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Periostin in ovarian carcinoma: from heterogeneity to prognostic value

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Abstract

Introduction. Periostin (POSTN), an extracellular matrix protein, is involved in tumor-associated extracellular matrix (ECM) remodeling. However, its potential value as a prognostic and/or predictive factor has not yet been confirmed. The present study aims to assess POSTN expression separately in tumor cells and stroma of different ovarian carcinoma (OC) histological types, and its relationship with clinicopathological features.

Material and methods. 102 cases of different histological OC subtypes were immunohistochemically investigated, for POSTN expression assessment in both epithelial tumor cells and tumor stroma. Statistical analysis was performed to correlate POSTN profile with clinicopathological characteristics, therapeutic response, and survival.

Results. POSTN expression in epithelial tumor cells was significantly correlated with POSTN expression in tumor stroma. The expression of POSTN in tumor cells was associated with histological type, tumor type (type I and II), tumor recurrence, progression-free survival (PFS), and overall survival (OS), whereas stromal POSTN expression was significantly correlated with age, histological type, tumor type, grade, and stage, residual disease, tumor recurrence, response to chemotherapy, and OS. Survival analysis revealed significant differences of PFS and OS in patients with high POSTN expression in tumor cells and negative stromal POSTN expression compared to patients with low POSTN expression in tumor cells and positive stromal POSTN expression (PFS: hazard ratio (HR) = 2.11, 95% confidence interval (CI): 1.33–3.37, P = 0.002; OS: HR = 1.78, 95% CI: 1.09–2.89, P = 0.019).

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Conclusions. The comparative assessment of POSTN immunoexpression in two tumor compartments: in tumor cells and stroma, by use of different scoring systems revealed that higher stromal POSTN levels are evidently correlated with unfavorable clinical features and poorer prognosis, while POSTN expression in tumor cells seems to be associated with a better patient outcome. (*Folia Histochemica et Cytobiologica 2023, Vol. 61, No. 1, 1–16*)

Keywords: ovarian cancer; periostin; epithelial tumor cells; stromal cells; therapy response; survival

Introduction

Ovarian cancer (OC), characterized by pathogenic and morphologic heterogeneity, is one of the most fatal gynecological malignancies due to its late diagnosis in the advanced stage, frequent relapse, and resistance to therapy [1–3]. The histopathologic spectrum of ovarian tumors comprises epithelial, germ cells, and sex cord-stromal tumors [4]. Nowadays, this classification is supplemented by the reconsideration of OC as two distinct entities, namely low-grade (type I) and high-grade (type II), based on the pathogenic dualistic model [5, 6]. These two types of OCs have different mechanisms of tumorigenesis due to distinct origins, molecular profiles, histology, epidemiology, and clinical behavior. Type I includes low-grade serous OC (LGSC), endometrioid, mucinous, and clear cell carcinomas, being considered as low-grade (defined as grade 1). They are typically slow-growing, originate from benign precursor lesions (ovarian surface, and fallopian tube epithelium or endometriotic implants), and show an indolent behavior [7–11]. Genetically, type I tumors harbor PTEN, BRAF, KRAS mutations, and have minor chromosomal instability [7–11]. Type II tumors include high-grade serous OC (HGSC), carcinosarcomas, and undifferentiated carcinomas, being considered high-grade (defined as grade 2 or 3), with aggressive behavior. Characteristically, they involve the ovary and, secondarily, the peritoneum, arising from the fimbriae of the fallopian tubes [7–11]. Genetically, they harbor BRCA-1, BRCA-2 or TP53 mutations and exhibit a high degree of genetic instability [7–11].

The current state of the art in OC provides a standpoint in understanding its pathogenesis, consisting in molecular and genetic events that can be responsible for the differences between the tumors' biological behavior. However, the intense research efforts for the identification of new biomarkers with prognostic and predictive values are far from being completed. Solid evidence shows that periostin (POSTN) could be one of these novel biomarkers. The focus on POSTN is partially based on its role in the communication between tumor cells and extracellular matrix (ECM), contributing to tumor progression and metastasis in various types of cancers [12–23].

The expression of POSTN, a molecule restricted to the periosteal/osteoblast and periodontal differentiation lines and generically known as osteoblast-specific factor 2 (OSF-2) [24], begins in embryonic life. The versatility of POSTN distribution in epithelial, endothelial, mesenchymal, and muscular cells accounts for its role in a multitude of processes, from differentiation and adhesion to migration and invasion [25, 26].

In terms of structure, POSTN protein is made up of a peptide N-terminal (constant) secretory domain and a C-terminal (variable) hydrophilic domain [24]. A cystein-rich EMILIN-like and four repetitive fasciclin domains are disposed of between these two terminal domains [24]. The N-terminal domain is indispensable for POSTN secretion that provides adhesion by the interaction between integrins and ECM *via* fasciclin domains [24, 27]. The C-terminal domain, responsible for the occurrence of eight isoforms, four of them being already sequenced and analyzed, provides the proteolytic cleavage of ECM proteins [24, 27, 28]. Thus, POSTN molecular structure is directly related to its activity in ECM remodeling, and, as a consequence, in tumor development and progression [25, 26]. ECM remodeling is amplified by the intervention of transforming growth factor β (TGF-β) [24], bone morphogenetic protein 2 (BMP-2) [29], fibroblast growth factor 1 (FGF-1), angiotensin II (ANG II) [30], interleukin 4 (IL-4), and IL-13 [31], along with cancer-associated fibroblasts (CAFs) infiltration and activation [26]. Using EMILIN-like domains, POSTN interacts with collagen type I [32], fibronectin [33] or Notch1 receptors [34], while it binds to BMP, by its Fas-1 domains [35].

Several reports have been focused on POSTN expression in different types of neoplasia, since the 2000's, such as lung [19, 20], breast [36], prostate [22], colon [23], head and neck [37], pancreatic [38], and esophageal carcinomas [18]. POSTN serum and tissue overexpression is correlated with unfavorable outcomes in non-small cell lung cancer [19, 20, 39] and poor prognosis in ovarian [12, 40, 41] and breast [36] cancer. A recent meta-analysis focused on POSTN prognostic value in solid cancers shows that its overexpression is associated with poor overall survival (OS) and disease-free survival (DFS), microvascular invasion, tumor differentiation, and lymph node metastasis [36].

Table 1. General characterization of the study group of patients with ovarian cancer

Pathogenic classification		**Tumor grade**			**International Federation of Gynecology and Obstetrics (FIGO) staging**			**Residual disease**		
Type I	Type II	G1	G2	G3	Stage I	Stage II	Stage III	NED	Optimal	Suboptimal
35	67	22	31	49	37	10	55	38	22	42
34%	66%	22%	30%	48%	36%	10%	54%	37%	22%	41%
n = 102		n = 102			n = 102			n = 102		

Abbreviations: NED — no evidence of disease on gross examination; Optimal — largest residual tumor between 0.1 and 1 cm; Suboptimal — largest residual tumor > 1 cm.

