Gynecologic Oncology 127 (2012) 217–222

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Cyclin I correlates with VEGFR-2 and cell proliferation in human epithelial ovarian cancer

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ARTICLE INFO

Article history:
Received 15 March 2012
Accepted 27 June 2012
Available online 5 July 2012

Keywords:
Cyclin I
VEGFR-2
Ovarian cancer
Prognostic factors
Survival
Angiogenesis

ABSTRACT

Objective. Ovarian cancer is the most lethal of all gynecologic malignancies. It is characterized by the spread of intraperitoneal tumors, accumulation of ascites, and formation of tumor blood vessels. Cyclin I has been linked with angiogenesis-related proteins, like vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR-2), in human breast cancer. We examined whether an association exists between expression of cyclin I, VEGFR-2, clinicopathologic parameters and survival of patients with epithelial ovarian cancer (EOC).

Methods. Cyclin I and VEGFR-2 expressions were analyzed by immunohistochemistry in 55 human primary EOC tissue specimens.

Results. Cyclin I immunoreactivity was significantly correlated with VEGFR-2 ($R=0.4587$, $P=0.0004$), and immunolabeling of cyclin I and VEGFR-2 significantly correlated with cancer cells' proliferative activity evaluated using cyclin A labeling index as a marker ($R=0.3107$, $P=0.0209$ and $R=0.4183$, $P=0.0015$, respectively). VEGFR-2 immunostaining was significantly higher in advanced, poorly differentiated, and suboptimally resected EOCs compared to their counterparts ($P<0.05$). Finally, higher VEGFR-2 expression was significantly associated with shorter disease-free survival ($P=0.0437$).

Conclusions. Our results indicate that elevated expression of cyclin I and VEGFR-2 is likely to provide a proliferative advantage to the EOC cells, and that cyclin I may be linked with angiogenesis in EOC. Higher expression of VEGFR-2 is associated with more advanced disease. Further investigation of cyclin I in ovarian cancer is needed to evaluate if cyclin I may become a novel target for an anticancer therapy.

Introduction

Ovarian cancer is the most lethal of all gynecologic malignancies with age-adjusted incidence rate 12.2 per 100,000 women (20,749 cases) and age-adjusted death rate 8.2 (14,621 deaths) in 2007, United States [1]. The malignant progression of this disease is characterized by the spread of solid intraperitoneal tumors, rapid accumulation of ascites, and depends on the formation of tumor blood vessels (angiogenesis) [2].

Cyclins are regulatory subunits of cyclin-dependent kinases (cdk), and complexes of cyclins and cdks control cell-cycle progression [3]. In contrast to the classical cyclins, cyclin I level does not fluctuate during cell cycle, and therefore it is believed that cyclin I has no role in regulating cell proliferation [4–6]. Recently, higher cyclin I immunostaining was found in human pancreatic carcinoma compared to healthy pancreas and benign pancreatic diseases, which indicates that cyclin I may play a role in carcinogenesis or progression of pancreatic cancer [7]. Moreover, cyclin I expression was correlated with angiogenesis-related proteins, like vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR-2), in human breast cancer, suggesting a link between cyclin I and angiogenesis [8]. The best-studied factors that stimulate angiogenesis are VEGFs which promote either physiologic or tumor vessel formation by binding to their receptors, VEGFR-1, -2, or -3. Among these three receptors, VEGFR-2 is generally recognized to have a principal role in mediating multiple signaling networks that result in cell survival, proliferation, migration, focal adhesion turnover, actin remodeling and vascular permeability [9]. In addition to targeting endothelial cells, the VEGF/VEGFR-2 system works as an essential autocrine–paracrine loop for enhanced cancer cell proliferation and survival in numerous human tumors, including ovarian cancer [10–12]. The aim of our study was to evaluate cyclin I and VEGFR-2 immunostaining in previously-untreated, primary

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doi:10.1016/j.ygyno.2012.06.038

epithelial ovarian cancer (EOC) tissue specimens, and to investigate whether an association exists in these tumors between expression of cyclin I, VEGFR-2, clinicopathologic parameters and patients' survival.

