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ORIGINAL RESEARCH

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Expression and clinical significance of osteocalcin in endometrial carcinoma

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Abstract

Background: Steocalcin (OCN) is a bone tissue-specific protein overexpressed in several human cancers. However, the physiological roles of OCN in endometrial cancer (EC) are unclear. This study aimed to investigate its prognostic impact on OCN expression in EC patients and their associations with clinicopathologic features. **Methods**: Between July 2010 and December 2013 at the Department of Obstetrics and Gynecology, Shanghai Pudong Hospital, we performed immunohistochemistry to detect OCN expression in 134 EC patients and 35 normal endometria (non-EC) specimens and analyze its correlation with clinicopathologic features. The enzyme-linked immunosorbent assay (ELISA) analyzed the OCN expression in serum. OCN expression was also detected in fresh tissues using real-time polymerase chain reaction (PCR) and western blotting. **Results**: OCN mRNA and protein expressions were significantly lower in EC tissues than adjacent normal tissues ($p < 0.001$). Low OCN expression was significantly associated with the International Federation of Gynecology and Obstetrics (FIGO) stage, lymph node metastasis, and depth of myometrial invasion ($p < 0.01$). Kaplan-Meier analysis exposed a significant difference in overall survival (OS) and disease-free survival (DFS) between EC patients with low and high OCN expression levels (log-rank, both $p < 0.001$). Moreover, multivariate Cox regression analysis showed that OCN expression was an independent predictive factor for overall survival (OS) ($p = 0.043$) and disease-free survival (DFS) ($p = 0.027$) in EC patients. Spearman's rank correlation analysis stated a positive correlation between serum OCN level and adiponectin ($r = 0.455$, $p < 0.001$) and a negative correlation between serum OCN level and leptin ($r = -0.307$, $p < 0.001$). **Conclusions**: The results speculate that the low OCN expression is associated with the progression and recurrence of endometrial cancer.

Keywords

Endometrial cancer; Osteocalcin; Prognosis; Immunohistochemistry

1. Introduction

Endometrial cancer (EC) originates from the endometrium epithelium, with adenocarcinoma derived from endometrial glands as the most common type [1]. EC is the most common gynecological cancer in developed countries, and its incidence is increasing in China [2]. Standard treatment is only useful for the early and intermediate phases of EC. Advanced EC remains unsatisfactory for treatment and shows a poor prognosis. Early diagnosis benefits EC patients to be treated by various methods, including surgery, adjuvant chemotherapy, or radiation [3]. Therefore, studying EC's pathogenesis and finding a new therapeutic target is essential to improve patients' prognosis and quality of life.

The leading causative hypothesis of endometrial cancer is the "non-antagonistic effect of estrogen", which signals through estrogen receptors and acts as an oncogenic signal [4]. Recently a series of studies have found insulin resistance and obesity are risk factors for EC [5, 6]. Adipose tissue is where androgens are transformed into estrogen in the peripheral tissues. It has metabolic and immunological activities and produces many proteins and hormones as "adipocytokines" [7]. Currently, adipocytokines such as adiponectin and leptin are EC's most selected early risk factors [8, 9]. Lower adiponectin or higher leptin in serum was associated with an increased risk of EC [10, 11]. However, it remains unclear whether other adipocytokines are related to the risk of EC.

Osteocalcin (OCN) is a type of noncollagenous protein that is produced and released by osteoblasts. OCN consists of fully carboxylated osteocalcin and undercarboxylated osteocalcin [12]. Most OCN is catalyzed by vitamin K to produce carboxylated OCN. A small amount of OCN is not carboxylated or incompletely carboxylated and is directly secreted into the blood to participate in energy metabolism and play a hormone-like role [12]. It has been corroborated that there is a close

relationship between OCN and glycolipid metabolism and insulin resistance. OCN knockout mice show symptoms such as hyperglycemia, hypoinsulinemia, and obesity [13]. Clinical studies also show that uncarboxylated forms of OCN were lower in prediabetic individuals than healthy volunteers and negatively associated with fasting insulin levels [14]. Further, OCN could prevent obesity and glucose intolerance by enhancing peripheral insulin sensitivity [15]. OCN is also a cancer biomarker; its serum levels were associated with the risk and osteonecrosis of prostate cancer [16, 17]. Moreover, OCN-positive cells in circulation were a diagnostic biomarker for breast cancer bone metastasis [18]. However, the possible effect of the OCN expression level in EC patients remains unexplored. Therefore, we first time reported the potential impact of OCN expression level associated with the progression and recurrence of EC.

In the present study, we investigated the expression of OCN in normal and malignant endometrial tissue by quantitative PCR, western blot, and immunohistochemistry (IHC). We then analyzed associations of OCN expression with clinicopathological features of EC patients. And we also examined the correlation between OCN expression and serum adiponectin and leptin levels of EC patients.