A number of studies have addressed POSTN expression and function in OC [12–14, 16, 17, 40–56] and published data show high POSTN levels in ovarian ascites [43, 45, 53], ovarian tumor tissue, and surrounding stroma [12, 16, 40–42, 45–50, 52], supporting the association between its expression and the therapeutic response [12, 13, 41, 42, 47, 55]. However, supplementary evidence is necessary to validate POSTN prognostic and predictive value in OC. Within this context, our study aims were: (i) the comparative assessment of POSTN expression in tumor cells and stroma of different OC histological types, and (ii) the analysis of the relationship between POSTN and clinicopathological features, therapy response, and patients' survival.

Materials and methods

Patients' characteristics. We conducted a retrospective study on paraffin-embedded samples obtained from 102 patients with primary epithelial OC diagnosed and treated in "Sf. Spiridon" Clinical Emergency County Hospital of Iasi and "Cuza Vodă" Obstetrics and Gynecology Hospital of Iasi, between 2006 and 2012. Borderline tumors, germline or sex-cord stromal tumors, and ovarian metastases were excluded. The study was approved by the Ethics Committee of "Grigore T. Popa" the University of Medicine and Pharmacy Iasi (no. 12378). The clinical information and follow-up were documented from the medical files and included data on age, histopathological diagnosis, residual disease, treatment and response to therapy, tumor recurrence, progression-free survival (PFS), and OS. The diagnosis of specimens was reviewed by two pathologists with expertise in gynecological pathology, according to the current World Health Organization (WHO) criteria and pathogenic dualistic model.

The patients median age at the time of diagnosis was 56.9 ± 9.9 years (range 30–80 years). The main clinicopathological characteristics of the investigated cases are summarized in Table 1.

OC samples included the following histological types: serous [67 cases: LGSC (9 cases) and HGSC (58 cases)], endometrioid (13 cases), clear cell (4 cases), mucinous (13 cases), Brenner tumor (1 case), carcinosarcoma (1 case), and undifferentiated carcinoma (3 cases).

Regarding the FIGO (International Federation of Gynecology and Obstetrics) tumor stage, we underline that there was no case included in stage IV because the patients did not meet the specific criteria at the time of diagnosis, more specifically: no signs of distant metastasis, liver or splenic parenchymal metastasis, no metastasis in extra-abdominal lymph nodes, and no transmural involvement of intestine or positive cytology.

Following the surgical treatment, 77 patients received front-line treatment with a standard platinum-based therapeutic scheme (platinum without taxanes in 16 cases; platinum and paclitaxel in 61 cases) while 25 patients were treated with other chemotherapeutic agents (bevacizumab or 5-fluorouracil). None of the patients received pre-operative chemotherapy. The response was evaluated using Response Evaluation Criteria in Solid Tumors [41], as follows: complete response in 64 patients, partial response in 8 patients, progressive disease in 22 patients, and stable disease in 8 patients. Consequently, 72 cases have been considered sensitive (responders) and 30 cases — resistant or refractory (non-responders) to chemotherapy. Tumor relapse was registered in 54 patients, while it was not detected in 48 patients.

During the median follow-up of 133 months, sixty tumor-related deaths were registered, 12 in patients with low-grade OC (type I) and 48 in patients with high-grade OC (type II).

Immunohistochemistry. For immunohistochemistry (IHC), sections of 4 μm thickness were cut from paraffin-embedded tissue blocks and placed on positively charged microscope slides. Tissue sections were deparaffinized in xylene at 58°C, for 45 min and rehydrated in successive baths of ethanol of decreasing concentrations (100%, 90%, and 70%). For antigen retrieval, the sections were treated with 0.01 M sodium citrate buffer (pH 6) in a water bath, at 97°C, for 30 min. After blocking the endogenous peroxidase activity in 3% hydrogen peroxide, the slides were incubated with the primary goat polyclonal antibody anti-POSTN (dilution 1:100; S-15, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) overnight, at 4°C. The slides were then incubated with the secondary biotinylated antibody and streptavidin-HRP complex (EnVision FLEX dual-link system, Dako, Agilent, Santa Clara, CA, USA), at room temperature, for 1 h. The slides were washed in 3 baths of phosphate-buffered saline (PBS), after each step. 3,3'-diaminobenzidine tetrahydrochloride chromogen (EnVision FLEX, Dako) was used to view the reaction. The staining pattern

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of tumor and stromal cells was a cytoplasmic type. Positive control was represented by normal colon specimens. The primary antibody was omitted in negative controls.

Semi-quantitative assessment. POSTN expression was assessed both in tumor epithelial cells and in the tumor stroma, using an original score, based on several previously reported scores [12, 19, 20, 41, 48, 57]. The scoring system of tumor cells took into consideration the intensity of the immunoreaction (0 — absent, 1 (+) — weak, 2 (++) moderate, and 3 (+++) — strong) and the percentage of positive tumor cells (0 — less than 10%, 1 — between 10–30%, 2 — between 30–60%, and 3 — more than 60% positive cells). The final score values, obtained by multiplication of intensity and percentage of positive cells, ranged from 0 to 9, being classified as low score (0 to 3) and high score (4 to 9). POSTN expression in tumor stroma was assessed as negative (absent immunostaining or positivity in less than 5% of stromal cells) and positive (immunostaining in more than 5% of stromal cells).

Statistical analysis. The statistical analysis was performed with IBM SPSS Statistics v. 29.0.0.0 (241) program (IBM Ireland Product Distribution Ltd., Dublin, Ireland). Continuous variables were assessed according to specific statistical indices [mean ± standard deviation (SD)]. The comparison tests applied for the continuous numerical variables were selected based on the distribution of series values and a number of cases included in the analysis. Thus, for continuous variables, the Student's *t*-test was applied when the value series had a normal distribution and the Wilcoxon test — when the value series did not have a normal distribution. A specific non-parametric test (Pearson Chi-square test) was used to analyze the correlation between the POSTN expression and clinicopathological characteristics. The Kaplan-Meier method was used to evaluate progression-free survival (PFS) and overall survival. We performed univariate and multivariate Cox proportional hazards regression analysis to evaluate the predictive factors of relapse or death (PFS and OS). The level of significance (P-value), which represents the probability of a type I error, was taken to be 0.05, indicating with a 95% confidence interval (CI) that the decision was correct. Thus, the threshold for statistical significance (P) was set at $P < 0.50$.

Results

Semi-quantitative assessment of POSTN expression of tumor cells and stroma

POSTN semi-quantitative assessment in tumor cells showed a high expression in 48 cases (47.1%) (Fig. 1A) and low or negative expression in 54 cases (52.9%) (Fig. 1B), whereas tumor stroma cells exhibited POSTN immunopositivity in 46 cases (45.1%) (Fig. 1C) and immunonegativity in 56 cases (55%) (Fig. 1D). Statistical analysis showed significant difference between POSTN expression in tumor cells and tumor stroma cells ($p = 0.03929$).

