025$) and multivariate (HR = 0.35, 95%CI:0.16–0.79, $P = 0.011$) Cox regression analyses demonstrate that high CYP7B1 protein expression predicts longer overall survival (OS). When considering only ER-positive patients ($n = 265$), CYP7B1 protein expression is more strongly associated with OS (HR = 0.20,95%CI:0.08–0.52, $P = 0.001$). The 3-year OS and 5-year OS in the low-CYP7B1 subgroup are 81.6% and 76.8%, respectively; while in the high-CYP7B1 subgroup are 93.0% and 92.0%, respectively ($P = 0.021$).

Conclusions High CYP7B1 protein expression predicted longer OS, suggesting that it may serve as an important molecular marker for EC prognosis.

Keywords Endometrial cancer, Estrogen receptor, CYP7B1, Overall survival

†Xiao-Fang Lu and Tao Huang contributed equally to this work.

*Correspondence:
Jia Lei
leijia33333@sina.com
Da-Lin Lu
dalinlu@jnu.edu.cn
[1]Department of Epidemiology, School of Medicine, Jinan University, No. 601, Huangpu Avenue West, Tianhe District, Guangzhou 510632, China
[2]Department of Gynecology, Wuzhou Red Cross Hospital, No. 3-1, Xinxing 1st Road, Wanxiu District, Wuzhou 543002, China
[3]Department of Pathology, Wuzhou Red Cross Hospital, Wuzhou, China
[4]Department of Pathology, Chinese People's Liberation Army Rocket Force Characteristic Medical Center, Beijing, China
[5]Department of Radiation Oncology, Shenzhen People's Hospital (The Second Clinical Medical College, Jinan University; The First Affiliated Hospital, Southern University of Science and Technology), Shenzhen, China

Background

Endometrial cancer (EC) is one of the most prevalent gynecological malignancies [1]. Immunotherapy, particularly immune checkpoint inhibitors, has made significant advances in the treatment of EC [2, 3]. The incidence of EC is on the rise with the increasing rate of obesity [4]. Studies have discovered a positive correlation between the cholesterol intake and the risk of EC [5, 6]. Notably, obesity promotes the synthesis of cholesterol in the body [7–11]. As a major metabolite of cholesterol, 27-Hydroxycholesterol (27HC) is the most abundant circulating oxysterol, and its plasma levels are strongly linked to cholesterol levels [12]. It was shown that 27HC, the first endogenous selective estrogen receptor modulator (SERM) to be discovered, increases the risk of EC development and progression by stimulating epithelial cell proliferation through an estrogen receptor (ER) dependent pathway [13, 14].

Two crucial enzymes determine the content of 27HC: cytochrome P450 oxidase (CYP27A1), which catalyzes the synthesis, and oxysterol 7α-hydroxylase (CYP7B1), which is accountable for breakdown [15, 16]. CYP7B1 contains 6 exons and 5 introns, with the gene's coding length being a minimum of 65 kb [17]. Compared to $CYP7B1^{+/+}$ mice, $CYP7B1^{-/-}$ mice had a significantly shorter tumor latency [18]. Reduced CYP7B1 expression in ER-positive breast tumors resulted in the accumulation of 27HC in tumors, which was linked to lower overall survival (OS) [19]. High CYP7B1 expression was correlated with longer progression-free survival in patients with ovarian cancer [20]. These studies indicate that CYP7B1 serves as an important biomarker for cancer progression and survival. Despite evidence in other types of cancer, the association between CYP7B1 and EC has not been established.

Therefore, we aimed to evaluate the prognostic value of CYP7B1 protein in patients with EC.

