Expression of Endocan in Tissue Samples from Prostate Adenocarcinoma and Prostate Hyperplasia: A Comparative Retrospective Study

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Purpose: In this study, we aimed to determine whether there is a significant difference in endocan expression levels between prostate adenocarcinoma and prostate hyperplasia tissues by using an immunohistochemical method.

Materials and Methods: All 51 patients, who were getting treatment for the last 5 years, participated in the study. 31 of 51 patients underwent transrectal sonography (TRUSG) -assisted prostate biopsy because of prostate adenocarcinoma as diagnosed with elevated PSA levels and histopathological examination. The remaining 20 patients comprised the control group. The control group included patients with benign prostate hyperplasia based on pathological examination.

Results: It was found that there was strong positive epithelial staining in 74.2% of patients with prostate cancer while in 5% of controls, indicating a statistically significant difference ($P < .001$). It was also found that the rate of strong positive endothelial staining was 77.4% in the patient group whereas 5% in the control group ($P < .001$). Also, the rate of strong positive stromal staining was 64.5% in the patient group while 5% in the control group ($P < .001$).

Conclusion: We found that tissue endocan expression level was statistically significantly higher in patients with prostate cancer compared to those with benign prostate hyperplasia by using an immunohistochemical method.

Keywords: benign prostate hyperplasia; endocan; expression; immunohistochemistry; prostate adenocarcinoma

INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men worldwide, with an estimated 1 276 000 new cancer cases and 359 000 deaths in 2018[(1)] The worldwide prostate cancer burden is expected to grow to almost 2.3 million new cases and 740 000 deaths by 2040 simply due to the growth and aging of the population.[(2)] In a study conducted in Iran in 2008-2010 that investigates the geographical frequency and degree of prostate cancer and evaluates its relationship with ethnicity, the average 3-year PCa incidence rate standardized for age was found to be 11.52 per 100,000 men.[(3)]

Given biological heterogeneity and clinical variability in localized PCa, an individualized approach is required for risk stratification and management.[(4)] To date, prostate-specific antigen (PSA) is the only biomarker approved for detection and prognostication of prostate cancer by the US Food and Drug Administration (FDA).[(5)] Initially, PSA allowed identifying more patients with PCa at early stages. However, PSA screening also causes overdiagnosis and overtreatment.[(6)] PSA showed severe limitations and inconsistencies as a diagnostic and prognostic marker for prostate cancer.[(7)]

In recent years, proteoglycans (PGs) have emerged as critical modulators of key cellular processes such as cell proliferation, adhesion, and migration, which are linked to several pathological conditions including inflammation, cancer, or infection.[(8)] Endocan (ESM-1 = Endothelial Cell-Specific Molecule 1) is one of the novel and promising biomarkers. Endocan is produced by many distinct types of the cell including prostatic epithelium. Endocan is a soluble dermatan sulfate (50 kDa in length) proteoglycan and it was first cloned from complementary DNA library of human umbilical vein endothelium by Lasalle et al. in 1996.[(9)] Endocan secreted by active vascular endothelial cells including tumor endothelium.[(10)] Also, endocan is present in the cell surface, extracellular matrix, and body fluids.[(9)] Endocan can be involved in molecular interaction in biologically active conditions such as cell adhesion, migration, proliferation, or neovascularization.[(11)] The excessive levels of endocan in sepsis, cancers, and inflammatory disorders suggest that it may play a role in the pathogenesis of these disorders.[(12-18)]

Some studies investigated endocan in bladder cancer, renal cell cancer, prostate cancer, and erectile dysfunction in the literature.[(19-22)] It was reported that serum endocan levels were significantly higher in patients who developed biochemical recurrence after radical prostatectomy in early prostate cancers.[(21)] There is a limited number of studies that investigated endocan expression in tissues from prostate cancer or benign prostate hyperplasia by using immunohistochemical techniques. In this study, we aimed to investigate whether there is

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Table 1. Data of patients with prostate cancer

