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ORIGINAL RESEARCH

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# Expression and clinical significance of CSAD in type I endometrial carcinoma

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## Abstract

The current study aimed to identify the expression and clinical significance of cysteine sulfinate decarboxylase (CSAD) in type I endometrial carcinoma (EC). A total of 350 patients with endometrial lesions were involved in the study at Zhejiang University's Women's Hospital. Immunochemical staining was performed on samples of normal endometrium (NE), atypical endometrial hyperplasia (AEH), and type I EC. The study evaluated the association of CSAD staining with clinical characteristics and patient survival. CSAD expression in type I EC was elevated in comparison with NE, and increased expression correlates with International Federation of Gynecology and Obstetrics (FIGO) stages, Lymph vascular space invasion (LVSI), and Lymph node metastasis (LNM). High CSAD levels are associated with poor disease-free survival and overall survival. small interfering RNAs (siRNAs) can decrease CSAD expression, inhibit cancer cell proliferation, and affect B-cell lymphoma-2 (BCL-2) and BCL2-Associated X (BAX) protein expression. Our research indicates that higher levels of CSAD expression in type I EC was significantly associated with some clinical variables indicating poor prognosis and survival rate in patients with type I EC. patients and could promote cancer cell growth by affecting cell apoptosis rate.

## Keywords

Cysteine sulfinate; Decarboxylase; Type I endometrial carcinoma; Prognosis; Proliferation; Apoptosis

## 1. Introduction

Endometrial carcinoma (EC) is a prevalent form of cancer in women, accounting for 7% of all cancer cases. In the USA, it is estimated that there are 65,620 new cases of EC and 12,590 fatalities attributed to this cancer per year [1]. Over the past few decades, there has been a steady increase in the occurrence of endometrial carcinoma. The projections showed that the number of cases will increase to 42.13 per 100,000 individuals in the USA by 2030 [2].

Over the past three decades, endometrial carcinoma has been broadly categorized into two subtypes: type I, which is hormone-receptor-positive, low-grade, endometrioid carcinoma and accounts for 80% of endometrial carcinoma, and type II, which is hormone-receptor-negative, high-grade and non-endometrioid carcinoma [3]. The rise in endometrial carcinoma incidence rates, especially type I EC (1.3% per year from 2007 to 2016), has been partially linked to the greater overall prevalence of obesity and metabolic syndromes in countries with rapid socioeconomic transitions, as well as the aging population [4, 5].

Most type I endometrial carcinomas (75%) are diagnosed at the early stages with optimal prognosis. The 5-year overall survival rate ranges from 74% to 91% in patients without metastasis, whereas it decreases to 20–66% in those with lymph node metastasis [3]. A better understanding of the mechanisms of endometrial carcinoma initiation and development and the identification of associated therapeutic targets would contribute to improving the prognosis of type I endometrial carcinoma patients.

Cysteine sulfinate decarboxylase (CSAD) was first discovered in the liver and serves as a vital rate-limited enzyme that controls the *in vivo* synthesis of taurine. CSAD regulates taurine synthesis via controlling of the partition of cysteine sulfinic acid between taurine synthesis and the production of pyruvate and sulfate [6, 7]. A previous study showed that the enzymatic activity and expression of CSAD increased after an ovariectomy procedure and decreased after estrogen replacement. Analysis of the CSAD promoter suggested three possible half-estrogen response elements (ERE) [8]. The correlation between CSAD and cancers has rarely been reported. Kishimoto *et al.* [9] discovered that the expression of CSAD messenger RNA (mRNA) and its protein was elevated during hepatocarcinogenesis. Currently, the expression and role of CSAD in type I endometrial tumorigenesis and progression have not been described. To better characterize the expression and clinical significance of CSAD in type I EC, we used immunohistochemistry to investigate the differential expression of CSAD in tissues from type I EC, atypical endometrial


  

hyperplasia and normal endometrium as well as its effect on cancer cell proliferation and apoptosis in human endometrial carcinoma cell lines human endometrial adenocarcinoma cell stage IA (HEC1A) and Ishikawa.

## 2. Materials and methods

### 2.1 Patients and tissue specimens

In this study, we analyzed 350 endometrial tissue samples, which consisted of 269 cases of type I endometrial carcinoma (EC), 26 cases of atypical endometrial hyperplasia (AEH), and 55 samples from normal endometrium (NE). The samples were collected between January 2006 and December 2013 at the Women's Hospital, School of Medicine, Zhejiang University. Table 1 shows a group of 26 patients with atypical endometrial hyperplasia specimens. The second group consists of 269 patients with type I endometrial carcinoma who underwent total hysterectomy, removal of both tubes and ovaries and pelvic lymph node dissection without any anticancer therapy or hormonal therapy before their surgery. Table 2 shows the clinicopathological characteristics of the patients with type I endometrial carcinoma. The last group enrolled 55 patients with normal endometrium with hysterectomies due to benign gynecologic diseases.