The POSTN expression in tumor cells (high and low) and stroma cells (negative and positive) allowed the stratification of the investigated cases into four classes, each class including both type I and type II OC (Table 2).

Relationships between tumor cells and stromal POSTN expression, clinicopathological factors, and survival parameters

The statistical analysis revealed correlations between POSTN immunoreactivity (high *vs.* low) in ovarian tumor cells and several clinicopathological and survival characteristics, with significant differences registered for histological type, tumor type, recurrences, PFS and OS (Table 3).

On the other hand, the statistical analysis showed that stromal POSTN immunoreactivity (negative *vs.* positive) was also associated with clinicopathological and survival characteristics, with significant differences for age, histological type, tumor type, grade, and stage, residual disease, tumor recurrence, response to chemotherapy, and OS (Table 4).

Differences in survival parameters according to POSTN expression in ovarian tumor cells and stroma cells

The survival parameters in the study group, stratified by POSTN immunoreactivity in tumor cells and stroma, are summarized in Table 5.

The comparative analysis of median values of PFS and OS in relation to the tumor cells and stromal POSTN immunoreactivity classes revealed that the maximum median value was registered in patients with high POSTN expression in tumor cells and negative stromal POSTN, so this group showed the minimum risk of an unfavorable event (Fig. 2 and Fig. 3).

Survival analysis revealed statistically significant differences in median values of PFS and OS only in patients who associated high POSTN immunoreactivity in tumor cells and negative stromal POSTN expression compared to patients that associated low POSTN immunoreactivity in tumor cells and positive stromal POSTN expression (PFS: hazard ratio (HR) = 2.11, 95% CI: 1.33–3.37, P = 0.002; OS: HR = 1.78, 95% CI: 1.09–2.89, P = 0.019) (Fig. 2 and Fig. 3).

The other classes defined by tumor cells and stromal POSTN immunoreactivity showed no differences in PFS and OS median values, *i.e.* cases with high POSTN tumor cells immunoreactivity and positive stroma POSTN expression *vs.* high POSTN tumor cells expression and negative POSTN stroma (PFS: HR = 1.33, 95% CI: 0.768–2.312, P = 0.307; OS: HR = 1.34, 95% CI: 0.770–2.357, P = 0.296), and cases with low POSTN immunoreactivity in tumor

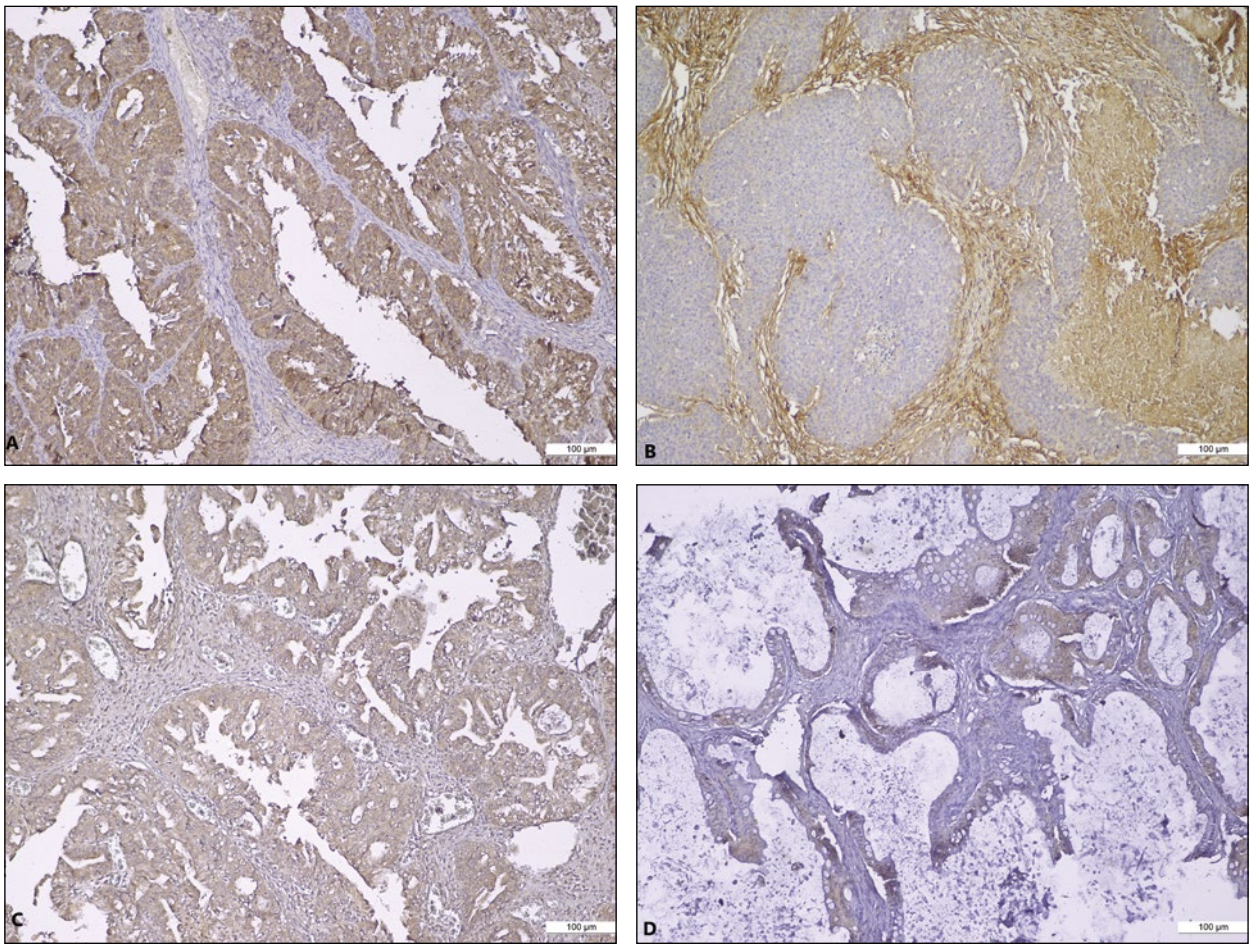

Figure 1. Periostin (POSTN) expression pattern in ovarian carcinoma. **A.** High-grade endometrioid carcinoma — high POSTN immunoreactivity in ovarian tumor cells and negative in stroma. **B.** High-grade serous carcinoma — low POSTN immunoreactivity in ovarian tumor cells and intensely positive expression in stroma. **C.** Low-grade serous carcinoma — high POSTN immunoreactivity in ovarian tumor cells and stroma. **D.** Mucinous carcinoma — low POSTN immunoreactivity in ovarian tumor cells and negative expression in stroma. Immunohistochemical stainings were performed and immunoexpression assessed as described in Methods. Magnification: 100×.

cells and negative POSTN stroma expression *vs*. high POSTN immunoreactivity in tumor cells and negative POSTN stroma expression (PFS: HR = 1.51, 95% CI: 0.935–2.466, P = 0.092; OS: HR = 1.44, 95% CI: 0.867–2.393, P = 0.159).