Patient number	Age (year)	PSA(ng/dL)	Gleason Score	Number of tumors monitored foci	Prostate size (mL)
1	63	6.06	6 (3+3)	5	48
2	61	11.49	6 (3+3)	8	86
3	69	17.96	6 (3+3)	4	78
4	56	8.75	6 (3+3)	2	110
5	47	7.84	6 (3+3)	4	56
6	72	9.29	6 (3+3)	5	86
7	70	6.51	6 (3+3)	3	94
8	59	4.59	6 (3+3)	3	105
9	61	9.64	6 (3+3)	5	65
10	65	11.84	6 (3+3)	3	65
11	70	4.78	5 (3+2)	3	82
12	68	8.52	6 (3+3)	8	92
13	65	10.91	6 (3+3)	4	54
14	61	4.89	6 (3+3)	6	120
15	64	6.08	6 (3+3)	5	88
16	77	40.79	7 (3+4)	3	64
17	60	5.40	7 (3+4)	11	75
18	79	100	7 (3+4)	11	50
19	62	100	7 (3+4)	9	45
20	71	14.63	7 (3+4)	6	78
21	77	100	7 (4+3)	10	44
22	77	90.32	8 (4+4)	5	86
23	56	30.47	8 (4+4)	6	54
24	61	9.07	8 (4+4)	3	74
25	60	8.23	8 (4+4)	5	56
26	75	43.28	8 (4+4)	12	130
27	65	41.00	8 (4+4)	8	56
28	76	100	9 (4+5)	12	44
29	59	50.59	9 (4+5)	11	78
30	83	27.42	9 (4+5)	12	120
31	**82**	**100**	**9 (5+4)**	**10**	**88**

a statistically significant difference in endocan expression level between PCa cancer and benign prostate hyperplasia (BPH) tissues by using an immunohistochemical method.

MATERIALS AND METHODS

This retrospective study was conducted in the Research and Training Hospital of Kırsehir Ahi Evran University. The study was approved by the local Ethics Committee (approval#2017-17/201). It was conducted by following the Helsinki Declaration. Between 2012 and 2017, 51 patients were included in this study. Of the 51 patients, 31 underwent transrectal ultrasonography (TRUSG)-assisted prostate biopsy because of prostate adenocarcinoma as diagnosed with elevated PSA level and a histopathological examination (**Table 1**). The remaining 20 patients comprised the control group. The patients in the control group were selected based on the benign surgical pathological results (**Table 2**).

Study Design

Individuals in the patient group had undergone 12-core biopsy and were diagnosed with prostate adenocarcinoma by examination of the first biopsy. A prostate needle biopsy was performed under local anesthesia for all patients. Under lateral decubitus position, the perianal region was cleaned with povidone-iodine. A local anesthetic gel with lidocaine was squeezed into the rectum. A TRUSG probe was inserted, and the measurements of the prostate gland were done. A periprostatic nerve block was done with lidocaine for all patients. A systematic 12 core biopsy from parasagittal and peripheral basal, middle and apical regions was performed. Gleason score was recorded in these cases. The patients who were diagnosed in the biopsy other than the first attempt and/or those who underwent biopsy by different core numbers other than 12, and those considered to have locally advanced or metastatic cancer were excluded. The remaining 20 patients comprised the control group. The patients in the control group were selected based on the benign surgical pathological results. Patients with chronic prostatitis were also excluded. Besides, patients with systemic comorbidity were excluded. 4 patients of the control group underwent transvesical prostatectomy due to recurrent urinary retention and a mean prostate size of 130 g . We performed transurethral prostatectomy due to recurrent retention and unresponsiveness to medical therapy in the remaining 16 individuals in the control group. We determined endocan expression in prostate tissue by using paraffin-embedded blocks from 51 patients in an immunohistochemical manner. The PSA levels of the cases (pre-biopsy and preoperative) included in the study in both groups were reached and recorded.

Immunohistochemistry

Tissue sections (4 µm in thickness) were cut from paraffin blocks, which were then deparaffinized and labeled by BenchMark XT Automated IHC/ISD slide staining system (Ventana, Medical Systems, Tucson, AZ) using recommended kits (Ultra View Universal DAB Detection Kit; Ventana Medical Systems Inc., Tucson, AZ). Immunohistochemical evaluations were performed by using an anti-endocan mouse monoclonal antibody (3 mg/mL; ab56914: Endocan antibody (anti-ESM1 antibody); 100 µg at 0.5 mg/mL; mouse monoclonal suitable for IHC-P, WB reacts with: Human) in formalin-fixed, paraffin-embedded tissues. Renal tissue was used as a positive control as recommended in the protocol (**Figure 1. A-B**). Immunostaining was assessed independently by a pathologist who was blinded to clinical findings and sample characteristics. Endothelial, epithelial, and stromal cells with brownish cytoplasm were considered as positive staining for en-