Materials and methods

Patient characteristics

The study group consisted of 55 primary EOC patients, diagnosed and treated at the First Department of Oncologic Gynecology and Gynecology, Medical University of Lublin, during the period of December 2004–October 2007. Median patient age was 56 years (range 23–85 years), and median ascites volume was 100 ml (range 0–7000). All 55 specimens underwent microscopic confirmation of diagnosis, histological type, and tumor grade, by experienced pathologist (B.J.), following established criteria [13]. The optimal debulking status after initial surgery was defined as residual tumors with maximal diameter of 1 cm or less [14]. Tumor specimens were collected from women during primary surgery and prior to the initiation of adjuvant therapy. After primary surgery, 42 patients (76.4%) received adjuvant chemotherapy

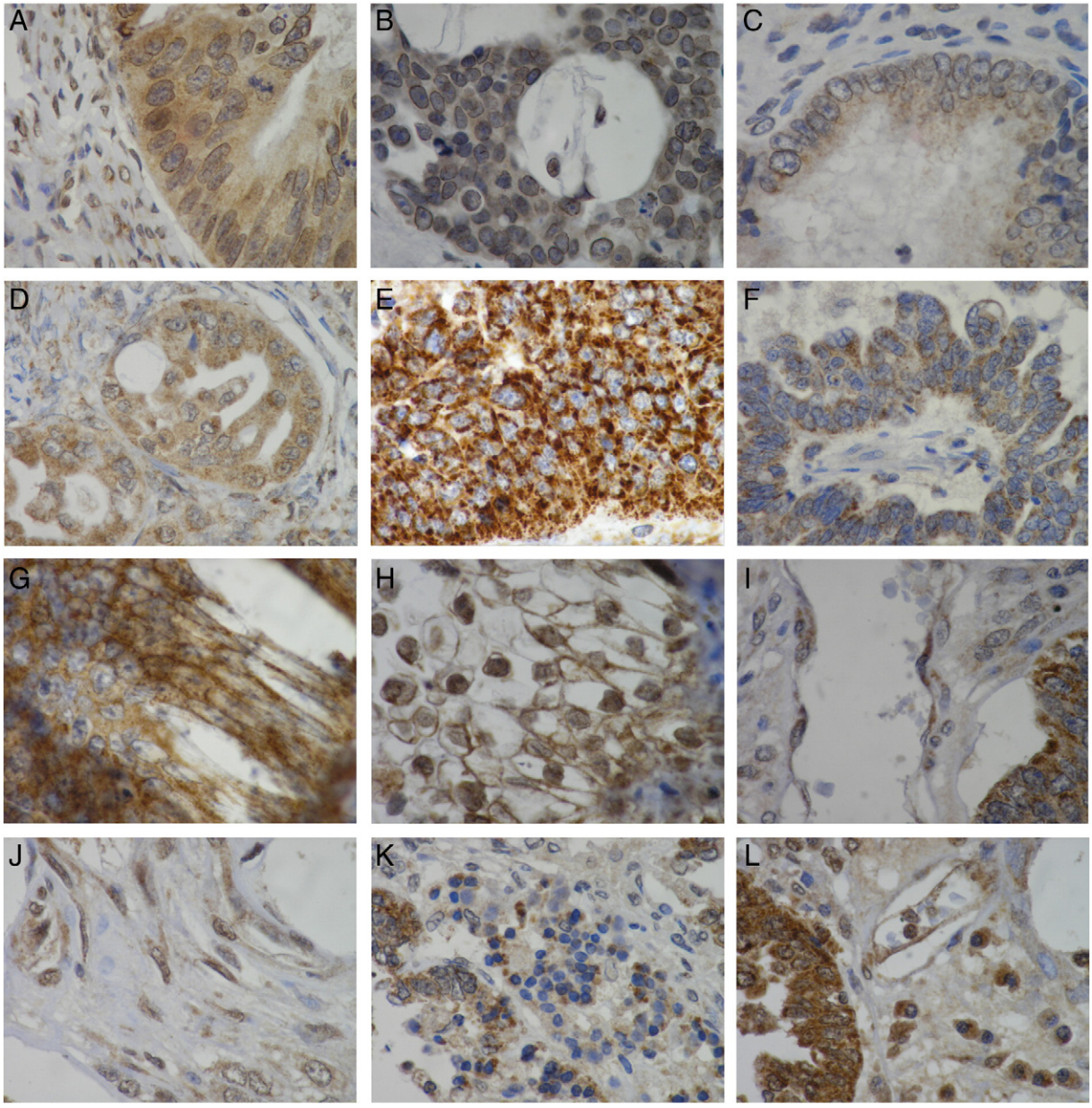

Fig. 1. Cyclin I (A–C) and VEGFR-2 (D–L) immunostaining in epithelial ovarian cancers. Panel A, mucinous ovarian adenocarcinoma (G2) with immunostaining of cyclin I in the cytoplasm and nuclei of tumor cells; staining index (SI) = 12. Immunolabeling in the cytoplasm and nuclei of fibroblasts on the left (400×). Panel B, endometrioid ovarian adenocarcinoma (G3) with immunoreactivity of cyclin I in the cytoplasm of tumor cells (400×; SI = 8). Panel C, serous ovarian adenocarcinoma (G3) with immunostaining of cyclin I in the cytoplasm of tumor cells (400×; SI = 2). Panel D, mucinous ovarian adenocarcinoma (G2) with immunolabeling of VEGFR-2 in the cytoplasm of tumor cells (400×; SI = 6). Panel E, endometrioid ovarian adenocarcinoma (G3) with immunostaining of VEGFR-2 in the cytoplasm of tumor cells (600×; SI = 12). Panel F, serous ovarian adenocarcinoma (G2) with immunoreactivity of VEGFR-2 in the cytoplasm of tumor cells (400×; SI = 3). Panel G, mucinous ovarian adenocarcinoma (G2) with membranous-cytoplasmic immunostaining of VEGFR-2 in tumor cells (600×). Panel H, serous ovarian adenocarcinoma (G1) with predominantly membranous immunolabeling of VEGFR-2 in tumor cells (600×). Panel I, serous ovarian adenocarcinoma (G2; right down corner of the photograph) with immunoreactivity of VEGFR-2 in the cytoplasm of vascular endothelial