### 2.2 Follow-up

The patients with type I EC were followed up postoperatively by either in-person interviews at the clinic or phone call. Regional tumor recurrence, distant metastasis, and patient survival were recorded and disease-free survival (DFS) and overall survival (OS) were calculated from the day of the surgery until recurrence or death. The last day of follow-up was May 2020. The median follow-up was 72 months (5–120 months). During follow-up, 35 patients (13.0%) had a clinical recurrence and 30 (11.2%) died of progressive disease.

### 2.3 Immunohistochemical staining

The tissues were immersed in paraffin, cut into sections that were 4 $\mu$m thick, placed on slides, treated with xylene for 15 minutes to remove wax, subjected to a series of solutions containing varying amounts of water and alcohol, and then rehydrated with distilled water. To restore antigen reactivity, the sections were heated in a 0.1 mol/L pH 6 citrate buffer for 20 minutes in a water bath at a temperature of 95 °C. After heating, the sections were cooled and left at room temperature for 30 minutes. The slices were incubated with diluted normal goat serum for 30 minutes to inhibit nonspecific binding.

The avidin-biotin-peroxidase method was used to perform the immunohistochemical staining using a SLABC kit (K5007, DAKO, Glostrup, Denmark). The slides were kept at room temperature for 2 hours and treated with CSAD (1:200, Invitrogen, PA5-58811, Carlsbad, CA, USA). The sections were then developed for 2 minutes with the enzyme-substrate 3,3′-diaminobenzidine chromagen (K3468, DAKO, Glostrup, Denmark) and counterstained with Mayer's hematoxylin for 30 s. After dehydration, the tissues were cover slipped with permount and subsequently examined.

### 2.4 Evaluation of immunoreactivity

Two experienced pathologists with no clinicopathological data information independently assessed immunohistochemical staining in five fields. If there was a difference of opinion, the pathologists engaged in discussions until they reached a consensus on the outcome. CSAD immunoreactivity was assessed by staining intensity and the distribution of positively stained tumor cells. Intensity was scored as negative (0), weakly positive (1), moderately (2) or strongly positive (3). The staining distribution was scored as 0 for $<$25%, 1 for 25 to $<$50%, and 2 for 50 to $<$75%, 3 for 75 to $<$100% positively stained cells. The product of both parameters yielded the immunohistochemical (IHC) score, which ranged from 0 to 9 points. A total score of $<$4 was considered as decreased expression and $\geq$4 as increased expression.

### 2.5 Cell culture

Human endometrial carcinoma cell lines HEC1A and Ishikawa were purchased from Wuhan Procell Life Technology (Wuhan, China). Cells were cultured in McCoy's 5a Medium (HEC1A) and dulbecco's modified eagle medium (DMEM) (for Ishikawa) supplemented with 10% fetal bovine serum (FBS).

### 2.6 Transfection with small interfering RNA (siRNA)

siRNAs were used to inhibit the CSAD expression in HEC1A and Ishikawa cells. Following the manufacturer's instructions, the cells were transfected with CSAD-specific siRNA using Lipofectamine™ RNAiMAX (13778150, Invitrogen, Carlsbad, CA, USA). The construction and sequences for the siRNAs were as follows:

si-CSAD-1:forward: 5′-CAGAAAGGAACCAGUGUCUTT-3′;

reverse: 5′-AGACACUGGUUCCUUUCUGTT-3′;

si-CSAD-2:forward: 5′-GUGAUUCGCUACAGUGUCATT-

**TABLE 1. The level of CSAD protein in 350 cases of type I endometrial carcinomas, atypical endometrial hyperplasia and normal endometrium.**

| Proteins | Expression level | Type I Endometrial cancers (n = 269) | Atypical endometrial hyperplasia (n = 26) | Normal endometrium (n = 55) |
|---|---|---|---|---|
| CSAD | | | | |
| | High | 83 | 4 | 8 |
| | Low | 186 | 22 | 47 |

*CSAD, Cysteine sulfinate decarboxylase.*

**TABLE 2. The correlation between the level of CSAD protein and the clinicopathological parameters in 269 cases with type I endometrial carcinomas.**