2. Materials and methods

2.1 Patients and specimens

In this retrospective study, we included 134 primary EC patients who underwent hysterectomy. Patients were enrolled from July 2010 to December 2013 at the Department of Obstetrics and Gynecology, Shanghai Pudong Hospital. The inclusion criteria are as follows: (1) Female EC patients with underwent a hysterectomy, (2) Ages between 37–78, (3) EC patients without previous chemotherapy or radiation treatments record. In this study, we included healthy female controls and non-EC patients who underwent a hysterectomy ages between 37–78. We excluded patients without undergoing a hysterectomy, ages outside between 37–78 and EC patients who have previous chemotherapy or radiation treatment records. Normal tissues were collected from surgical samples of 35 non-EC patients who underwent a hysterectomy due to hysteromyoma or uterine prolapse. The EC patients were divided into four stages according to the criteria of the FIGO [19], and they were also divided into three histological grades according to World Health Organization (WHO) histopathological grading system standards [20]. Fresh tissues were collected from 5 EC patients and 4 healthy people and stored at −80 °C. A venous blood sample was collected from patients pre-operatively on the 10–25th day of the menstrual cycle from each patient and healthy controls and underwent centrifugation at 1500 × g for 5 min, then separated serum was stored at −20 °C.

2.2 Real-time quantitative PCR

Total RNA was extracted from EC and normal tissues using TRIzol® reagent (15596026, Invitrogen, Life Technologies, Shanghai, China) and reversely transcribed into cDNA using Superscript II reverse transcriptase (18064022, Toyobo, Osaka, Japan). The PCR reaction system contained 2 μL of the cDNA sample solution, 10 μL of SYBR-Green PCR master mix, 0.5 μL of forward and reverse primers (1 μM), and 7.5 μL of water (H_2O) and PCR was performed in Applied Biosystems® 7300 system (ABI 7300, Thermo Fisher Scientific, Waltham, MA, USA). The primer sequences were as follows: OCN (forward: 5′-ATG AGA GCC CTC ACA CTC CTC-3′; reverse: 5′-GCC GTA GAA GCG CCG ATA GGC-3′). And glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5′-ACC ACA GTC CAT GCC ATC AC-3′ and reverse 5′-TCC ACC ACC CTG TTG CTG TA-3′. The amplification condition was denaturation at 95 °C for 5 min, followed by 40 cycles at 95 °C for 10 s and 58 °C for 45 s. The mRNA levels of Osteocalcin were analyzed by the $2^{-\Delta\Delta Ct}$ method and normalized to those of GAPDH.

2.3 Western blotting

Five EC and four normal tissues were frozen and then lysed at 4 °C for 30 min with radioimmunoprecipitation assay (RIPA) buffer (Abcam, Cambridge, MA, USA). Proteins concentrations were measured by the Bicinchoninic acid (BCA) protein assay kit (P0012S; Beyotime, Shanghai, China) and separated by 10% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Then proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (EMD Millipore, Burlington, MA, USA), which was blocked with 5% skim milk for 2 h. Membranes were incubated overnight at 4 °C with primary antibodies against osteocalcin (1:1000; Abcam, Cat# ab133612) and GAPDH (1:2000; Abcam, Cat# ab9485). After washing three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20, membranes were incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:5000; Abcam, Cat# ab7090) for 2 h at room temperature. Protein bands were detected using enhanced chemiluminescence (Thermo Fisher Scientific, USA). The experiments were repeated three times.

2.4 Immunohistochemistry

A total of 134 formalin-fixed, paraffin-embedded EC and 35 non-tumor tissues were collected, and these paraffin specimens were cut into 4-μm sections. Sections were deparaffinized with xylene and treated with a graded series of alcohol. Each section was boiled for 5 min in 0.01 M citrate buffer (pH 6.0), incubated with 0.3% hydrogen peroxide (H_2O_2) in methanol for 30 min to block endogenous peroxidase activity and incubated with blocking solution (BSA) for 20 min. The slides were incubated with a rabbit polyclonal to osteocalcin (1:200; ab93876, Abcam, UK) at 4 °C overnight, and then the slides were washed with PBS, followed by incubation with the secondary antibody. The colour reaction was visualized by staining with diaminobenzidine (DAB), followed by counterstaining with hematoxylin. Two independent individuals observed the staining under a microscope, according to the scoring method described in a previous publication [21]. The intensity of staining was graded on a scale of 0–3 (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining), and the distribution of stained tumor cells was graded on a scale of 0–4 (0, negative; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%). Then, OCN protein expression levels were semi-quantitatively

classified by multiplying two scores. Samples with a 0–3 indicate low expression, and a score of 4–12 indicates high expression. The negative control of immunohistochemistry was a buffer that replaced the osteocalcin antibody, and human osteosarcoma tissue other than endometrial carcinoma was used as a positive control.