cells (center of the photograph; 600×). Panel J, serous ovarian adenocarcinoma (G2) with immunostaining of VEGFR-2 in the cytoplasm of fibroblasts (neoplastic cells are not shown; 600×). Panel K, serous ovarian adenocarcinoma (G2; right upper part and left down corner of the photograph) with immunolabeling of VEGFR-2 in lymphocytes (center of the photograph; 600×). Panel L, serous ovarian adenocarcinoma (G2; left down corner of the photograph) with immunoreactivity of VEGFR-2 in the cytoplasm of plasmocytes with eccentric nuclei (to the right from neoplastic cells; 600×).

(paclitaxel–platinum or cyclophosphamide–cisplatin) and 6 patients (10.9%) received radiation therapy. Paclitaxel–platinum chemotherapy was administered to 32 patients (58.2%) and consisted of paclitaxel (175 mg/m^2) and carboplatin (6 AUC) or cisplatin (75 mg/m^2). Ten women (18.2%) received cyclophosphamide–cisplatin adjuvant chemotherapy which consisted of cyclophosphamide (750 mg/m^2) and cisplatin (70 mg/m^2). All six patients, who were treated with radiation therapy, had FIGO IA cancers and received external beam radiotherapy to the pelvis with 46.0 Gy/23 fractions. Moreover, seven women (12.7%) did not receive adjuvant therapy (three of them died within 2 months after surgery, two were disqualified from adjuvant therapy due to poor general status and comorbidities, and another two had FIGO IA stage of disease). The patients' response to chemotherapy was assessed according to RECIST criteria ver. 1.1 [15] and platinum sensitivity of tumors was determined as described previously [14]. All women were followed until death or the end of follow-up period (January 31, 2012). Overall survival (OS) was defined as the time interval from the date of primary surgery to the date of death (failure) or to the end of follow-up for women who were alive (censored). Disease-free survival (DFS) was defined as the time elapsed from the date of primary surgery to the appearance of disease recurrence or progression (failure) or the last follow-up for women who were alive with no evidence of disease recurrence or progression (censored). Ethical approval to carry out the study was granted by the Research Ethics Committee of Medical University of Lublin.