| Characteristic | No. | CSAD, n (%) | | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| | | Low | High | | |
| Age (yr) | | | | | |
| ≤60 | 201 | 139 (51.7) | 62 (23.0) | $3.2 \times 10^{-5}$ | 0.995 |
| >60 | 68 | 47 (17.5) | 21 (7.8) | | |
| Menopause | | | | | |
| No | 103 | 77 (28.6) | 26 (9.7) | 2.464 | 0.116 |
| Yes | 166 | 109 (40.5) | 57 (21.2) | | |
| FIGO stage | | | | | |
| <II | 185 | 135 (50.2) | 50 (18.6) | 4.069 | 0.044 |
| ≥II | 84 | 51 (19.0) | 33 (12.3) | | |
| Differentiation | | | | | |
| Well/moderate | 214 | 148 (55.1) | 66 (24.5) | $9.5 \times 10^{-5}$ | 0.992 |
| Poor | 55 | 38 (14.1) | 17 (6.3) | | |
| Myometrial invasion | | | | | |
| <1/2 | 208 | 149 (55.4) | 59 (21.9) | 2.665 | 0.103 |
| ≥1/2 | 61 | 37 (13.8) | 24 (8.9) | | |
| Tumor size | | | | | |
| <4 cm | 217 | 144 (53.5) | 73 (27.1) | 4.083 | 0.043 |
| ≥4 cm | 52 | 42 (15.6) | 10 (3.7) | | |
| Peritoneal lavage cytology | | | | | |
| Negative | 264 | 183 (68.1) | 81 (30.1) | 0.191 | 0.662 |
| Positive | 5 | 3 (1.1) | 2 (0.7) | | |
| LVSI | | | | | |
| No | 238 | 170 (63.2) | 68 (25.3) | 5.048 | 0.025 |
| Yes | 31 | 16 (5.9) | 15 (5.6) | | |
| LNM | | | | | |
| No | 241 | 173 (64.3) | 68 (25.3) | 7.559 | 0.006 |
| Yes | 28 | 13 (4.8) | 15 (5.6) | | |

*CSAD, Cysteine sulfinate decarboxylase; FIGO, International Federation of Gynecology and Obstetrics; LVSI, Lymph vascular space invasion; LNM, Lymph node metastasis.*

3′;

reverse: 5′-UGACACUGUAGCGAAUCACTT-3′

The siRNAs were synthesized at Tsingke Biotechnology Co., Ltd. (Hangzhou, China). Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and western blot determined the transfection efficiency of CSAD.

## 2.7 Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted using total RNA extractor (TRIzol) Reagent (15596018CN, Invitrogen, Carlsbad, CA, USA), and amplification of the complementary DNA (cDNA) (1 $\mu$g per sample) was performed using the Prime Script Kit (RR014A, TaKaRa Bio Inc., Otsu, Japan) according to the manufacturer's indication. Synergy Brands (SYBR) Green fluorescent-based assay (638320, TaKaRa Bio Inc., Otsu, Japan) was used to run real-time PCR in triplicate on a ViiATM7 RT-PCR system (Applied Biosystems, Carlsbad, CA, USA). The primers for real-time PCR were as follows:

CSAD: forward: 5′-CTTCTCCAGGATACCTCGAACC-3′;

reverse:5′-CAGAGCCACATCGTAGAACTTG-3′;

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward: 5′- GGAGCGAGATCCCTCCAAAAT-3′;

reverse: 5′-GGCTGTTGTCATACTTCTCATGG-3′;

Relative mRNA expression levels were calculated by the $2^{-(\Delta\Delta Ct)}$ method and were normalized to the internal control (GAPDH), $\Delta$Ct = Ct (targeting gene) − Ct (GAPDH), and $\Delta\Delta$Ct = $\Delta$Ct (treated) − $\Delta$Ct (control).

## 2.8 Western blot analysis

Cells containing protease and phosphatase inhibitor cocktail were lysed in radio immunoprecipitation assay (RIPA) lysis (P0013B, Beyotime, Shanghai, China). Protein concentration was determined using the Bicinchoninic Acid Assay (BCA) protein assay kit (P0012S, Beyotime Biotechnology, Shanghai, China). Protein samples were separated by 10% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto 0.2 $\mu$m Polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA) under a constant 300 mA. The membrane was then incubated with the primary antibodies at 4 °C overnight after being blocked in TBST (Tris-buffered saline (TBS) with 0.5% Tween) containing 5% skim milk at 37 °C for 1 h.

After incubating with a Horseradish Peroxidase (HRP)-conjugated secondary antibody for 1 h at 37 °C, the membrane was washed in TBST and prepared for signal detection. The signals were automatically visualized using the FUSION FX6 (vilber, Eberhardzell, BW, Germany) and quantitatively analyzed with Image Lab software (6.1, Bio-Rad, Hercules, CA, USA). GAPDH protein expression was used as the internal control. The following antibodies were used: CSAD (1:2000, A13845, Wuhan, Hubei, China), HRP Goat Anti-Rabbit IgG (H + L) (1:5000, AS014, Wuhan, Hubei, China), HRP Goat Anti-Mouse IgG (H + L) (1:5000, AS003, Wuhan, Hubei, China) from ABclonal; BCL2 (1:1000, 26593-1-AP, Wuhan, Hubei, China), BAX (1:1000, 50599-2-Ig, Wuhan, Hubei, China) from Proteintech; GAPDH (1:5000, db11729, Hangzhou, Zhejiang, China) from Diagbio.

## 2.9 Cell proliferation

The cells were placed in 96-well plates for 24 h and transfected with siR-CSAD and siR-control. The proliferation was measured by the cell counting kit (CCK)-8 kit (40203ES60, Yeasen Biotech, Shanghai, China) according to the manufacturer's protocol.