Materials and methods

Patients

Patients diagnosed with EC at Wuzhou Red Cross Hospital between January 2009 and December 2020 were retrospectively studied. The Ethics Committee of Jinan University and Wuzhou Red Cross Hospital have approved this work (Ethics approval number: LL2022-160). This study was based on the patient's prior written informed consent. The inclusion criteria were: (1) patients with initial surgery; (2) patients older than 18 years; (3) patients with primary EC. The exclusion criteria were as follows: (1) failed to undergo surgery; (2) patients with other malignancies; (3) patients diagnosed with endometrial carcinosarcoma; (4) unavailable tumor tissue sections; (5) lost to follow-up.

Follow up

Patients were followed up by reviewing medical records and conducting telephone interviews. Data were collected until death or September 2022. OS was defined as the period from the surgery to either the time of death or the time of the last follow-up. Recurrence-free survival (RFS) was defined as the duration between surgery and either recurrence or the last follow-up.

Immunohistochemistry

The Department of Pathology provided the paraffin-embedded sections of EC tissues needed for this study. The tissue sections underwent immunohistochemical staining before the quantitative measurement of CYP7B1. Briefly, paraffin sections were first dewaxed and hydrated. Sections were subjected to microwave antigen repair with sodium citrate retrieval solution at pH 6.0, cooled and then treated with 3% H_2O_2 for 10 min to block endogenous peroxidase activity. Next, sections were incubated with rabbit anti-human CYP7B1 antibody (primary antibody) at 4 °C for 17 h and with the goat anti-rabbit lgG antibody (secondary antibody) at 37 °C for 20 min (all purchase from Yong Jin Biotech). 3,3'-diaminobenzidine (DAB) was used for color development and hematoxylin was used for redyeing. Finally, the sections were sealed with neutral balsam.

Detection of CYP7B1 expression via image analysis

Positive expression was determined by the presence of brown-yellow granules in the nucleus/cytoplasm of EC. All sections were photographed using a microscope under the same conditions. All photographs were processed and analyzed using image analysis software Image-Pro Plus 7.2. Measurement data for relevant indicators was obtained for statistical analysis. Briefly, new images were added to the software, and an optical density correction was performed. The desired depiction tool was used to select the measurement area and the color parameters were adjusted. The measurement indicators to be selected included the integral optical density (IOD) and the total area of the measurement area (Area). The software was run to obtain the raw data of the measurement indicators. The IOD/Area ratio, which is the average optical density (AOD), was calculated. The AOD was then statistically analyzed.

Statistical analyses

All immunohistochemical images were analyzed by Image-Pro Plus 7.2 and statistically analyzed using AOD values. Continuous variables were represented by means and standard deviations, while categorical variables were summarized by frequencies and percentages. The Wilcoxon rank sum test was applied to assess correlations between binary categorical variables and CYP7B1

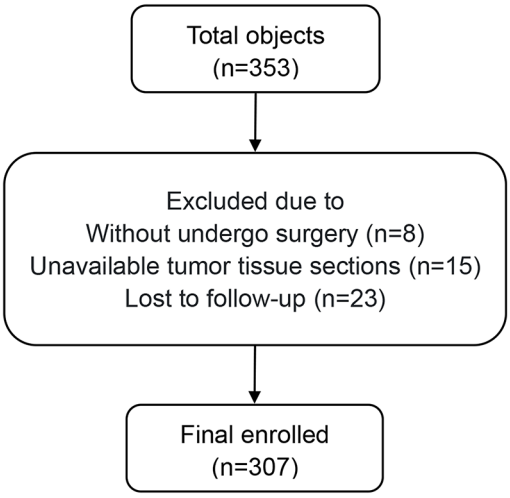

Fig. 1 A flow chart describing the process applied to identify the eligible patients to be enrolled in the study