Table 2. Data of the patients with benign prostatic hyperplasia

Patient number	Diagnostic Method	Age (year)	PSA (ng/dL)	Prostate size (mL)
1	TUR-P	55	1.78	44
2	TUR-P	61	3.41	56
3	TUR-P	60	4.39	74
4	TUR-P	77	2.66	66
5	TVP	82	8.66	166
6	TVP	71	8.82	185
7	TVP	76	1.97	136
8	TUR-P	73	1.70	78
9	TUR-P	61	3.90	74
10	TUR-P	54	3.13	62
11	TUR-P	83	1.09	50
12	TUR-P	69	1.58	76
13	TVP	75	5.17	140
14	TUR-P	78	4.27	63
15	TUR-P	72	0.44	44
16	TUR-P	55	4.06	56
17	TUR-P	64	5.61	86
18	TUR-P	84	1.67	48
19	TUR-P	55	26.52	96
20	TUR-P	81	10.85	78

Abbreviations: TUR-P, transurethral resection of the prostate; TVP, transvesical prostatectomy

docan. Endocan expression was assessed in epithelial, endothelial, and stromal cells in the tissue samples from patient and control groups. Sections with spotted staining were classified into 4 groups by using a semi-quantitative scoring system based on the intensity of spotted staining. 0, negative; 1, weak cytoplasmic staining in more than 50% of tumor cells; 2, moderate staining in more than 50% of tumor cells and 3, strong staining in more than 50% of tumor cells.[23] Given better visualization, endocan expression in epithelial cells was rated according to staining intensity: 0; negative, 1; weak, 2; moderate, and 3; strong.[16] Endocan expression in endothelial and stromal cells was also rated according to staining intensity: 0; negative, 1; weak, 2; moderate, and 3; strong.

Statistical analysis

Data were analyzed using SPSS version 20.0 (Armonk, New York, USA). Chi-square test was used for categorical variables. For group comparisons, t-test or Mann-Whitney *U* test was used in independent groups, depending on whether the assumptions were met or not. P values under 0.05 were considered statistically significant. G-power 3.1 (Department of Psychology, University of Düsseldorf, Germany) was used for post power analysis.

RESULTS

Mean age was 66.9 ± 1.6 years as a mean ± standard error of the mean (M ± SEM) in the patient group whereas 68.3 ± 2.2 years in the control group. Study and control groups were found to be similar in terms of age ($P = .355$). We found that there was strong positive epithelial staining in 74.2% of patients with PCa while in 5% of controls (BPH patients), indicating a statistically significant difference ($P < .001$) (**Figure 2A, 2B, 2C, 2D blue arrow**). Also, we found that the rate of strong positive endothelial staining was 77.4% in the patient group whereas 5% in the control group ($P < .001$) (**Figure 2A, 2B, 2C, 2D red arrow**). Besides, the rate of strong positive stromal staining was 64.5%