## 2.10 Apoptosis assay

Cell apoptosis was examined using a FACSVerse (BD Biosciences, Franklin Lakes, NJ, USA) flow cytometer. Yeasen Biotech's Annexin V-FITC/PI Apoptosis Detection Kit (40302ES20, Yeasen Biotech, Shanghai, China) was used to evaluate apoptosis following the manufacturer's instructions. The following steps were taken to prepare the cells for analysis: $1 \times 10^5$ cells/mL of cells were resuspended in 1× binding buffer. Then, 5 $\mu$L of Annexin V-FITC reagent and 10 $\mu$L of PI reagent were added to a tube. The mixture was incubated at room temperature in the dark for 15 minutes.

## 2.11 Statistical analysis

Statistical analyses were performed using IBM SPSS 24.0 for Windows (IBM Corp., Armonk, NY, USA). The correlation of CSAD expression with clinicopathological characteristics and the significance of differences in CSAD expression were determined by Pearson's chi-square test. Survival curves were generated using the Kaplan-Meyer method, and survival rates were compared using the log-rank test. The Cox proportional hazard method was employed in univariate and multivariate studies to identify independent determinants of survival. *In vitro* assay, a student's test was used to determine the statistical differences between the two groups. All statistical tests were two-sided, and $p$-values $< 0.05$ were considered statistically significant.

# 3. Results

## 3.1 The Evaluation of CSAD expression in endometrial tissue samples

Three hundred fifty (350) endometrial tissue samples were collected for immunohistochemistry to evaluate CSAD expression, comprising 269 type I EC, 26 AEH and 55 NE. The mean age of patients was 54.39 years for type I EC, 48.38 years for AEH, 46.58 years for NE. According to the 2009 report by the International Federation of Obstetrics and Gynecology, 185 individuals with type I endometrial carcinoma were in Stage I, while 84 were in Stage II–IV. 214 cases were well-moderately differentiated (G1/G2), and 55 cases were poorly differentiated (G3). Of 269 cases, 28 (10.41%) showed evidence of lymph node metastasis (LNM). Lymphatic vascular space invasion (LVSI) was detected in 31 (11.52%) of 269 cases. Immunohistochemical staining of CSAD expression is shown in Fig. 1 and described below.

## 3.2 CSAD expression

Most type I EC tissue samples exhibited uniform and intense CSAD staining, mainly localized in the cytoplasm and cell membrane. CSAD immunoreactivity was less observed in either the cytoplasm or cell membrane of NE tissues. Table 1 demonstrates that 30.86% (83/269) of type I EC samples had a significant level of CSAD expression (score $\geq$4). However, increased expression of CSAD was seen in 15.38% (4/26) AEH specimens and 8 of 55 NE specimens (14.55%). The expression of CSAD in Type I EC was significantly higher than in NE and AEH (NE *vs.* Type I EC, $p = 0.014$; AEH *vs.* Type I EC, $p = 0.099$), although there was no statistical difference compared to the AEH group. Additionally, there was no statistically significant difference in CSAD expression between NE and AEH (NC *vs.* AEH, $p = 0.921$).

## 3.3 The correlation of CSAD expression with clinicopathological characteristics in Type I EC

The correlation of CSAD expression with clinicopathological characteristics of Type I EC tissues is shown in Table 2. Tissue samples from 83 Type I EC patients (30.86%) exhibited an elevated CASD (score $\geq$4), while the remaining 186 samples (69.14%) showed reduced staining (score $<$4). Increased CSAD expression was significantly associated with FIGO stages ($p = 0.044$), lymph vascular space invasion (LVSI) ($p = 0.025$) and lymph node metastasis (LNM) ($p = 0.006$). In contrast, a decrease in CSAD expression showed a strong correlation with larger tumor sizes ($p < 0.05$).

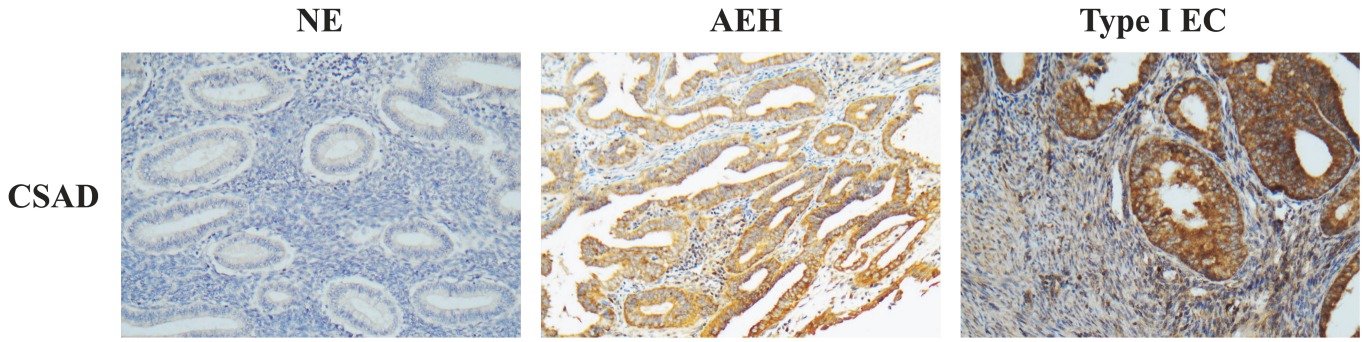


**FIGURE 1. Representative immunohistochemical staining showing CSAD expression in normal endometrium (NE), Atypical endometrial hyperplasia (AEH) and Type I Endometrial carcinoma (Type I EC) tissues using serial section technique.** Increased CSAD expression was observed in one representative case with type I EC. Magnifications, ×200. CSAD, Cysteine sulfinate decarboxylase.