Table 1 Clinicopathological characteristics of the patients

Characteristics	Patients($n=307$)
Age (years)	
Mean ± SD	52.6 ± 8.0
BMI (kg/m^2)	
Mean ± SD	24.1 ± 3.5
Menopausal status, n (%)	
Premenopausal	156(50.8)
Postmenopausal	151(49.2)
Histological type, n (%)	
Type I	281(91.5)
Type II	26(8.5)
FIGO stage, n (%)	
I	201(65.5)
II	60(19.5)
III	43(14.0)
IV	3(1.0)
Histological grade, n (%)	
1	37(12.0)
2	151(49.2)
3	119(38.8)
Myometrial infiltration, n (%)	
≤ 1/2	224(73.0)
> 1/2	83(27.0)
ER, n (%)	
Negative	42(13.7)
Positive	265(86.3)
PR, n (%)	
Negative	52(16.9)
Positive	255(83.1)

Abbreviations BMI, body mass index; ER, estrogen receptor; FIGO, International Federation of Gynecology and Obstetrics; PR, progesterone receptor; SD, standard deviation

expression data. The surv_cutpoint function was used to obtain the optimal cut-off value for CYP7B1 [21]. Univariate and multivariate Cox regression analyses were conducted to evaluate the association between CYP7B1 expression and the prognosis of EC. Hazard ratios (HRs) and 95% confidence intervals (CIs) were reported. Kaplan-Meier curves were used to describe OS and RFS, and the log-rank test was used to compare survival differences. Statistical analyses were performed using R software (version 4.2.2). A two-tailed P value <0.05 was considered statistically significant.

Results

Clinicopathological characteristics

Of the 353 patients with EC, 307 patients who conformed to the inclusion and exclusion criteria were finally enrolled in this study (Fig. 1). The average age of patients at diagnosis was 52.6 ± 8.0 years. 46 patients (15.0%) died, and 29 (9.4%) experienced recurrence. The median follow-up times for OS and RFS were 5.0 (range 0.7–13.6) years and 4.4 (range 0.3–13.6) years, respectively. Patients were divided into FIGO stage I (65.5%), stage II (19.5%), stage III (14.0%) and stage IV (1.0%). More detailed information is shown in Table 1.

Correlations between clinical factors and CYP7B1

A representative image of CYP7B1 expression is shown in Fig. 2A. The result shows that CYP7B1 protein is expressed in both the cytoplasm and the nucleus, with significantly higher expression in the cytoplasm than in the nucleus ($P<0.001$, Fig. 2B). CYP7B1 protein expression is reduced in women aged ≥ 55 years compared with in those aged < 55 years. CYP7B1 expression is positively correlated with both ER and PR expression. ER-positive ($P=0.028$) and PR-positive ($P<0.001$) patients tend to express higher levels of CYP7B1 protein (Table 2).

Associations of CYP7B1 levels with patient outcomes

The optimal cut-off value for CYP7B1 protein is determined to be 0.0040 using the surv_cutpoint function of the R package survminer in the R programming language, which effectively separates the expression of the CYP7B1 protein into two distinct groups: low-CYP7B1 and high-CYP7B1.

Univariate Cox regression analysis reveals that high CYP7B1 expression is associated with longer OS (HR = 0.41, 95% CI: 0.18–0.90, $P=0.025$) as presented in Table 3. Furthermore, multivariate Cox regression analysis shows that high CYP7B1 expression is an independent prognostic factor for OS, eliminating the effects of other factors (HR = 0.35, 95%CI:0.16–0.79, $P=0.011$) as presented in Table 4. When only ER-positive patients are considered, this association persists (HR = 0.20, 95%CI:0.08–0.52, $P=0.001$) as presented in