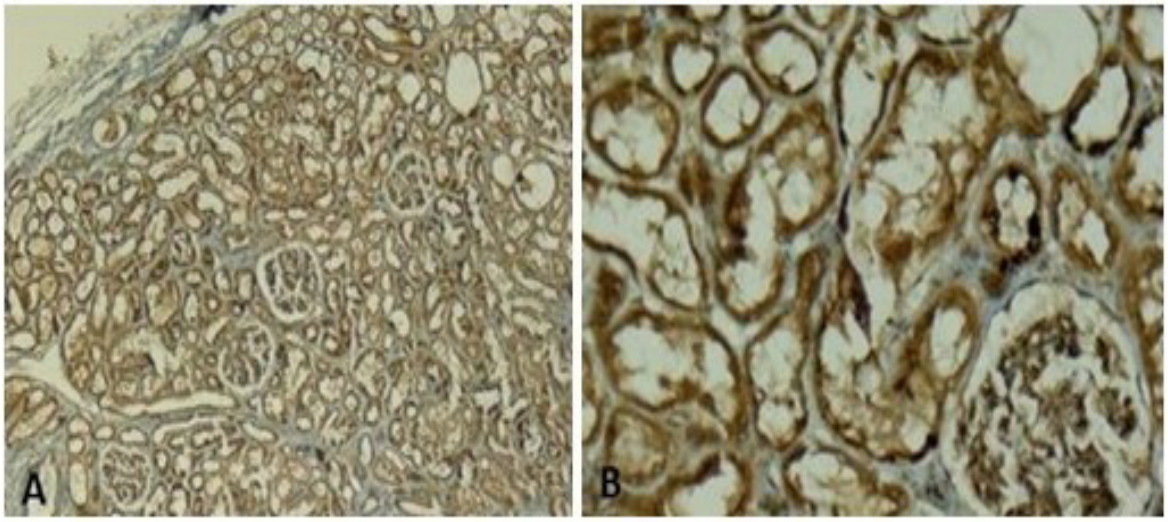

Figure 1. Immunostaining with endocan in kidney tissue (positive control) *[Ax50, Bx200]*

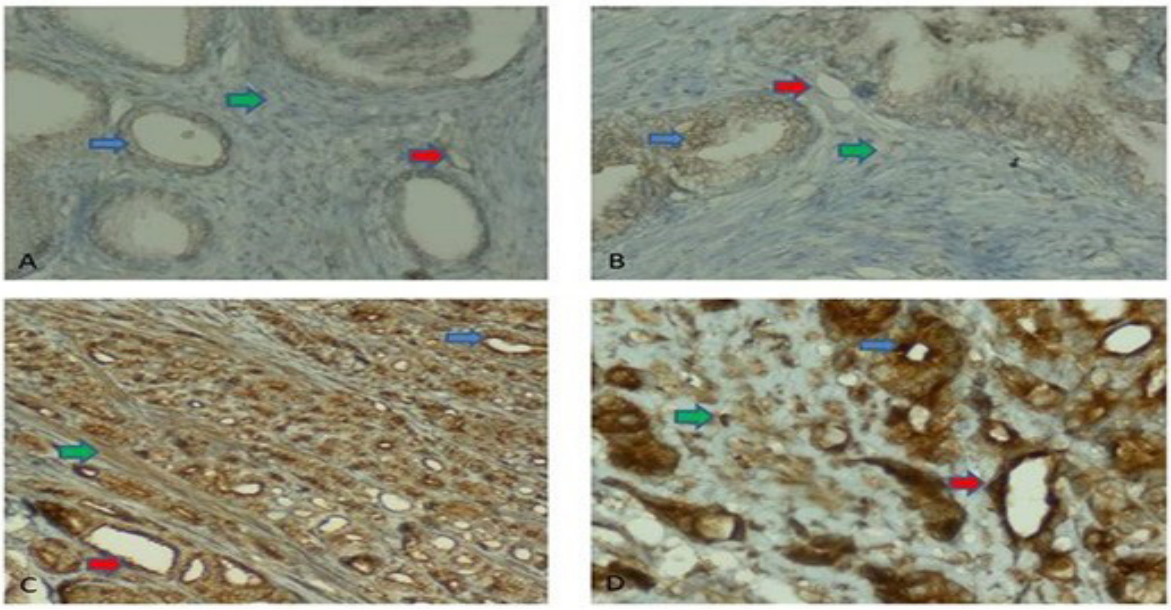

Figure 2. Weak staining with endocan (the blue arrow shows epithelium, the red arrow shows endothelium and the green arrow shows stroma) in benign prostate hyperplastic tissue samples **(A-B)** *[Ax100, Bx200]*. Strong staining with endocan (the blue arrow shows epithelium, the red arrow shows endothelium and, the green arrow shows stroma) in prostate adenocarcinoma tissue samples **(C-D)** *[Ax200, Bx400]*.

in the patient group whereas 5% in the control group ($P < .001$) (**Figure 2A, 2B, 2C, 2D green arrow**). Endocan expression levels in epithelial, endothelial, and stromal cells were presented in Figure 3. Mean PSA levels were 5.5 ± 1.3 ng/mL in the control group while 31.8 ± 6.6 ng/mL in the PCa group ($P < .05$). Study and control groups were compared in terms of PSA and prostate size values; The mean PSA in the biopsy group was found to be statistically significantly higher than the control group ($P < .001$). In terms of prostate size values, there was no significant difference between the study and control groups ($P = .469$).