### 3.4 Survival analysis

Kaplan-Meier analysis was used to investigate the prognostic value of the CSAD expression in type I EC. The survival curves in Fig. 2 illustrate the association of CSAD expression with disease-free survival (DFS) and overall survival (OS) in 269 patients diagnosed with type I EC. Significance was tested in univariate and multivariate Cox regression models. The Kaplan-Meier analysis showed that increased CSAD expression was significantly associated with shorter DFS ($p = 0.044$) and OS ($p = 0.045$). In addition, Cox univariate proportional hazards analysis showed that menopause status, International Federation of Gynecology and Obstetrics (FIGO) stage, histological differentiation, myometrial invasion, LVSI, LNM and CSAD expression were significant predictors of shorter DFS and OS. There was also an association between poor OS and peritoneal lavage cytology. Furthermore, multivariate analysis showed that only myometrial invasion, LVSI and LNM were independent predictors of shorter DFS. LVSI and LNM indicated poor OS in patients with type I EC (Table 3).

### 3.5 Down-regulation of CSAD expression using siRNA targets CSAD, inhibits Endometrial carcinoma cell growth and induces cell apoptosis

The siRNAs were used to inhibit CSAD expression in Endometrial carcinoma cells. The results from qRT-PCR and Western blot analysis demonstrated that siRNA targeted at CSAD could significantly reduce mRNA and protein levels of CSAD in HEC1A and Ishikawa (IK) cells (Fig. 3). Further studies found down-regulation of CSAD expression markedly inhibited cell growth ($p < 0.05$) in both HEC1A and Ishikawa cells (Fig. 4). Additionally, deceased CSAD expression markedly downregulated apoptosis-related protein BCL-2 and upregulated BAX expression. This indicates that silence CSAD suppressed cell growth by regulating apoptosis progression ($p < 0.05$) (Fig. 3A,B). Flow cytometry was used to measure apoptosis rates after reduced expression of CSAD. The results showed that the silence of CSAD could induce apoptosis compared with negative controls in HEC1A and IK cells (Fig. 5) ($p < 0.05$).

## 4. Discussion

Cysteine sulfinic acid decarboxylase (CSAD) was first identified in the liver as one of the primary rate-limiting enzymes in taurine biosynthesis [10, 11]. Since then, it has been discovered that CSAD is expressed in the liver and the kidney [12], brain [13] and male reproductive organs. CSAD functioned as an enzyme for the decarboxylation of cysteine sulfinic acid to produce hypotaurine, which is then oxidized to taurine [14] and played a vital role in anti-inflammatory and immunoregulatory effects [15]. CSAD exhibits a more direct relationship with taurine concentration than any other rate-limiting enzyme [11, 16]. Nevertheless, there is still a lack of correlation between CSAD and tumor initiation and progression. The mechanism by which CSAD is involved in carcinogenesis and cancer progression in the uterus has not been described.

The findings of this study indicate that CSAD levels were higher in individuals diagnosed with type I endometrial carcinoma (EC) compared to patients with atypical endometrial hyperplasia (AEH) or normal endometrium (NE). No statistical difference was observed between the AEH group and the type I EC group due to a few cases in the AEH group. In addition, the expression of CSAD does not exhibit any notable distinction between the AEH and NE groups. Collectively, these data indicate that abnormal expression of CSAD may play a role in endometrial carcinogenesis.

Previous studies have highlighted discrepancies between the preoperative diagnosis of AEH and the final histology, with a rate of grade 1 EC in hysterectomy specimens reaching 42% [17]. From a clinical perspective, the differentiation between AEH and EC was crucial to determining the therapeutic strategy [18]. Therefore, it is essential to accurately distinguish the EC from AEH to choose the appropriate therapeutic strategy. Our study adds to the existing evidence that CSAD can be a useful biomarker and that immunostaining could assist in distinguishing EC from AEH.