2.5 ELISA assay

Serum undercarboxylated osteocalcin (Cat# DSTCN0), adiponectin (Cat# DRP300), and leptin (Cat# DLP00) were measured using a sandwich ELISA assay (R&D Systems, MN, USA). The absorbance was measured at a wavelength of 450 nm by a microplate reader. The concentrations of osteocalcin, adiponectin, and leptin were calculated according to the standard curve.

2.6 Statistical analysis

The Chi-square or Fisher's exact test was performed to analyze the between-group differences in category parameters. A *t*-test was performed to compare the difference between the two groups. Kaplan-Meier method and log-rank test were performed for survival analysis. A Multivariate Cox regression model was used to test the independent prognostic factors. Spearman's rank correlation analysis was used to estimate the correlations between serum OCN levels and adipocytokine markers. Data for quantitative reverse transcription (qRT)-PCR and western blot were presented as mean ± standard deviation (SD). Statistical analysis was performed by statistical package for the social sciences (SPSS) software (SPSS version 20.0, IBM, Chicago, IL, USA).

3. Results

3.1 OCN expression was decreased in EC patients

Fresh frozen tissues from 5 EC and 4 normal endometrial tissues were used to analyze the OCN mRNA and protein expressions by reverse transcription (RT)-qPCR and Western blot. *t*-test analysis showed that the OCN mRNA and protein levels were significantly lower in EC tissues than in normal endometrial tissues ($p < 0.001$; Fig. 1A–C).

3.2 OCN expression and clinicopathological characteristics

A total of 134 paraffin-embedded EC samples were analyzed by immunohistochemistry. OCN expression was detected using immunohistochemistry in 134 EC tissues and 35 non-tumor tissues. We found that OCN had strong staining in various non-EC samples and high/weak staining in EC samples (Fig. 2). Besides, the positive osteocalcin staining was mainly observed in the cytoplasm. The Chi-square test showed that 36.6% (49/134) of EC patients have a low expression of OCN, whereas only 15.4% (8/52) of normal endometrium specimens showed low OCN expression ($p = 0.005$, Table 1). Based on OCN expression, patients were divided into two subgroups: OCN high expression (n = 85) and OCN low expression (n = 49). Chi-square test analysis showed that low expression of OCN was associated with high FIGO stage ($p < 0.001$), lymph node metastasis ($p = 0.001$), and deep myometrial invasion ($p = 0.011$; Table 2).

3.3 Association between OCN expression and prognostic parameters

Kaplan-Meier analysis suggested that their patients with low OCN expression had significantly lower OS and DFS (Fig. 3). Univariate Cox regression model analysis showed a strong correlation between OCN expression and OS and DFS in EC patients (OS: $p = 0.005$, DFS: $p = 0.003$; Table 3). Multivariate Cox regression analysis showed that OCN expression and lymph node metastasis were independent prognostic factors for both OS and DFS (OS: $p = 0.043$, DFS: $p = 0.027$; Table 4).

3.4 Correlation between OCN expression and serum adiponectin and leptin levels of EC patients

To investigate associations between OCN expression in EC specimens and serum adipocytokine level, an ELISA assay was performed to measure the serum levels of OCN, adiponectin, and leptin, and the OCN was evaluated in serum undercarboxylated form. Serum level of OCN was significantly lower in patients who died during follow-up (n = 32) than in patients who were surviving (n = 102) (Fig. 4A). Compared to patients with high osteocalcin expression, patients with low osteocalcin expression had significantly lower serum levels of OCN and adiponectin and higher leptin level (all $p < 0.001$; Fig. 4B–D). Then Spearman's rank correlation analysis was performed between serum OCN and adiponectin or leptin. Results showed that serum OCN level positively correlated with adiponectin ($r = 0.455$, $p < 0.001$) and negatively correlated with leptin ($r = -0.307$, $p < 0.001$) (Fig. 4E,F).

4. Discussion

In this study, we investigated the expression of OCN in EC tissues and its associations with clinicopathological characteristics and prognosis. The study results demonstrated (1) the OCN mRNA and protein levels were significantly lower in EC tissues than in normal endometrial tissues ($p < 0.001$); (2) low expression of OCN was associated with high FIGO stage ($p < 0.001$), lymph node metastasis ($p = 0.001$), and deep myometrial invasion; (3) OCN expression and lymph node metastasis were independent prognostic factors for both OS and DFS (OS: $p = 0.043$, DFS: $p = 0.027$); and (5) serum OCN level positively correlated with adiponectin ($r = 0.455$, $p < 0.001$) and negatively correlated with leptin ($r = -0.307$, $p < 0.001$). Therefore, OCN expression level could be associated with the progression and recurrence of endometrial cancer.