Analysis of POSTN expression and clinicopathological factors, as independent predictive factors of clinical outcome of ovarian carcinoma patients

Our study showed that several features were significantly associated with PFS.

Patients with high POSTN immunoreactivity in tumor cells and stromal POSTN positive expression had a higher risk for recurrence (HR = 1.608, 95% CI = 1.133–2.281, P = 0.008) than those with low immunoreactivity in tumor cells and negative expression in stroma cells (HR = 1.440, 95 %CI = 1.023–2.027, p = 0.037), in univariate analysis. Despite that the PFS was shorter when POSTN was intensely expressed in tumor cells and stroma, its value as an independent predictive factor was not confirmed by multivariate analysis. Only three clinicopathological factors were validated by multivariate analysis, namely age, FIGO stage, and response to chemotherapy. Patients over 55 years old had a shorter PFS than those under 55 years (HR = 1.496, 95% CI = 1.023–2.190, P = 0.038). The advanced FIGO stage has been associated with a two-fold increase of the relapse risk (HR = 1.997, 95% CI = 1.192–3.346, P = 0.009). The resistant/refractory status to chemotherapy led to a fourfold higher recurrence risk as compared to sensitivity status (HR = 3.970, 95% CI = 2.669–5.906, P ≤ 0.001).

Patients' overall survival was not influenced by high POSTN immunoreactivity neither in tumor or stroma cells. We found a significant association with

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Table 2. Different types of periostin expression in ovarian cancer

Ovarian carcinoma type	Immunoreactivity of periostin			
	High in tumor cells & negative in stroma (n, %)	High in tumor cells & positive in stroma (n, %)	Low in tumor cells & negative in stroma (n, %)	Low in tumor cells & positive in stroma (n, %)
Type I	16 (53.33%)	7 (38.88%)	9 (34.61%)	3 (10.71%)
Type II	14 (46.66%)	11 (61.11%)	17 (65.38%)	25 (89.28%)
Total	30	18	26	28

Table 3. Periostin expression in tumor cells along with clinicopathological and survival characteristics of ovarian cancer patients

Characteristics	POSTN expression in tumor cells		P-value (95% CI)
	High (n = 48; 47.10%)	Low (n = 54; 52.90%)	
Age: years, mean ± SD†	56.90 ± 9.90	56.60 ± 11.90	0.8544
Age: years‡ < 55 ≥ 55	 24 (52.20%) 24 (42.90%)	 22 (47.80%) 32 (57.10%)	0.1846
Histological type‡ Serous Non-serous	 27 (40.30%) 21 (60%)	 40 (59.70%) 14 (40%)	0.00744*
Tumor type‡ I II	 23 (65.70%) 25 (37.30%)	 12 (34.30%) 42 (62.70%)	0.00609*
Grade‡ 1-2 3	 27 (50.90%) 21 (42.90%)	 26 (49.10%) 28 (57.10%)	0.24734
FIGO stage‡ Early stage (I–II) Late stage (III)	 25 (53.20%) 23 (41.80%)	 22 (46.80%) 32 (58.20%)	0.10449
Residual disease‡ NED < 1cm > 1cm	 22 (57.90%) 9 (40.90%) 17 (40.50%)	 16 (42.10%) 13 (59.10%) 25 (59.50%)	0.05712
Tumor recurrence‡ Not registered Registered	 28 (58%) 20 (37%)	 20 (42%) 34 (63%)	0.031*
Response to chemotherapy‡ Sensitive Resistant/refractory	 32 (50%) 16 (42.10%)	 32 (50%) 22 (57.90%)	0.34355
OS status‡ Alive Died	 23 (56.10%) 25 (41%)	 18 (43.90%) 36 (59%)	0.03379*
PFS status‡ Not registered Registered	 22 (61.10%) 26 (39.40%)	 14 (38.90%) 40 (60.60%)	0.00476*

Continuous variables were expressed as: mean ± standard deviation; categorical variables: number (%); Abbreviations: OS — overall survival; PFS — progression-free survival. †Student's t-test for continuous variables when the value series had a normal distribution; ‡Chi-square test (Pearson test); *Marked effects are significant at $P < 0.05$.

the risk of death for six clinicopathological factors (age, histological type, tumor grade, FIGO stage, residual disease, and response to therapy). However, the multivariate analysis demonstrated that only age (HR = 1.847, 95% CI = 1.248–2.733, P = 0.030), advanced FIGO stage (HR = 1.882, 95% CI = 1.125––3.149, P = 0.016), and resistance to chemotherapy (HR = 3.089, 95% CI = 2.056–4.642, $P \leq 0.001$) were significant predictors of death.

Discussion

Periostin, a molecule encoded by POSTN gene, located on 13q13.3 chromosome, is present both in a soluble and secretory form in collagen-rich ECM [26, 58]. Increasing evidence shows that POSTN is upregulated in various non-tumor lesions, such as asthma [59, 60] and myocardial infarction [61–63], and different malignancies [18–20, 22, 23, 36–39]. However, up

Table 4. Periostin immunoexpression in the tumor stroma of ovarian cancer patients, along with clinicopathological and survival characteristics

Characteristics	POSTN expression in tumor stroma		P-value (95% CI)
	Positive (n = 46; 45.10%)	Negative (n = 56; 54.90%)	
Age: years, mean ± SD†	57.48 ± 10.80	56.14 ± 11.30	0.3907
Age: years‡ < 55 ≥ 55	 17 (37%) 29 (51.80%)	 29 (63%) 27 (48.20%)	0.03364*
Histological type‡ Serous Non-serous	 35 (52.20%) 11 (31.40%)	 32 (47.80%) 24 (68.60%)	0.00720*
Tumor type‡ I II	 10 (28.60%) 36 (53.70%)	 25 (71.40%) 31 (46.30%)	0.00104*
Grade‡ 1–2 3	 18 (34%) 28 (57.10%)	 35 (66%) 21 (42.90%)	0.01825*
FIGO stage ‡ Early stage (I–II) Late stage (III)	 17 (36.20%) 29 (52.70%)	 30 (63.80%) 26 (47.30%)	0.02590*
Residual disease‡ NED < 1cm > 1cm	 15 (39.50%) 7 (31.80%) 24 (57.10%)	 23 (60.50%) 15 (68.20%) 18 (42.90%)	0.01055*
Tumor recurrence‡ Not registered Registered	 17 (35%) 29 (54%)	 31 (65%) 25 (46%)	0.0639
Response to chemotherapy‡ Sensitive Resistant/refractory	 25 (39.10%) 21 (55.30%)	 39 (60.90%) 17 (44.70%)	0.03549*
OS status‡ Alive Died	15 (36.60%) 31 (50.80%)	26 (63.40%) 30 (49.20%)	0.04433*
PFS status‡ Not registered Registered	 13 (36.10%) 33 (50%)	 23 (63.90%) 33 (50%)	0.05556

Continuous variables were expressed as: mean ± standard deviation; categorical variables: number (%);
†Wilcoxon rank-sum test for continuous variables when the value series did not have a normal distribution;
‡Chi-square test (Pearson test); * Marked effects are significant at $P < 0.05$.