Moreover, our study demonstrated a substantial correlation between elevated CSAD expression and specific clinical characteristics, such as FIGO stages, LVSI, and LNM, which indicated enhanced tumor invasiveness. Prior studies have repeatedly proven that aberrant expression of CSAD might be involved in muscle-invasive bladder cancer metasta-

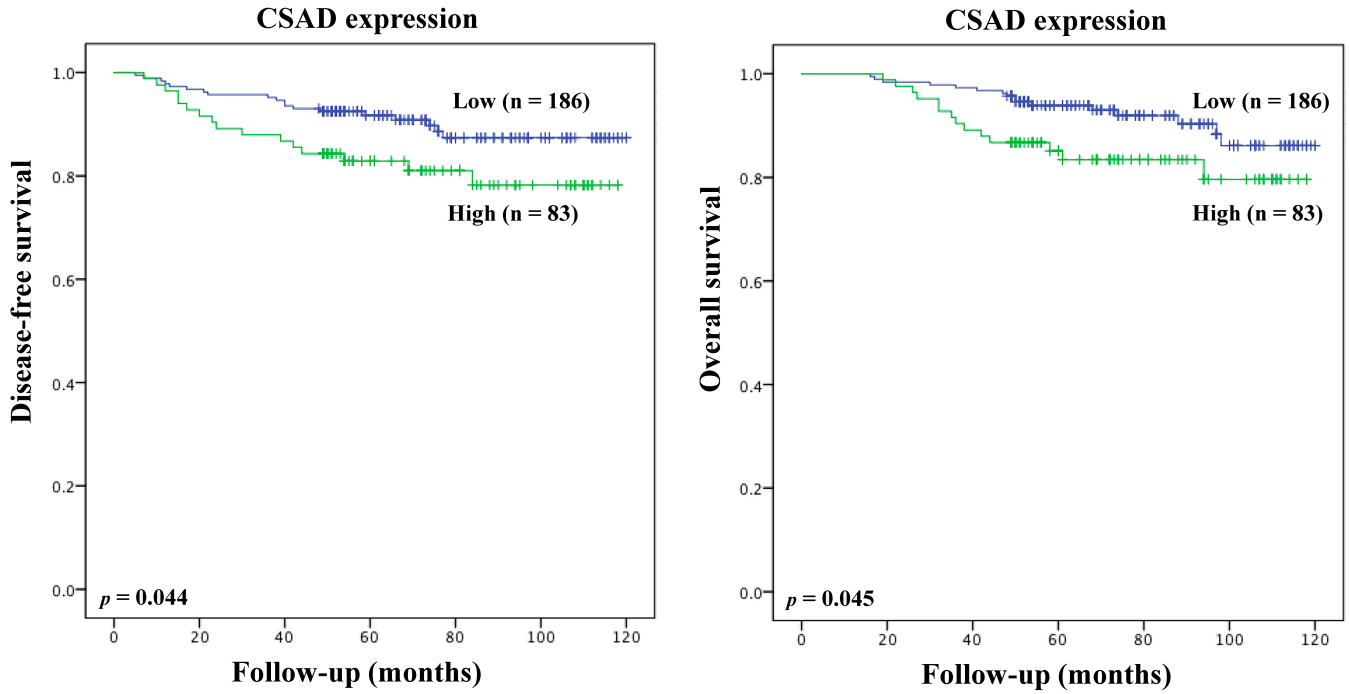


**FIGURE 2. Kaplan-Meyer curves showing the association of aberrant expression of CSAD and patient disease-free survival (DFS) and overall survival (OS).** Increased CSAD was significantly associated with shorter DFS and OS. CSAD, Cysteine sulfinate decarboxylase.

**TABLE 3. Univariate and multivariate analysis of the correlation between prognostic value and disease-free survival (DFS) and overall survival (OS) in 269 patients with type I endometrial carcinomas.**

| Characteristic | Disease-free survival | | | Overall survival | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* | HR | 95% CI | *p* |
| Univariate analyses | | | | | | |
| Age | 1.590 | 0.791–3.196 | 0.193 | 1.267 | 0.580–2.768 | 0.552 |
| Menopause | 2.655 | 1.159–6.078 | 0.021 | 2.718 | 1.110–6.656 | 0.029 |
| FIGO stage | 2.995 | 1.533–5.852 | 0.001 | 2.401 | 1.169–4.934 | 0.017 |
| Differentiation | 3.463 | 1.780–6.735 | $2.5 \times 10^{-4}$ | 3.583 | 1.747–7.347 | $4.9 \times 10^{-4}$ |
| Myometrial invasion | 8.187 | 4.061–16.504 | $4.2 \times 10^{-9}$ | 7.204 | 3.417–15.189 | $2.1 \times 10^{-7}$ |
| Tumor size | 1.065 | 0.465–2.438 | 0.882 | 1.260 | 0.540–2.940 | 0.593 |
| Peritoneal lavage cytology | 4.147 | 0.988–17.408 | 0.052 | 5.114 | 1.204–21.725 | 0.027 |
| LVSI | 10.760 | 5.528–20.946 | $2.7 \times 10^{-12}$ | 10.563 | 5.145–21.685 | $1.3 \times 10^{-10}$ |
| LNM | 12.530 | 6.397–24.548 | $1.7 \times 10^{-13}$ | 13.729 | 6.661–28.299 | $1.3 \times 10^{-12}$ |
| CSAD expression | 1.954 | 1.005–3.801 | 0.048 | 2.049 | 1.000–4.200 | 0.050 |
| Multivariate analyses | | | | | | |
| Menopause | 1.984 | 0.833–4.724 | 0.122 | 1.771 | 0.686–4.569 | 0.237 |
| FIGO stage | 0.806 | 0.321–2.024 | 0.646 | 0.458 | 0.161–1.298 | 0.142 |
| Differentiation | 1.504 | 0.716–3.159 | 0.281 | 1.805 | 0.809–4.027 | 0.149 |
| Myometrial invasion | 2.939 | 1.229–7.025 | 0.015 | 2.068 | 0.765–5.591 | 0.152 |
| Peritoneal lavage cytology | - | - | - | 1.095 | 0.229–5.234 | 0.910 |
| LVSI | 3.342 | 1.391–8.025 | 0.007 | 3.433 | 1.328–8.876 | 0.011 |
| LNM | 3.406 | 1.253–9.258 | 0.016 | 6.470 | 2.028–20.645 | 0.002 |
| CSAD expression | 1.131 | 0.562–2.280 | 0.730 | 1.287 | 0.588–2.817 | 0.527 |