We analyzed the association between OCN expression levels and several clinicopathologic features. We showed low OCN expression is associated with advanced FIGO stage, more cases with lymph node metastasis and infiltration in a deep muscular layer in endometrial cancer. Besides, patients with low OCN expression had shorter overall survival (OS) and disease-free survival (DFS) than patients with high OCN expression. Our results follow another report: OCN expression was reduced

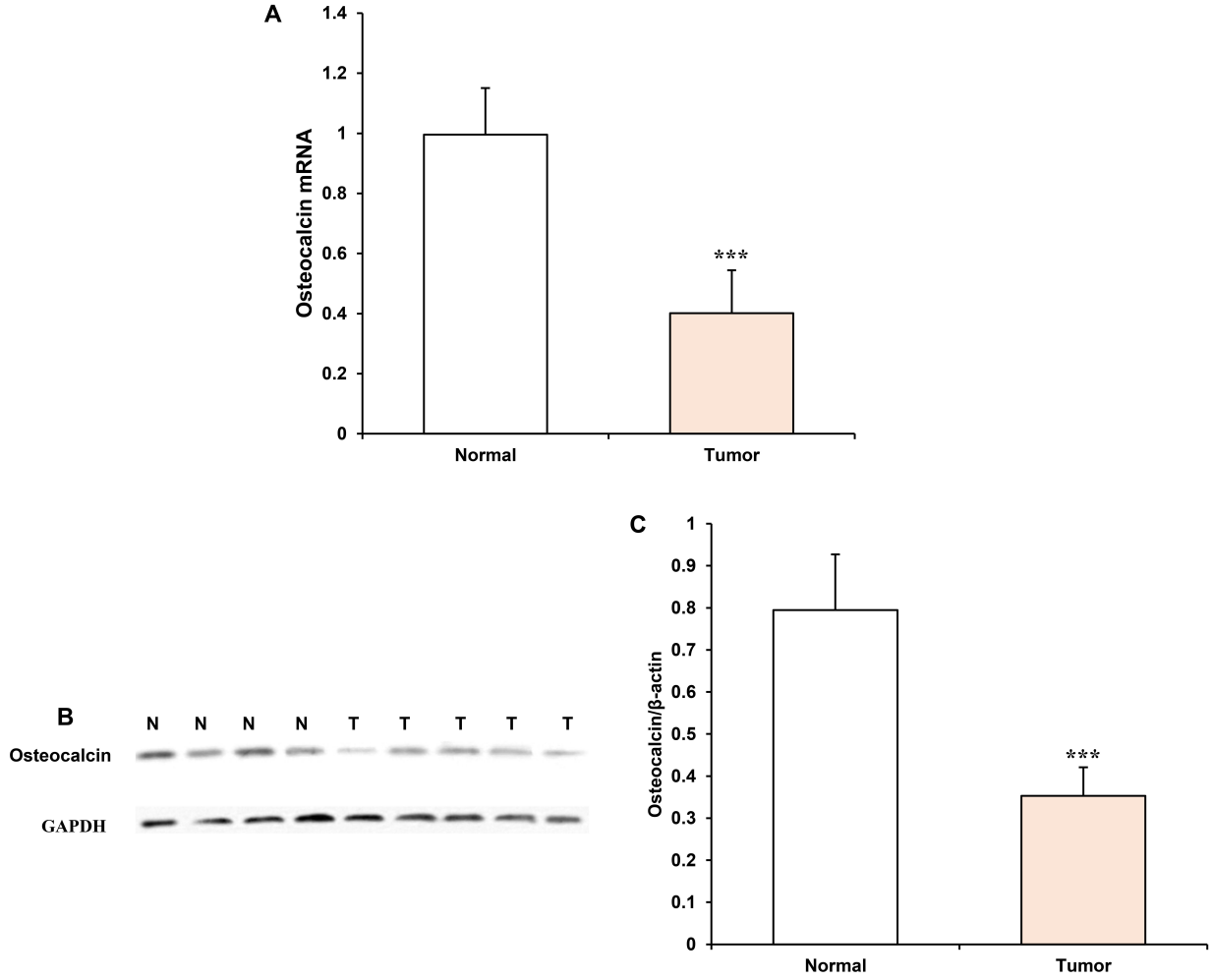

FIGURE 1. Expression of OCN in EC tissues. (A) mRNA expression of OCN in normal endometrial tissues (n = 4) and EC tissues (n = 5). (B) Representative OCN protein bands of Western blot in endometrial tissues (N) and EC tissues (T). (C) Quantitative analysis shows that OCN protein level is lower in EC tissues as compared to normal endometrial tissues. The data are presented as mean ± SD. ***$p < 0.001$ *vs.* normal endometrial tissues. OCN, osteocalcin; EC, endometrial cancer; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