Table 5. Periostin expression classes and survival parameters

POSTN expression classes (n)	Survival parameters	
	PFS (95% CI)	OS (95% CI)
High POSTN tumor cells & positive POSTN stroma (n = 18)	25 months (19.60–30.40)	70 months (47.30–87.60)
High POSTN tumor cells & negative POSTN stroma (n = 30)	40 months (26.40–53.60)	81 months (72.60–89.70)
Low POSTN tumor cells & positive POSTN stroma (n = 28)	21 months (11.50–30.40)	43 months (41.20–54.79)
Low POSTN tumor cells & negative POSTN stroma (n = 26)	32 months (29.90–34.01)	64 months (51.60–73.30)

Abbreviation: n — number of cases.

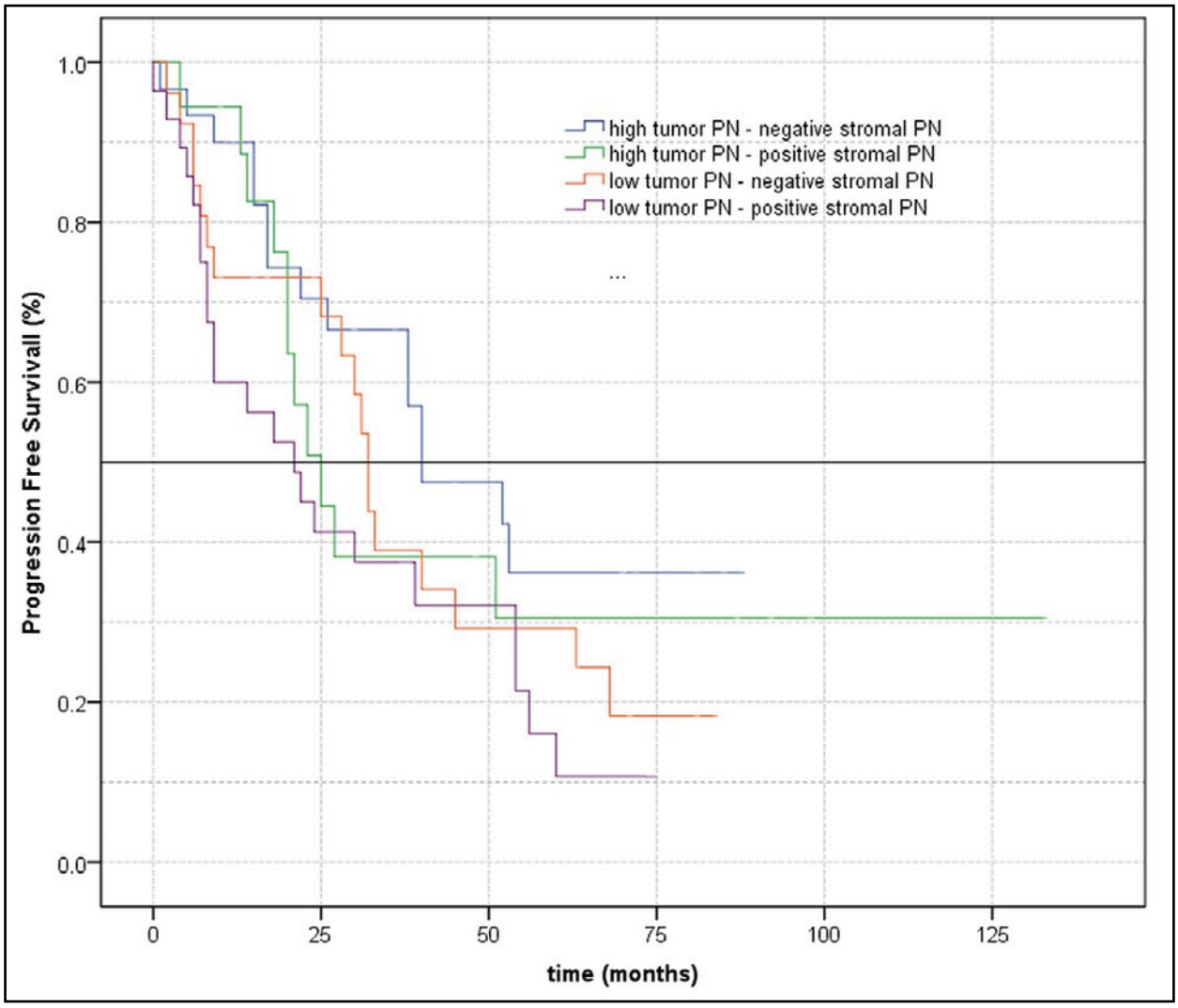

Figure 2. Kaplan-Meier curves for progression-free survival (PFS) of ovarian cancer patients according to expression patterns of periostin (PN) assessed by immunohistochemistry in tumor cells and tumor stroma (n = 102).

to date, only less than 20 papers focused on POSTN expression in ovarian tumors. POSTN expression was demonstrated in OC cell lines [12–14, 17, 46, 64] and in human ovarian tumor tissue [12, 16, 54, 56]. High POSTN expression is associated with advanced tumor stage and unfavorable prognosis in OC patients [12, 45, 49, 54, 56].

The published reports regarding POSTN in OC showed a great variability of the available POSTN antibodies, most of them being able to recognize different isoforms [25] through their affinity for the variable C-terminal domain, involved in ECM organization. Contrary, our approach has been to use an antibody against the N-terminal domain that promotes POSTN secretion and regulates cell functions, aiming to identify the capability of both tumor and stromal cells to synthesize POSTN.

It is to be noticed that not only the anti-POSTN antibodies are variable in OC studies, but also the method of semi-quantitative evaluation. Most of the used scores in POSTN expression evaluation are common for both tumor and stromal cells [40, 41, 45, 48]; moreover, the threshold values, as well as the score values set by authors are different. To the best of our knowledge, the different quantification of POSTN expression in tumor cells and stroma has been reported only recently [12], considering only the percentage of positive cells, but not the intensity of the immunostaining.