*CSAD, Cysteine sulfinate decarboxylase; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymph vascular space invasion; LNM, lymph node metastasis; HR, hazard ratio; 95% CI, 95% confidence interval.*

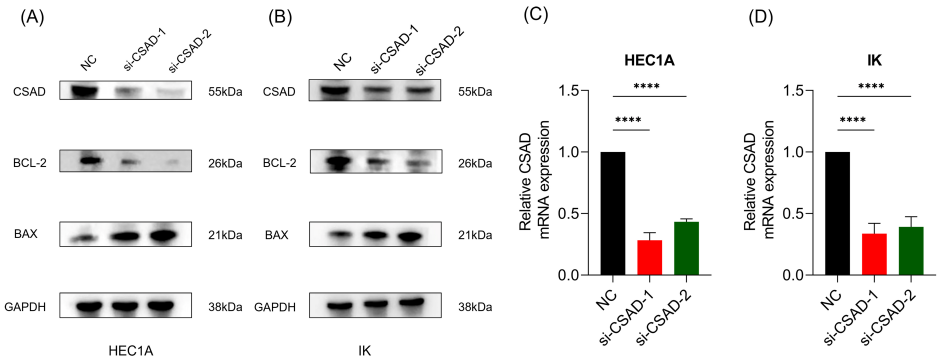


**FIGURE 3. Western-Blot and qRT-PCR showing the expression of CSAD were inhibited by siRNAs.** (****, $p <$ 0.0001). CSAD, Cysteine sulfinate decarboxylase; IK, Ishikawa; si, small interfering; BCL-2, affect B-cell lymphoma-2; BAX, BCL2-Associated X; GAPDA, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; HEC1A, human endometrial adenocarcinoma cell stage IA.

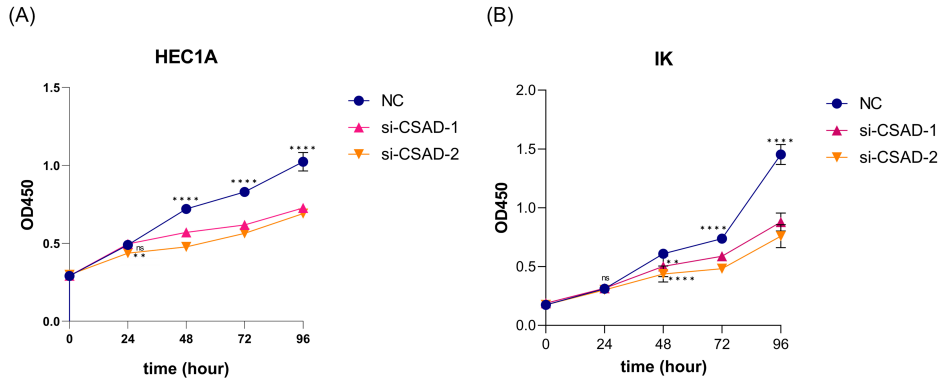


**FIGURE 4. Silence of CSAD markedly suppressed the cell growth in both HEC1A and IK cells.** (ns, $p > 0.05$; **, $p = 0.0014$ (HEC1A), $p = 0.0038$ (IK); ****, $p < 0$ .0001). CSAD, Cysteine sulfinate decarboxylase; IK, Ishikawa; si, small interfering; OD, optical density; HEC1A, human endometrial adenocarcinoma cell stage IA; NC, negative control.