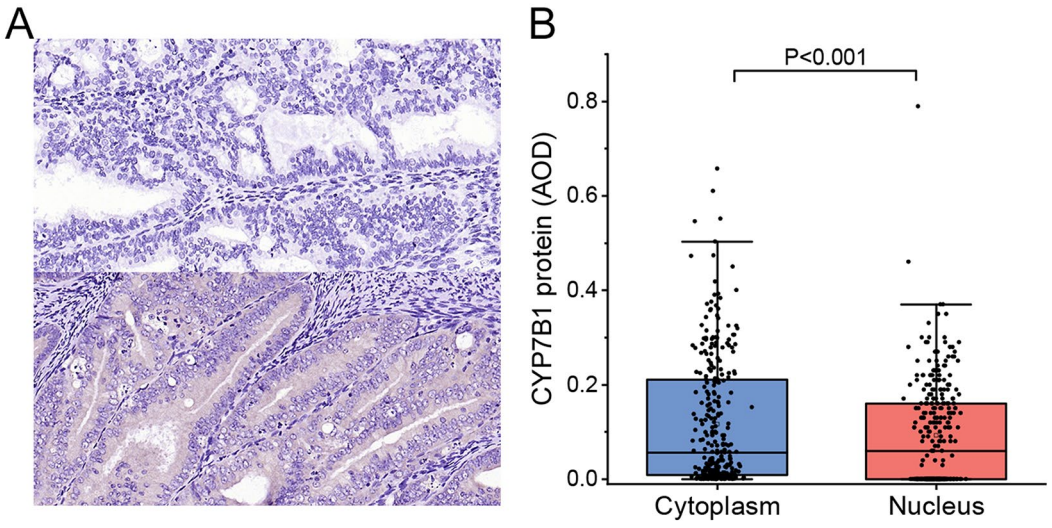

Fig. 2 CYP7B1 protein expression in endometrial cancer tissues **(A)** A representative image of CYP7B1 expression. **(B)** Comparison of CYP7B1 protein expression in cytoplasm and nucleus. *Abbreviations* AOD, average optical density

Table 2 Relationship between the expression of CYP7B1 and clinicopathological characteristics

	N	CYP7B1 protein		*P*
		median	interquartile	
Age (years)				
< 55	192	0.107	0.011–0.281	0.040
≥ 55	115	0.043	0.003–0.265	
BMI (kg/m^2)				
< 25	192	0.077	0.010–0.278	0.796
≥ 25	115	0.106	0.006–0.267	
Menopausal status				
Premenopausal	156	0.108	0.101–0.278	0.200
Postmenopausal	151	0.058	0.005–0.270	
Histological type				
Type I	281	0.085	0.009–0.277	0.468
Type II	26	0.066	0.005–0.231	
FIGO stage				
I-II	261	0.080	0.009–0.277	0.965
III- IV	46	0.098	0.010–0.252	
Histological grade				
1–2	188	0.092	0.007–0.269	0.727
3	119	0.068	0.010–0.284	
Myometrial infiltration				
≤ 1/2	224	0.087	0.009–0.278	0.906
> 1/2	83	0.074	0.007–0.254	
ER				
Negative	42	0.032	0.004–0.135	0.028
Positive	265	0.102	0.009–0.289	
PR				
Negative	52	0.014	0.004–0.081	< 0.001
Positive	255	0.120	0.010–0.296	

Abbreviations BMI, body mass index; ER, estrogen receptor; FIGO, International Federation of Gynecology and Obstetrics; LNM, lymph node metastasis, LVSI, lymph-vascular space involvement; PR, progesterone receptor

Table 4. The beneficial effect of high CYP7B1 expression on ER-positive tumors appears to be more significant for women aged ≥ 55 years (HR = 0.13, 95%CI:0.03–0.59, *P* = 0.009) as seen in Table 4. Interestingly, high CYP7B1 expression extends OS in postmenopausal ER-positive patients (HR = 0.11, 95%CI:0.03–0.46, *P* = 0.003) but did not influence OS in premenopausal patients (HR = 0.43, 95%CI:0.07–2.52, *P* = 0.349) as seen in Table 4. Nevertheless, both univariate (HR = 0.80, 95%CI: 0.38–1.65, *P* = 0.540) and multivariate (HR = 0.83, 95%CI: 0.37–1.86, *P* = 0.647) analyses show no significant difference in RFS between the high-CYP7B1 group and the low-CYP7B1 group as seen in Tables 3 and 4. A statistically significant association between CYP7B1 and RFS (HR = 0.50, 95%CI: 0.10–1.27, *P* = 0.144) is still not found when only ER-positive patients are taken into account. When further stratifying ER-positive patients according to age and menopausal status, the difference in RFS between the high-CYP7B1 and low-CYP7B1 groups is still not statistically significant (*P* > 0.05) as presented in Table 4.