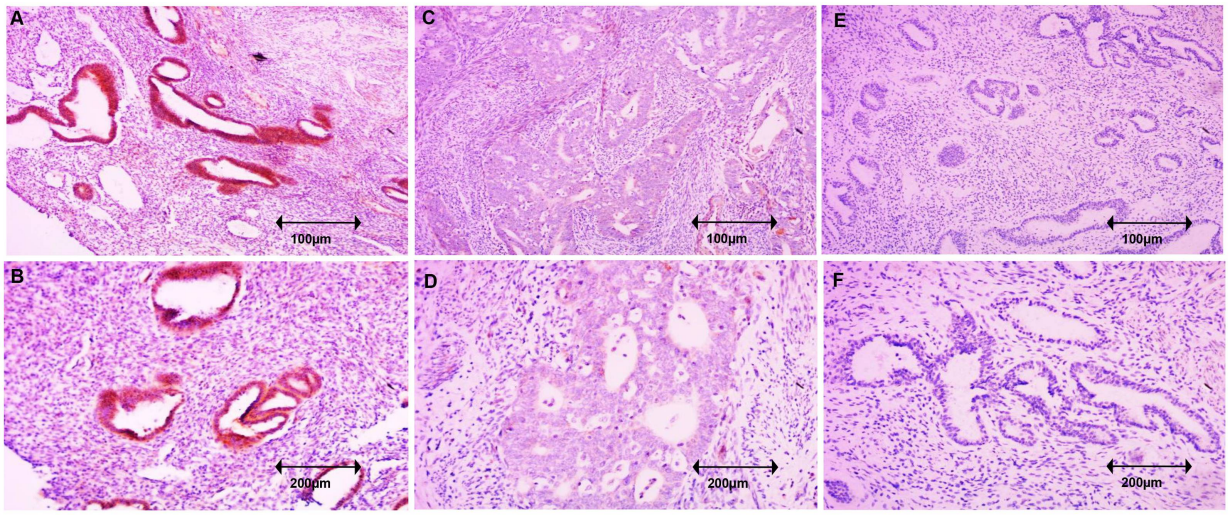

FIGURE 2. Immunohistochemical staining of OCN in EC tissues. (A,B) High expression of OCN in normal endometrial tissues (×100; ×50). (C,D) High/Weak expression of OCN in EC (×100; ×50). (E,F) Low expression of OCN in EC (×100; ×50).

TABLE 1. Expression levels of osteocalcin in endometrial carcinoma.

Groups	Sample	OCN expression				*p*-value
		High	Percentage (%)	Low	Percentage (%)	
Endometrial cancer	134	85	63.43	49	36.57	0.005
Normal endometrium	52	44	84.62	8	15.38	

Chi-square test was performed. OCN, osteocalcin.

TABLE 2. Correlation between OCN expression and clinicopathological characteristics of EC.

Variables	Patients	OCN expression				*p*-value
		High	Percentage (%)	Low	Percentage (%)	
Age (yr)						
<60	91	62	68.13	29	31.87	0.125
≥60	43	23	53.49	20	46.51	
Histological grade						
G1	53	35	66.04	18	33.96	
G2	48	31	64.58	17	35.42	0.715
G3	33	19	57.58	14	42.42	
FIGO stage						
I	81	60	70.07	21	25.93	
II	25	16	64.00	9	36.00	<0.001
III	19	8	42.11	11	57.89	
IV	9	1	11.11	8	88.89	
Lymph node metastasis						
No	121	83	68.60	38	31.40	0.001
Yes	13	3	23.08	10	76.82	
Depth of myometrial invasion						
<50%	63	47	74.60	16	25.40	0.011
≥50%	71	38	53.52	33	46.48	

Chi-square test was performed. EC, endometrial cancer; OCN, osteocalcin; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; FIGO, International Federation of Obstetricians and Gynecologists.

in canine osteosarcoma samples and was associated with poor prognosis [22]. Thus, OCN could be a valuable biomarker demonstrating anti-tumor effects on endometrial cancer.

A growing number of reports have shown that OCN plays a multifaceted role in cancer. However, there are conflicting results about the parts of OCN in cancer progression and prognosis. Serum OCN levels were increased in breast cancer patients with bone metastasis than in non-metastatic patients [23]. One study using rat osteoblasts reported that estrogen receptor activation enhances osteoblast proliferation and OCN production [24]. However, serum undercarboxylated OCN was positively associated with advanced-stage and high-grade prostate cancer [16]. On the other hand, one report showed that serum OCN levels were decreased in Hepatitis B Virus (HBV)-related hepatocellular carcinoma (HCC) patients compared with healthy controls [25]. It seems that the function of OCN is dependent on tumor types. There are two types of OCN, namely, carboxylated or undercarboxylated OCN.