PCa patients were divided into 3 groups according to their PSA values as PSA ≤ 10, 11 < PSA < 20 and PSA > 20 ng / dL. No statistically significant difference was found between PSA groups and Stroma ($P = .308$), endothelial ($P = .966$), and epithelial ($P = .747$) groups in terms of density and prevalence.

PCa patients were divided into 3 groups as Gleason Score < 7, = 7 and > 7. No statistically significant difference was found between Gleason Score groups and Stroma ($P = .131$), endothelial ($P = .782$), and epithelial ($P = .454$) groups in terms of density and prevalence.

Post-power analysis was performed using the G-Pow-

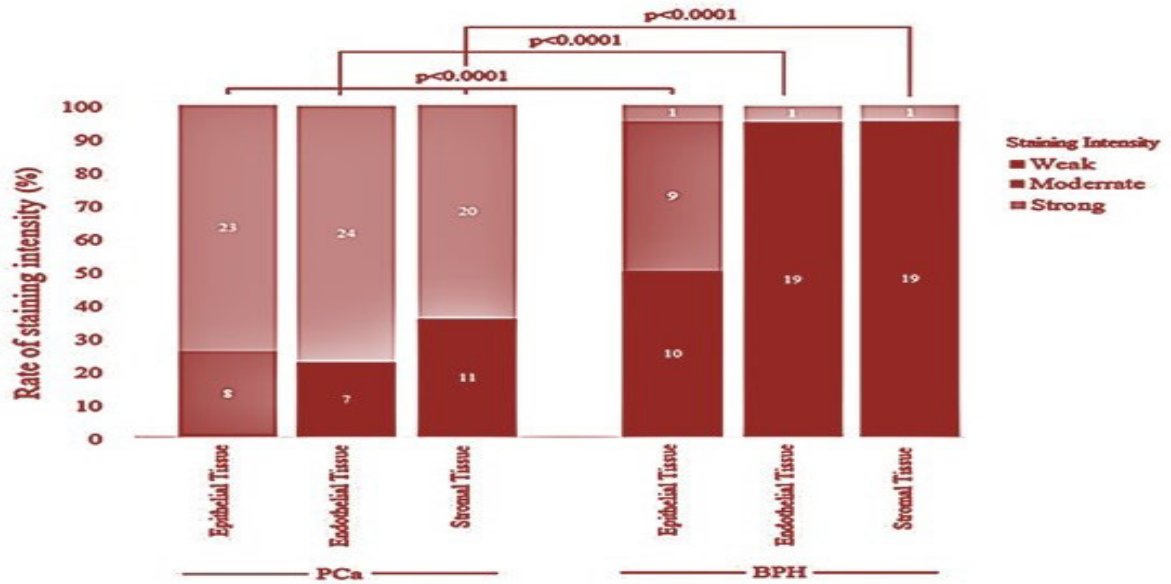

Figure 3. Endocan staining intensity levels of epithelial, endothelial, stromal tissues among groups. The number of tissues in each subgroup (n) was presented inside the column.

er 3.1 to determine the power of the study. According to the results of the study in which a large effect size was achieved between the endothelial, epithelial, and stroma groups and the study groups, the power of the study was found to be 98% at the end of the study with a sample size of 31 people with an error margin of 5%.

DISCUSSION

Considering the strong evidence in the literature regarding the association between endocan and malignant disorders, we hypothesized that endocan may have a role in the pathogenesis of PCa. Our study showed higher strong staining of endocan in tissues from PCa patients which supports our hypothesis. We could not measure tissue or plasma endocan concentrations which could contribute to our study. Nevertheless, our results have the potential to contribute to the existing literature on the topic of using endocan as a biomarker for PCa and distinguishing PCa from BPH.