Kaplan-Meier curves of CYP7B1 protein expression show that the 3-year OS is 81.6% (95%CI:69.8–93.3) in the low-CYP7B1 subgroup and 93.0% (95%CI:89.8–96.2) in the high-CYP7B1 subgroup. The 5-year OS is 76.8% (95%CI:62.4–91.1) in the low-CYP7B1 subgroup and 92.0% (95%CI:88.5–95.5) in the high-CYP7B1 subgroup (*P* = 0.021, Fig. 3A). On the other hand, the 3-year RFS presents as 82.3% (95%CI:71.4–93.2) and 87.7% (95%CI:83.6–91.8), respectively. The 5-year RFS is 82.3% (95%CI:71.4–93.2) in the subgroup of patients with low CYP7B1and 84.5% (95%CI:79.8–89.2) in the subgroup of patients with high CYP7B1 (Fig. 3B). However, the difference in 3-year RFS between the low-CYP7B1 and

Table 3 The univariate COX regression analysis of factors predicting OS and RFS of endometrial cancer

	OS		RFS	
	HR (95%CI)	***P***	**HR (95%CI)**	***P***
Age (years)				
≥ 55 vs. <55	1.78(0.86–3.70)	0.121	1.58(0.8802.83)	0.123
BMI (kg/m^2)				
≥ 25 vs.<25	0.90(0.42–1.94)	0.787	0.90(0.49–1.66)	0.739
Menopausal status				
Postmenopausal vs. Premenopausal	1.62(0.77–3.39)	0.203	1.47(0.82–2.63)	0.197
Histological type				
Type II vs. Type I	4.00(1.71–9.37)	0.001	3.15(1.52–6.52)	0.002
FIGO stage				
III- IV vs. I-II	6.91(3.33–14.33)	< 0.001	6.88(3.85–12.31)	< 0.001
Histological grade				
3 vs. 1–2	3.98(1.76–8.99)	< 0.001	4.11(2.16–7.81)	< 0.001
Myometrial infiltration				
> 1/2 vs. ≤1/2	5.65(2.63–12.16)	< 0.001	6.78(3.66–12.57)	< 0.001
ER				
Positive vs. Negative	0.32(0.15–0.71)	0.005	0.51(0.25–1.02)	0.057
PR				
Positive vs. Negative	0.31(0.14–0.65)	0.002	0.42(0.22–0.78)	0.006
CYP7B1				
High vs. Low	0.41(0.18–0.90)	0.025	0.80(0.38–1.65)	0.540

Abbreviations BMI, body mass index; ER, estrogen receptor; FIGO, International Federation of Gynecology and Obstetrics; OS, overall survival; PR, progesterone receptor

Table 4 Multivariate COX regression analysis of associations between CYP7B1 and OS and RFS

	OS_CYP7B1 (Ref. Low)		RFS_ CYP7B1 (Ref. Low)	
	HR (95%CI)	***P***	**HR (95%CI)**	***P***
All patients (*n* = 307)	0.35(0.16–0.79)	0.011	0.83(0.37–1.86)	0.647
ER positive patients (*n* = 265)	0.20(0.08–0.52)	0.001	0.50(0.10–1.27)	0.144
ER positive/ <55 years (*n* = 170)	0.16(0.04–0.67)	0.012	0.63(0.17–2.39)	0.499
ER positive/ ≥55 years (*n* = 95)	0.13(0.03–0.59)	0.009	0.35(0.09–1.38)	0.134
ER positive/ Premenopausal (*n* = 139)	0.43(0.07–2.52)	0.349	1.02(0.18–5.60)	0.985
ER positive/ Postmenopausal (*n* = 126)	0.11(0.03–0.46)	0.003	0.33(0.10–1.13)	0.076

Adjusted for age, BMI, menopausal status; histological type, FIGO stage, histological grade, myometrial infiltration, ER and PR where applicable

Abbreviations ER, estrogen receptor; OS, overall survival; Ref, reference; RFS, recurrence-free survival

high-CYP7B1 groups is not statistically significant, nor is the difference in 5-year RFS (P=0.539).