The carboxylated OCN confers a high affinity for bone matrix, whereas the undercarboxylated one plays a role in energy metabolism [26]. Both carboxylated or undercarboxylated OCN promoted average prostate epithelial cell growth. However, the development of prostate cancer cells was accelerated by carboxylated OCN but suppressed by undercarboxylated OCN [27]. Moreover, undercarboxylated OCN also suppressed melanoma growth *in vitro* and *in vivo* through cellular immunostimulatory effects [28]. Therefore, OCN is not merely a cancer biomarker, and its undercarboxylated form might exert an anti-tumor effect. OCN expression is restricted to osteoblasts. Osteocalcin protein was undetectable in primary prostate tumors or lymph nodes. It was still highly expressed in bone-metastasized prostate tumors, and serum OCN level was elevated by releasing from osteoblastic lesions in metastatic bone tumors [29]. Our current study explains why serum OCN level is increased in breast and prostate cancer patients with bone metastasis. Our study first showed decreased OCN expression in endometrial cancer.

Moreover, decreased serum OCN was associated with poor overall survival, lower serum adiponectin, and higher serum leptin. As there was no bone metastasis in patients of this

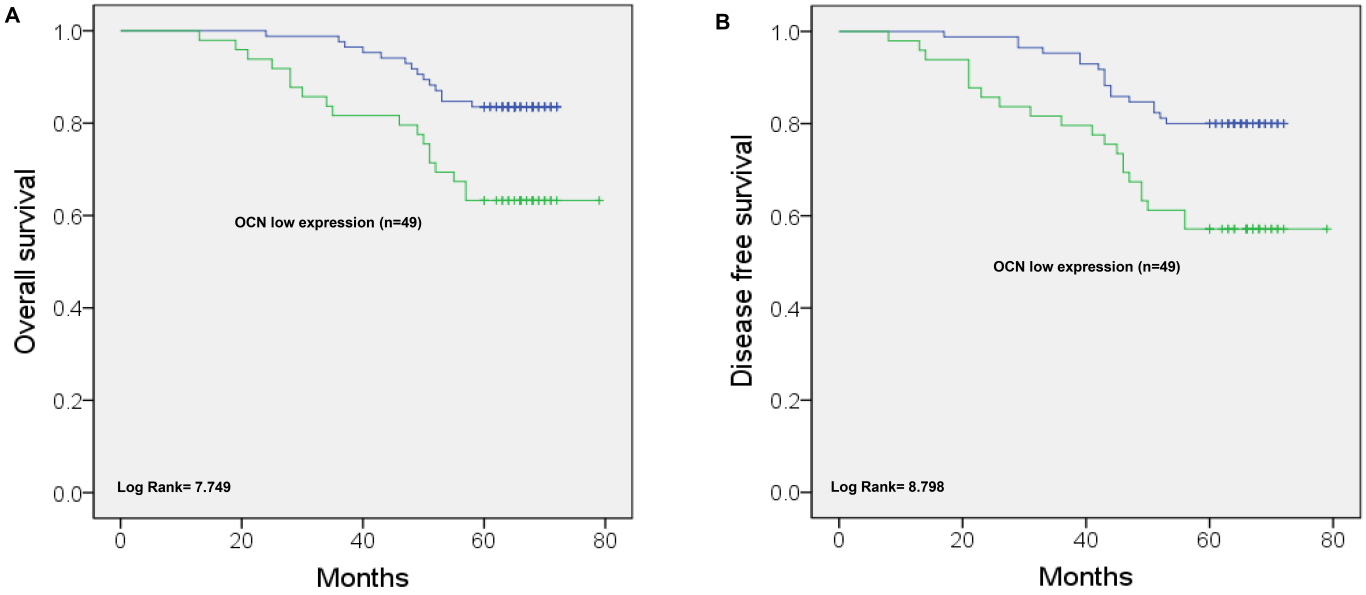

FIGURE 3. Survival of EC patients is dependent on OCN expression. (A) Kaplan-Meier analysis of OS. Patients with low OCN expression have a more inferior OS than those of patients with high OCN expression. (B) Kaplan-Meier analysis of DFS. Patients with low OCN expression have a poorer DFS than those of patients with high OCN expression. OS, overall survival; DFS, disease-free survival.

TABLE 3. Univariate survival analysis of OS and DFS in EC patients.