The novelty of our approach to assessing the immunoreactivity of POSTN in OC is the concomitant assessment of the expression both in tumor cells and stroma, using two individual scores – thus complementing our preliminary communicated results [65]. The score for POSTN expression in tumor cells is a variant

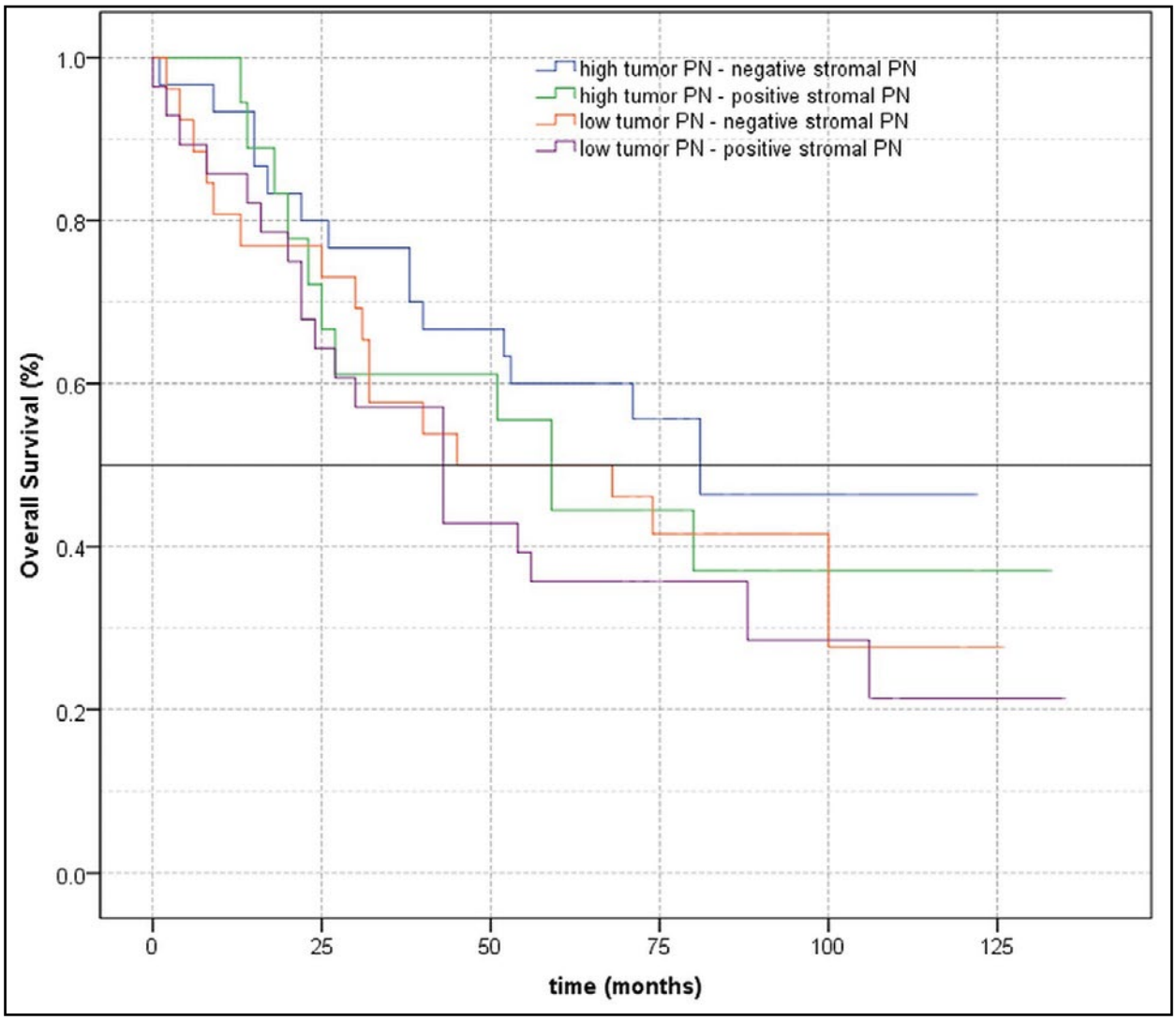

Figure 3. Kaplan-Meier curves for overall survival (OS) of ovarian cancer patients according to expression patterns of periostin (PN) assessed by immunohistochemistry in tumor cells and tumor stroma (n = 102).

of a classical immunoreactivity score, proposed for estrogen-receptor detection in breast cancer [57], and recently applied for POSTN analysis in non-small cell lung cancer [19, 20]. We considered that the POSTN complex evaluation in tumor cells, taking into account not only the percentage but also the immunoreaction intensity, which reflects the variable amount of intracellular POSTN, supports a refined classification in low and high score values that could be correlated with tumor aggressiveness. The score for POSTN expression in stromal cells relies on a threshold of 5% and of the general immunoexpression of stromal cells, without any correlation with the intensity differences, considering that ECM histological structure involves reaction-diffusion towards ground substance and ECM fibers (as shown in [12]), when synthesis is triggered. Having these in mind, we considered that the classification into negative and positive POSTN expression would allow a correlation with tumor aggressiveness.

High immunoreactivity of POSTN was noticed in almost half of the study group: in tumor cells in 48 cases (47.1%), being assessed as high score, and in stromal cells in 46 (45.1%) positive cases. Our results are relevant as they show not only a high level of stromal POSTN expression but also a high level of POSTN expression in tumor cells — validated by only two studies that confirm its presence in the epithelial tumor compartment [12, 41]. Moreover, we identified POSTN positivity in the normal ovarian tissue adjacent to the tumor, in the ovarian surface epithelium, and in stromal cells located at the periphery of the ovary cortex, respectively. This observation is important, as POSTN is currently reported as absent in both epithelial and stromal cells in normal control ovaries [48].

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The brief review of the literature shows a large heterogeneity of data regarding POSTN expression in OC — a feature that can be related to consistent differences in the design of these studies, including the employed scoring systems. Thus, by applying a common score for stromal and tumor cells, Choi *et al.* [48] found a positive POSTN immunoexpression in stromal cells in 53% of cases — a percentage close to our data, but weak, insignificant, rarely detected POSTN expression in tumor cells [48]. Another study, also using a single score for both types of cells, led to the classification in low and high groups of immunoreactivity, as follows: high POSTN stromal expression in 30.2% of cases and a low one in 69.8% of cases, along with a high POST expression in tumor cells in 37% of cases and low expression in 63% of cases [41]. In a study essentially focused on POSTN gene expression profiling in a particular group of OC (including platinum-sensitive primary tumors, platinum-resistant primary tumors, and platinum-resistant recurrent tumors), POSTN immunoreaction was also assessed as present or absent [45]. The results revealed no detectable or minimal POSTN stromal expression in platinum-sensitive primary tumors, and a constant POSTN positivity in CAFs, in platinum-resistant primary and recurrent tumors, while POSTN expression was completely negative in tumor cells [45]. On the other hand, it is worth mentioning the work of Kujawa *et al.*, [12], based on individual scores for the evaluation of POSTN expression in tumor cells and stroma [12]. Through quantification of the percentage of POSTN-positive tumor cells, 25.9% of cases showed a weak immunoreaction while 74.1% of cases were strongly positive. POSTN expression was assessed in the tumor stroma, using a 3-stage scale, and the results indicated 56.5% of cases with score 1, 37% with score 2, and 6,5% with score 3 [12]. Comparing our results with those obtained by Kujawa *et al*., [12], we noticed discrepancies in POSTN expression in tumor cells — with a lower percentage of cases with high score/strong positivity in our group (47.1% *vs.* 74.1%), and similarity in POST expression in stromal cells (45.1% positive cases *vs.* 43.5% cases with score 2 and 3).