Discussion

Considering the etiology of estrogen-mediated EC, the role of 27HC remains a significant area of research [22–25]. 27HC has been shown to be linked with growth and progression in cancer [18–20]. CYP7B1 is the enzyme responsible for the breakdown of 27HC. Hypermethylation of the CYP7B1 promoter leads to an elevated 27HC concentration, which promotes the proliferation of ER-positive breast cancer cell [26]. However, there are few studies on the relationship between CYP7B1 and EC [14, 27]. Therefore, it is important to evaluate whether CYP7B1 can be used as a prognostic indicator for patients with EC.

We have gathered new evidence regarding the expression of CYP7B1, the enzyme required for breaking down 27HC. Our findings show that CYP7B1 protein is detected in EC tissues, with notably higher levels in the cytoplasm compared to the nucleus. The expression level of CYP7B1 protein in PR-positive tumors is higher than that in PR-negative tumors. These findings appear to parallel those reported for breast cancer, where a higher proportion of PR-positive tumors was observed in CYP7B1-positive tumors compared to CYP7B1-negative tumors [28]. Furthermore, the present study shows that CYP7B1 protein expression decreases as age increases, suggesting that 27HC accumulation may occure in older patients. A significant positive correlation has been reported between the circulating levels of 27HC and cholesterol, and plasma 27HC levels tend to rise with hypercholesterolemia [19, 29, 30]. Research on breast cancer revealed that the rise of 27HC in tumors was caused by a decrease in CYP7B1 expression [19].

Given the role of CYP7B1 in 27HC metabolism, we proposed that CYP7B1 may influence the outcomes of EC. Our study confirms that CYP7B1 protein expression is an independent predictor of OS, with higher OS