Variables	n	OS		*p*-value	DFS		*p*-value
		Mean ± standard error (SE)	95% confidence interval (CI)		Mean ± SE	95% CI	
Age (yr)							
<60	91	66.2 ± 1.3	63.7–68.7	0.204	64.0 ± 1.6	60.9–67.0	0.204
≥60	43	66.7 ± 3.1	60.7–72.8		63.5 ± 3.5	56.6–70.3	
Histological grade							
G1	53	70.5 ± 2.2	66.1–74.8		66.5 ± 2.7	61.3–71.8	
G2	48	65.5 ± 2.1	61.5–69.6	0.532	63.6 ± 2.4	58.9–68.4	0.622
G3	33	62.5 ± 2.6	57.5–67.6		60.6 ± 3.0	54.7–66.5	
FIGO stage							
I	81	74.4 ± 1.3	71.8–77.1		72.2 ± 1.6	69.0–75.5	
II	25	62.2 ± 2.9	56.4–67.9	0.003	58.8 ± 3.5	52.0–65.6	0.007
III	19	57.1 ± 4.6	48.1–66.2		53.9 ± 5.3	43.5–64.3	
IV	9	54.7 ± 6.0	42.8–66.5		50.2 ± 6.7	37.2–63.3	
Lymph node metastasis							
No	121	72.6 ± 1.3	70.1–75.0	<0.001	70.1 ± 1.5	67.1–73.1	<0.001
Yes	13	45.2 ± 5.9	33.6–56.8		39.8 ± 6.3	27.5–52.2	
Depth of myometrial invasion							
<50%	63	68.0 ± 1.0	65.9–70.0	0.004	66.6 ± 1.3	64.0–69.2	0.002
≥50%	71	65.9 ± 2.4	61.2–70.5		62.0 ± 2.7	56.7–67.3	
OCN expression							
High	85	67.7 ± 1.1	65.5–69.9	0.005	65.8 ± 1.4	63.0–68.6	0.003
Low	49	64.3 ± 3.0	58.4–70.2		60.3 ± 3.4	53.7–66.9	

OS, overall survival; DFS, disease-free survival; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; FIGO, International Federation of Obstetricians and Gynecologists; OCN, osteocalcin.

TABLE 4. Multivariate survival analysis of OS and DFS in EC patients.

Variables	OS		*p*-value	DFS		*p*-value
	Exp (B)	95% confidence interval (CI)		Exp (B)	95% CI	
Lymph node metastasis	4.915	2.216–10.902	<0.001	4.583	2.177–9.651	<0.001
OCN expression	2.103	1.020–4.316	0.043	2.098	1.087–4.047	0.027

OS, overall survival; DFS, disease-free survival; OCN, osteocalcin.