In our study, the statistical analysis indicated that POSTN expression in tumor cells (low score *vs.* high score) correlates to stromal POSTN expression (negative *vs.* positive) ($P < 0.039$), similar to the data reported by Sung *et al.* [41] which studied both cellular compartments but using a single score system [41]. This correlation suggests common sequences in the mechanism of action of the two cell types that, together with the cross-talk developed in EMC framework, determine their interference [66]. A possible hypothesis is that several signaling molecules released by stromal cells, specifically by CAFs [26, 67] and tumor-associated macrophages — TAMs [68, 69] may influence tumor cells, stimulating POSTN synthesis [44] and, as a consequence, may lead to the promotion of tumor growth, migration and invasion.

Literature data support our observation that POSTN high expression in tumor cells is higher than in normal and benign ovary, being significantly correlated to the advanced stage, high grade, and recurrent tumors [40], while POSTN stromal expression is correlated to the number of mitoses, FIGO stage, surgery debulking, recurrences, and metastasis, but not to the histological type or tumor grade [41]. Without pointing out the cellular or stromal location, a review article sustains a higher POSTN expression in FIGO stages II–IV compared to stage I, in grades 2 and 3 compared to grade 1, in resistant or refractory patients to first-line chemotherapy compared to sensitive ones [43]. The relationship between POST expression and survival parameters reveals better OS in patients with negative POSTN stromal expression compared to those with positive POSTN stroma [41, 48], that also have a shorter PFS [41]. POSTN in-depth study, using gene expression profiling, shows that the POSTN gene is one of the three upregulated genes in the peritumoral stroma in epithelial OC (EOC), being responsible for chemoresistance [45]. Thus, stromal POSTN is considered a valuable marker for poor survival and platinum resistance [41, 45], with high POSTN expression in CAFs being a valuable predictor for shorter PFS, in patients with first-line chemotherapy [45]. The hallmarks of several studies on POSTN immunoreactivity in OC are summarized in Table 6.

In agreement with the literature data, our results show that stromal POSTN expression, rather than POSTN expression in tumor cells, may be regarded as a prognostic and predictive biomarker in OC [41, 48, 49]. This assumption is supported by the statistically significant differences between stromal POSTN immunoreactivity (positive *vs.* negative) and almost all clinicopathological factors (age, histological type, pathogenic subtype, tumor grade, FIGO stage, residual disease, tumor recurrence, response to therapy, and OS). On the other hand, for tumor cells POSTN expression (low score *vs.* high score) we registered statistically significant differences only for the histological and pathogenic type, along with tumor recurrence, OS, and PFS.

A special comment has to be made about POSTN expression in different tumor histological types. Out of 67 serous OC, 35 cases (52%) had positive stromal POSTN and only 27 cases (40.3%) had high POSTN expression in tumor cells, whereas for the other 35 non-serous OC, stromal POSTN was positive in 11

cases (31.4%) and high POSTN tumor cells expression was registered in 21 cases (60%). These data indicate an obvious relationship between stromal POSTN expression and serous histological type of OC, with repercussions in tumor aggressiveness. The critical analysis of the studies regarding POSTN expression in OC reveals that the histological structure of different study groups is not usually specified, being limited to their grouping into two major diagnostic classes, namely OC or EOC, respectively. Consequently, the literature includes little data regarding the relationship between POSTN and different histological types of OC. The study by Sung *et al*. [41], was conducted on 308 OC samples, 99 of them were diagnosed as serous adenocarcinoma, 61 as endometrioid adenocarcinoma, 63 as clear cell adenocarcinoma, 48 as mucinous carcinoma, 14 as borderline tumors, 23 as other types of tumors and paired normal tissues, reported no differences between histological groups [41]. The histological diversity of our cases allowed us to classify them into two categories, serous and non-serous, with statistically significant differences between POSTN expression in tumor cells and stroma, between the two groups. Nevertheless, the high number of cases of serous OC with high POSTN expression in tumor cells cannot be overlooked. This finding shows, in our opinion, the reciprocal potentiating mechanism of tumor and stromal components, which leads to a general increase of tumor POSTN expression. Additionally, POSTN predominant expression in tumor cells compared to stroma in non-serous OC may raise the possibility of different POSTN synthesis regulation, dependent on the cell type and its interactions with ECM.

Focusing on POSTN immunoreactivity in both tumor cells (low *vs.* high) and tumor stroma (positive *vs.* negative), we noted differences between OC pathogenic types, defined according to the pathogenic dualistic model. Our data showed that more than half of the cases included in type II presented positive stromal POSTN expression, compared to only a third of cases comprised in type I. To the best of our knowledge, no other study on POSTN expression addressed the analysis of the study group in relation to the pathogenic classification of OC. These results enable us to consider an association between type II OC, with high grade due to rapid growth and unfavorable prognostic, stromal POSTN expression, and tumor aggressiveness — POSTN playing an important role in invasion and metastasis as has been previously suggested [12, 40, 42].

The stratification of the analyzed cases in relation to the tumor and stromal POSTN expression allowed the identification of four distinct OC classes, comparable to those set by Sung *et al.* [41], with variable clinical courses. Our results are in agreement with their data only in relation to stromal POSTN expression but not to POSTN expression in tumor cells. The shortest PFS and OS are associated, in our study, to low score POSTN in tumor cells and positive stromal POSTN, although they have been previously associated to both high tumor cells and stromal POSTN expression [41]. On the other hand, we found the longest PFS and OS in patients with high POSTN expression in tumor cells and negative stromal POSTN, whereas these intervals have been correlated to both tumor and stromal low POSTN expression [41]. These different results could be attributed to a higher degree of precision in the assessment of tumor cells and stromal POSTN expression performed by using independent scores compared to a previous study [41]. However, in the evaluation of these inconsistencies, we have to consider the racial genetic characteristics, the study analyzed a group of 308 Chinese patients, whose genetic profile may show some differences when compared to Caucasian patients. Nevertheless, our study confirms that there are statistically significant differences of PFS and OS between the cases with high POSTN expression in tumor cells and negative stromal POSTN expression, and those with low POSTN expression in tumor cells and positive stromal POSTN. Supplementary, our data also show that high POSTN expression in tumor cells and stromal POSTN positivity correlate to a higher risk for recurrence than low POSTN expression in tumor cells and stromal POSTN negativity. Similar Kaplan-Meier curves were also found in other malignancies, considering that the patients diagnosed with prostate or breast cancer with low stromal and positive epithelial POSTN expression showed the lowest mortality risk as opposed to the patients with either high or absent POSTN expression in both cell compartments, that had the highest mortality rates [70, 71].