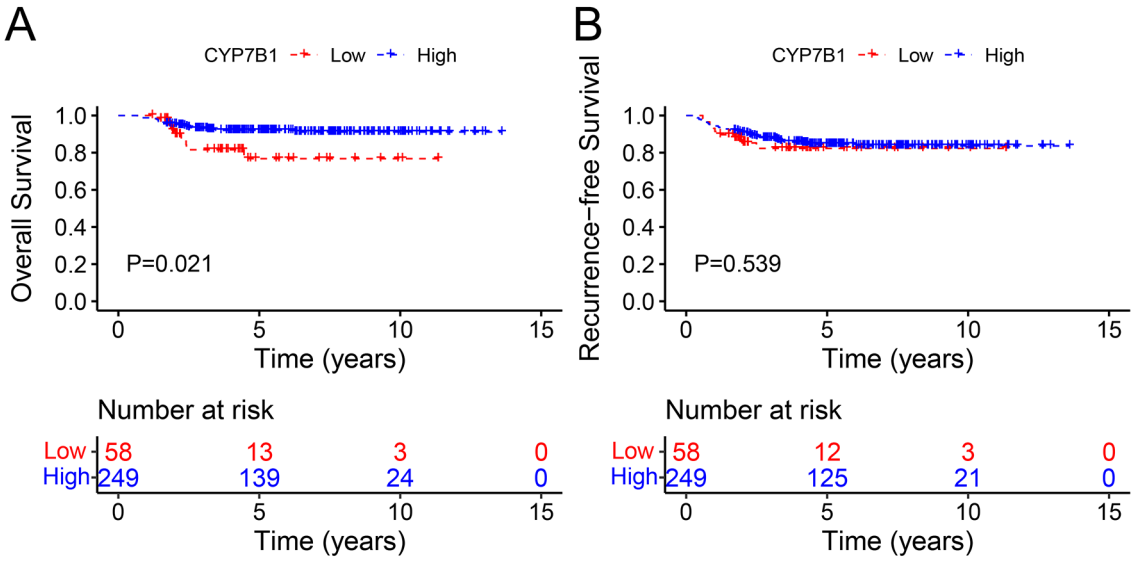

Fig. 3 Kaplan-Meier survival curves of different CYP7B1 expression groups. **(A)** Overall survival in different CYP7B1 expression groups. **(B)** Recurrence-free survival in different CYP7B1 expression groups

in the high-CYP7B1 group compared to the low-CYP7B1 group. Consistent with our results, previous studies have shown that CYP7B1 mRNA expression was downregulated in EC tissues compared to normal tissues, and its expression level was markedly lower in poorly differentiated cancers than in moderately differentiated cancers [14, 27]. ER regulates many physiological functions and plays a crucial role in the development of various types of tumors [31–34]. The absence of ER significantly correlated with advanced stage and higher grade, hence the poor outcome of EC, while its high expression served as a predictor of a better prognosis [35–38]. The present study discovers higher levels of CYP7B1 protein in ER-positive tumors. The results of subgroup analyses of ER-positive patients still support a favorable association between high CYP7B1 protein expression and OS. Interestingly, CYP7B1 protein is more protective in patients aged ≥ 55 years compared to ER-positive patients aged < 55 years. Furthermore, the protective effect of CYP7B1 protein seems to be limited to postmenopausal ER-positive patients. The association between CYP7B1 and prognosis varies in different subgroups, which may be related to the levels of estrogen. Estrogen levels decrease significantly, and serum 27HC increases in postmenopausal women [29, 39]. The promoter of CYP7B1 contains putative response elements for half palindromic hormone response elements (HREs) in the nucleotide sequence from −738 to −758 [17]. Estrogen up-regulates CYP7B1, a target gene for estrogen-mediated regulation [40, 41]. Estrogen has contrary effects on CYP7B1 due to ER status [42]. Estradiol stimulates the catalytic activity and mRNA expression of CYP7B1 when ER is present. On the contrary, estradiol inhibits CYP7B1 activity when ER is absent. Tang et al. have explored the mechanism by which estrogen mediates the regulation of the CYP7B1 gene promoter [41]. However, CYP7B1 expression varies in different cancer tissues. In prostate cancer, local methylation of the CYP7B1 promoter leads to increased expression of the CYP7B1 gene [43]. In contrary, increased methylation of the CYP7B1 promoter in breast cancer cells downregulates its expression [26]. Therefore, more research is required to clarify the precise mechanisms of CYP7B1 in EC.

Our research has some noteworthy limitations. Firstly, the retrospective nature of our study restricts our conclusions to associations rather than causal relationships, and the influence from selection bias cannot have been ruled out. Secondly, this study was conducted at a single center and had a relatively small sample size, which limits the generalizability of our findings. Therefore, future large multicentric well-conducted studies are necessary.

Conclusion

In summary, our results supported that CYP7B1 is an independent predictor of EC survival, especially ER-positive EC, suggesting that CYP7B1 may serve as a predictor of EC follow-up.

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Author contributions

Study conception and design: all authors. Data collection: X-FL, TH, CC and JL. Data analysis: X-FL, JZ and X-YF. Drafting the manuscript: X-FL and D-LL. Project supervision: BC, Y-YZ, JL, and D-LL. D-LL and JL are the co-corresponding authors. All authors have reviewed and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by Jinan University and Wuzhou Red Cross Hospital Ethics Committee and all patients provided informed consent for the use of their clinical information.

Competing interests
The authors declare no competing interests.

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