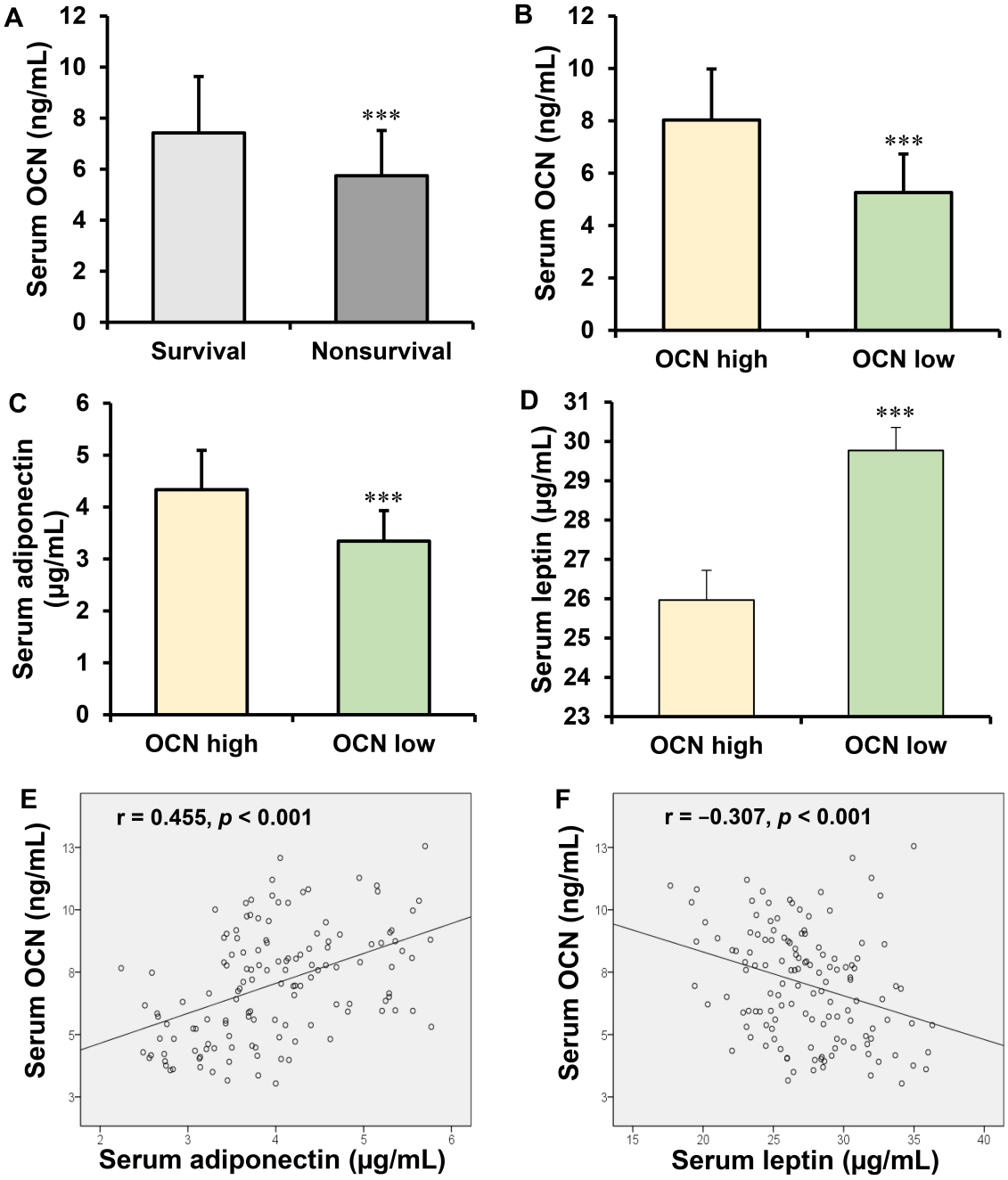

FIGURE 4. Associations of OCN with serum adiponectin and leptin, and the OCN was evaluated in serum undercarboxylated form. Patients were divided into two groups according to OCN expression. ELISA assay was performed. (A) Serum OCN level was lower in patients who died than patients who were survival. Compared to the OCN high group, OCN low group has significantly lower serum OCN (B) and adiponectin (C) and significantly higher serum leptin (D). Pearson correlation analysis shows serum OCN positively correlates with adiponectin (E) and a negative correlation with leptin (F). ***$S < 0.001$ *vs.* patients with high OCN. OCN, osteocalcin.

study, this implies that serum OCN might be released from intact bone, mainly undercarboxylated OCN. Therefore, OCN act as an anticancer molecule in endometrial cancer.

In the present study, serum OCN showed a positive correlation with serum adiponectin and a negative correlation with serum leptin. Patients with endometrial cancer had lower serum adiponectin levels and higher serum leptin levels than healthy controls [10]. Serum adiponectin level was negatively correlated with EC [30], suggesting that adiponectin may be a potential tumor suppressor [31]. Low adiponectin and high leptin indicate insulin resistance [32], which is associated with an increased risk of endometrial cancer [5]. Moreover, OCN is a negative regulator of insulin resistance. Serum undercarboxylated OCN levels were inversely associated with insulin resistance [33] and the risk of developing type 2 diabetes mellitus [34]. OCN suppresses insulin resistance through various pathways. Undercarboxylated OCN effectively improved resistance in hepatic tissue and white adipose tissue.

Furthermore, OCN increases the secretion of undercarboxylated OCN into circulation by improving insulin resistance [35, 36]. This indicates mutual regulation between insulin resistance and OCN: OCN inhibits insulin resistance, and insulin resistance reduces OCN gene expression in human osteoblast-like cells [37]. Therefore, OCN may represent a novel therapeutic target in reversing insulin resistance of endometrial cancer.

Our study also has several limitations. Firstly, the sample size was relatively small, and a more significant number of EC patients should validate a more definite conclusion. Secondly, this is a prospective study, and a prospective study is also needed to validate the predictive effect of OCN on the progression of endometrial cancer. Thirdly, OCN can increase testosterone secretion from Leydig cells and be inversely associated with estrogen [38, 39]. Since the "non-antagonistic effect of estrogen" is the central causative hypothesis of endometrial cancer, whether OCN downregulates estrogen signal in endometrial cancer remains unclear and needs further investigation.

5. Conclusions

The study results show that OCN expression is decreased in EC tissues, and low OCN expression is closely correlated with cancer progression and poor outcomes in EC patients. OCN may serve as a new biomarker and potential therapeutic target of EC.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

JSG and LMG—performed experiments and wrote the first draft of manuscript; HYH and HZ—collected clinical data, analyzed the data, and performed statistical analysis; YCD—designed and supervised the study and revised it. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and was approved by Shanghai Pudong Hospital Ethics Committee (Date of Issue April 2020; NO. WZ-009). All participants provided written consent to participate in this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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