Unfortunately, our results could not validate POSTN expression (neither stromal nor tumor) as an independent predictive factor for OC survival. However, the multivariate analysis performed in order to evaluate the influence of clinicopathological characteristics on the patient's clinical course, added to that of POSTN expression, confirmed the prognostic value only in terms of age, FIGO stage, and therapeutic response.

The major limitation of our research consists in POSTN analysis exclusively performed by IHC technique. Supplementary methods, deepening the study to the level of proteomic analysis, may well support our data. Thus, the application of laser microdissection could provide direct access to the tumor and stromal cell population, respectively, allowing their POSTN protein quantification by Western Blot analysis. Despi-

Table 6. Summary of the important studies on periostin immunoreactivity in ovarian carcinoma

Study (authors, year, country, reference)	**Main traits**	
Zhu *et al.*, 2010, USA [4]	**Material** 138 samples (119 EOC, 19 normal ovary & benign tumors) cell lines	**Methods** IHC (score: intensity), WB, *in vitro* invasion assay, *in vivo* tumorigenicity
	POSTN expression significantly higher in primary tumors than in normal and benign samples significantly lower in primary tumors than in recurrent tumors significantly higher in FIGO stages III–IV than in FIGO stages I–II associated with clinical stages and recurrence status	
Choi *et al.*, 2011, Republic of Korea [48]	**Material** 132 samples (66 primary EOC*, 26 borderline, 20 benign, 10 normal ovary) — 1998–2005 *28 serous, 13 mucinous, 10 endometrioid, 15 clear type grade cell lines	**Methods** IHC (score: 3-point scale, only percentage, tumor cells & stroma), IF, WB, de-adhesion assay, cell migration assay, invasion assay, RT-PCR
	POSTN stromal expression 53% cases EOC, absent in normal ovary increases from benign to EOC correlated with mitoses, FIGO stage, tumor recurrence, distant metastases and poor survival significantly lower OS than in no POSTN stromal expression independent prognostic factors POSTN expression in tumor cells absent (weak staining, rarely observed)	
Ryner *et al.*, 2015, USA [45]	**Material** 85 EOC (high grade serous & endometrioid) = discovery set 136 EOC (high grade serous & endometrioid) = validation set	**Methods** ISH IHC — no score
	POSTN stromal expression absent/ minimal in platinum-sensitive-primary tumors; present in platinum-resistant-primary- and platinum-resistant recurrent tumors POSTN expression in tumor cells absent	
Sung *et al.*, 2016, Taiwan [41]	**Material** 308 samples (99 serous ADK, 61 endometrioid ADK, 63 clear cell ADK, 48 mucinous carcinoma, 14 borderline, 23 others plus paired normal tissues) — 2000–2008 cell lines	**Methods** IHC (score: 4-point scale, intensity & percentage, tumor cells & stroma, 2 classes: low, high), WB, cell proliferation assay
	POSTN stromal expression high 30.2% cases, low 69.8% cases significant predictor for PFS prognostic factor for clinical outcome indicator for platinum response **High POSTN stromal expression** significantly higher in advanced FIGO stage correlated with higher tumor recurrent rate associated with high POSTN expression in tumor cells significantly lower OS and PFS compared with low POSTN stromal expression compared with low POSTN stromal expression: no differences between age, histological groups, tumor grading, CA-125 pre-operatively or after 1st chemotherapy **POSTN expression in tumor cells** high 37% cases, low 63% cases **High POSTN expression in tumor cells** no significant prognostic value in OS and PFS compared to low expression POST expression (low, high) in stromal & tumor cells = 4 groups high expression in both stroma and tumor: shortest OS and PSF among others groups	

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Study (authors, year, country, reference)	**Main traits**	
Kujawa *et al.*, 2020, Poland [12]	**Material** 108 samples (97 serous OC, 106 grade 3, 36 peritoneal metastases) tissue microarrays: EOC, fallopian tube	**Methods** IHC (score: separate scale for tumor cells & stroma)
	POSTN stromal expression score 1: 56.5% cases, score 2: 37% cases, score 3: 6,7% cases significantly lower in primary tumors than in peritoneal metastases **High POSTN stromal expression** associated with higher degree of desmoplastic reaction significantly shorter OS than in low stromal expression no correlation with clinicopathological parameters **POSTN expression in tumor cells** weak: 25.9% cases, strong: 74.1% cases associated with calcifications no correlation with clinicopathological parameters no correlation with OS, DFS	

Abbreviations: ADK — adenocarcinoma; EOC — epithelial ovarian carcinoma; FIGO — International Federation of Gynecology and Obstetrics; IF — immunofluorescence; IHC — immunohistochemistry; ISH — *in situ* hybridization; OS — overall survival; PFS — progression free survival; POSTN — periostin; RT-PCR — reverse transcription — polymerase chain reaction; WB — Western blotting.

te the limits, our study provides substantial evidence for the significant stromal POSTN influence on tumor behavior, not only in the tumor microenvironment but also on tumor cells, by a possible change of their secretory phenotype. These changes are still difficult to understand, because the positive stromal POSTN may be associated to high or low POSTN expression in tumor cells, and the absence of stromal POSTN does not exclude the presence of POSTN in tumor cells. These differences suggest the involvement of other mechanisms/factors which may lead to variable POSTN patterns. Our data open wide perspectives for a deeper knowledge of the intracellular and intercellular signaling pathways occurring in the complex mechanism of ovarian carcinogenesis and metastasis. Undoubtedly, POSTN's role in these molecular pathways' loop deciphering would add significant value to the prediction of OC heterogeneous behavior.

In summary, we applied a novel approach to study POSTN immunoreactivity in OC by using distinct scoring systems to assess the expression in two tumor compartments: in tumor cells and tumor stroma. This approach provides a more accurate image of POSTN dynamics in OC and allows the correlation of its expression, in tumor cells and stroma, in relationship with several clinicopathological features and survival parameters. Our data support the findings that stronger stromal POSTN expression is evidently correlated with unfavorable clinical features and worse prognosis, while concomitantly higher POSTN expression in tumor cells seems to be associated with a better course of the disease.

Conflict of interest

The authors declare no conflict of interest.

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