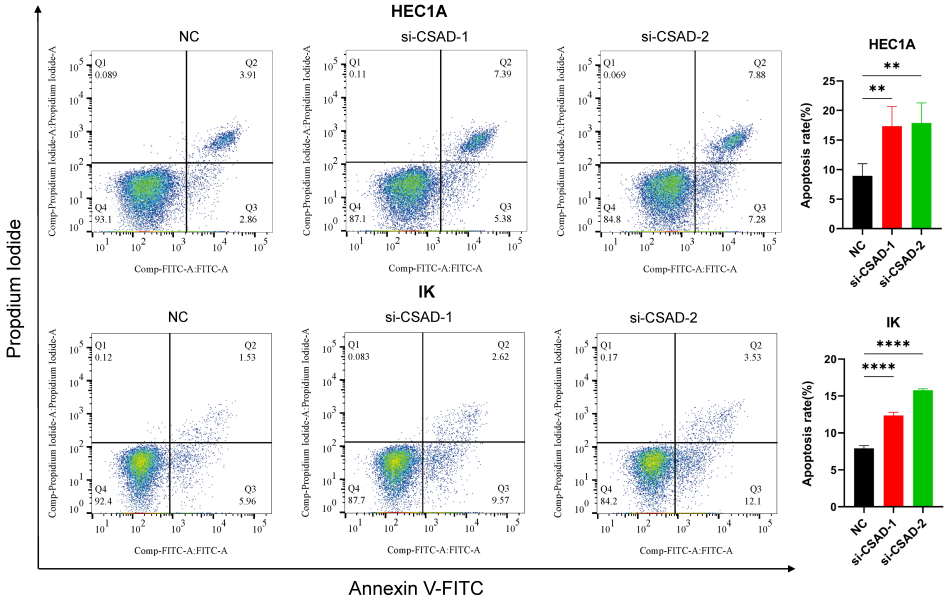


**FIGURE 5. The silence of CSAD could induce apoptosis compared with negative controls in HEC1A and IK cells.** (**, $p = 0.0030$ (HEC1A, nc *vs.* si-CSAD-1), $p = 0.0021$ (HEC1A, nc *vs.* si-CSAD-2); ****, $p < 0.0001$ (IK)). CSAD, Cysteine sulfinate decarboxylase; IK, Ishikawa; si, small interfering; NC, negative control; V-FITC-A, Annexin V-FITC; HEC1A, human endometrial adenocarcinoma cell stage IA.

sis. Stubendorff *et al*. [19] identified CSAD as a methylation marker suitable to distinguish between patients with positive and negative lymph nodes in muscle-invasive bladder cancer. This allows for a risk assessment to be made for patients who potentially benefit from extended lymph node resection as well as from neo-adjuvant chemotherapy. Immune infiltration is widely regarded as a critical factor in developing endometrial carcinoma [20]. By conducting subgroup analysis on the status of immune infiltration, we hope to provide more in-depth insights. However, we recognize the lack of effective standards to accurately assess the state of immune infiltration, which is a limitation in our research. In this study, we included 55 normal endometrial samples, 26 atypical hyperplasia samples, and 269 type I endometrial cancer samples. Nonetheless, the limited number of samples may result in bias in the subgroup analysis of immune infiltration or other related conditions.

Our vitro experiments showed that decreased expression of CSAD inhibited cell proliferation, promoted cell apoptosis, and affected apoptosis-related protein BCL-2 and BAX expression in HEC1A and Ishikawa cells, implying that aberrant expression of CSAD might contribute to the initiation and development of type I EC. Additional *in vivo* and *in vitro* research in type I EC cell lines and animal models are required to investigate the mechanism of aberrant CSAD expression in carcinoma and cancer.

Endometrial carcinoma poses an enormous threat to the health of females worldwide. Currently, the prognostic methods for endometrial carcinoma are limited. Several risk stratification systems (RSS) have been developed that consider the clinical risk factors such as FIGO stages, histology types, pathological grade, age, tumor size, and lymphatic vascular clearance involvement (LVSI) [20–23].

However, these RSS measurements' accuracy remains unsatisfactory [24]. So far, there is a scarcity of tumor biomarkers with high sensitivity and specificity to predict the prognosis and recurrence of endometrial carcinoma [25]. Our study showed a strong correlation between increased CSAD expression and shorter DFS and OS. Univariate analysis further verified that CSAD expression was a predictor of shorter DFS (hazard ratio (HR) = 1.954, 95% confidence interval (CI) = 1.005–3.801) and OS (HR = 2.049, 95% CI = 1.000–4.200) in patients with type I EC. However, the multivariate analysis indicated that CSAD expression did not serve as an independent predictor. The reason may be attributed to the absence of advanced-stage patients in our series. Our findings suggest that CSAD may serve as a biomarker for poor prognosis in individuals with type I EC.

## 5. Conclusions

In summary, elevated levels of CSAD expression in type I endometrial carcinoma was found to be significantly correlated with certain clinical factors suggestive of a poor prognosis and reduced survival among patients with type I endometrial cancer and may enhance cancer cell proliferation by affecting the rate of cell apoptosis.

## AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

## AUTHOR CONTRIBUTIONS

HH, MNW, XJC, QC and YF—designed the study, collected the samples, performed the experiments, did statistical analysis and drafted the manuscript. BHL—conceived the study, participated in its design and coordination, and revised the final manuscript. All the authors read and approved the final version of the manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was approved by the Institutional Review Board, Women's Hospital, School of Medicine, Zhejiang University (Approval no. 20150100). The informed consent has been given and obtained from all individual participants included in the study.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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