Previous studies have shown that endocan is associated with the regulation of major processes such as cell adhesion, inflammatory disorders, or tumor progression. (24) Although regulatory mechanisms for endocan production haven't been fully understood, recent studies indicated that numerous signaling pathways and bioactive mediators play a role. Inflammatory cytokines such as vascular endothelial growth factor-A (VEGF-A), VEGF-C, interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), transforming growth factor-ß1, fibroblast growth factor-2 (FGF-2) increase while phosphatidylinositol-3-kinases (PI3K) and interferon-γ decrease during endocan production and secretion.[9,10] In a study investigating the prognostic value of endocan, serum endocan levels were studied.[21] The study included 86 patients who underwent Radical Prostatectomy (RP) due to localized prostate cancer and 80 control patient with the normal digital rectal examination and PSA levels. Serum endocan and PSA levels were measured before the procedure. Biochemical recurrence was defined as serum PSA level > 0.2 ng/mL at the end of year one. The mean serum endocan level was 3.14 ng/mL in the RP group whereas 2.98 ng/mL in the control group. The RP group was stratified into two groups according to serum endocan levels: ≥ 1.8 ng/mL and < 1.8 ng/mL. Gleason score and biochemical failure rate were found to be significantly higher in patients with endocan level ≥1.8 ng/mL. The time of biochemical recurrence was 38 months (31-42 months) and 56 months (46-65 months) in patients with endocan levels ≥ 1.8 ng/mL and < 1.8 ng/mL, respectively (P = .041). This study revealed that elevated serum endocan level (≥ 1.8 ng/mL) is an important marker for biochemical progression-free survival. Elevated serum levels may be due to systemic disorders and/or inflammation from an unknown source. Thus, endocan studies at target tissue may provide more accurate results compared to serum endocan studies. There is a need for prospective studies which will evaluate serum and target tissue endocan levels simultaneously.

In another study, expressions of VEGF (VEGF-A and VEGF-C) and their receptors were measured in neoplastic tissues and corresponding stroma from RP specimens by using tissue micro-array assays and immunohistochemical methods from 535 Norwegian patients. [25] High VGEFR-2 expression in stroma and epithelium was associated with a high incidence of PCa recurrence (P = .038). High expression of VGEF-A, VGEFR-2, or both in the stroma was independently associated with high biochemical failure incidence (P = .011). This study emphasizes the prognostic value of stromal VEGF-A and VGGFR-2 expressions. Since endocan is stimulated by these factors, the results of this study support our findings.

Asgari M et al. studied endothelin-1 expression for the determination of prognosis in patients with prostate adenocarcinoma.[23] The authors assigned 83 patients who underwent RP into 2 groups: 43 patients without extra-prostatic extension (EPE) and 40 patients with EPE. Endothelin-1 staining was performed on paraffin-embedded blocks obtained from preoperative core biopsies. Endothelin-1 expression was increased in 72% of patients in the EPE group (P < .001). Serum PSA levels were found to be higher in the group with endothelin-1 positivity (P = .039). Also, endothelin-1 expression was positive in 67% of patients with perineural invasion (P < .001). According to this study, endothelin-1 positivity can effectively predict EPE in patients with PCa (OR = 5.46, P = .010). The authors recommend using the endothelin-1 expression as a complementary factor in the assessment of core biopsies in prostate adenocarcinoma.

Chung-yu Lai et al. investigated the relationship between endocan and androgen receptor (AR) expressions.[26] They measured the Gleason score of human PCA tissues. Also, they evaluated endocan and AR expressions in prostate tissues from healthy individuals and patients with PCa using immunohistochemistry. This study found that endocan expressions were higher in tissues from prostate tumors than normal prostate tissues (P < .01). Besides, they found that endocan expressions in tumor tissues were associated with Gleason score (P < .016) and Gleason grade (P < .013). It was found that endocan expressions were higher in tumor tissues with higher Gleason score and grade (P < .001 for each) and endocan expressions were correlated to AR expressions (R = 0.727, P < .001). The authors proposed considering endocan as a marker for the diagnosis of PCa which supports our study's results.

In a study by Taghavi A et al., the frequency of the polyomavirus hominis 1, better known as BK virus (BKV) infection was found to be higher in PCa patients compared to BPH patients.[27] It has been stated that BKV may be a predisposing factor for Pca.

The retrospective design and the low sample size are the limitations of this study.

CONCLUSIONS

Our study showed strong epithelial staining of endocan in PCa tissues compared to BPH tissues. To our knowledge, the present study is the first study that showed elevated endocan expression levels in PCa tissues compared to BPH tissues. This finding may help to distinguish PCa from BPH. This finding also suggests that endocan may have a role in the pathogenesis of prostate adenocarcinoma. Many studies in the literature showed that endocan is closely related to the recurrence of PCa. We found that endocan expression was statistically significantly higher in patients with prostate cancer than those with benign prostate hyperplasia by using an immunohistochemical method. The interactions between endocan and other cytokines and growth factors in prostate adenocarcinoma development are still unclear, and

their clarification requires further studies.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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