Immunohistochemistry

Tissue sections (4 μm) from formalin-fixed and paraffin-embedded ovarian samples were deparaffinized, rehydrated and subjected to heat-induced antigen retrieval in DakoCytomation Target Retrieval Solution, Citrate, pH 6.0 (DAKO, Carpinteria, USA) at 99 °C for 20 min in water bath. Endogenous peroxidase was blocked using Dako REAL™ Peroxidase-Blocking Solution (DAKO) for 10 min, and then slides were incubated for 30 min in Protein Block Serum-Free (DAKO) to inhibit non-specific staining. Afterwards, the sections were incubated with the primary antibody for 30 min. All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and their dilutions were cyclin I (1:100, rabbit polyclonal antibody, clone H-279), VEGFR-2 (1:200, mouse monoclonal antibody, clone A-3), and cyclin A (1:200, mouse monoclonal antibody, clone 6E6). After being washed, the specifically bound antibodies were detected with the sensitive polymer-based system (Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse) according to the manufacturer's instructions. Finally, the slides were counterstained in hematoxylin and coverslipped. A positive control section was included in each staining run. Prostate cancer, colon adenocarcinoma, and breast carcinoma specimens were used as positive controls for cyclin I, VEGFR-2, and cyclin A respectively. Negative controls were performed by omitting the primary antibody and no staining was detected in negative control sections.

Microscopic assessment of cyclin I, VEGFR-2, and cyclin A immunostaining

The pathologist (B.J.) and researcher (M.C.) independently evaluated immunohistochemically stained slides without knowledge of the clinicopathologic data of the patients, and in cases of disagreement, the slides were reviewed simultaneously. Immunolabeling of cyclin I and VEGFR-2 was graded according to the staining intensity of cancer cells as follows: negative = 0, weak = 1, moderate = 2, or strong = 3. Moreover the proportion of immunostained cancer cells was recorded as follows: 0% = 0, 1–25% = 1, 26–50% = 2, 51–75% = 3, or 76–100% = 4. Thus, a staining index (SI) of cyclin I and VEGFR-2 (values 0–12) was obtained by multiplying the staining intensity (scale 0–3) with the staining proportion (scale 0–4). Furthermore, VEGFR-2 immunoreactivity in the membranes of cancer cells and in the cells of stromal compartment was evaluated separately, as described above. The best predictive cut-off score for cyclin I and VEGFR-2 in cancer cells was a SI of 6, and therefore a SI≥6 was considered to indicate high expression of cyclin I and VEGFR-2. Additionally, cancer cells' proliferative activity was evaluated by calculating cyclin A labeling index (LI). Cyclin A LI was determined by observing about 1000 nuclei in the areas of the section with the highest labeling rates using basic imaging software cell^B (Olympus, license No #A5320700). Only cancer cells with strong nuclear staining with cyclin A antibody were considered positive. The LI was obtained by dividing the number of positive cells by the sum of positive and negative ones and expressed as a percentage.

Table 1
Cyclin I and VEGFR-2 immunostaining in cancer cells as related to clinicopathologic variables, patients' response and epithelial ovarian cancers' sensitivity to platinum-based chemotherapy in 55 patients with epithelial ovarian cancer.

Variable	N	Cyclin I high expression (SI≥6)		VEGFR-2 high expression (SI≥6)	
		N (%)	P	N (%)	P
Age					
<60 years	34	11 (32.4)	0.0943[a]	26 (76.5)	0.7505[a]
≥60 years	21	12 (57.1)		17 (81.0)	
Menopausal status					
Before	20	6 (30.0)	0.2573[a]	15 (75.0)	0.7401[a]
After	35	17 (48.6)		28 (80.0)	
FIGO stage					
I and II	28	9 (32.1)	0.1765[a]	18 (64.3)	**0.0200**[a]
III and IV	27	14 (51.9)		25 (92.6)	
Histology					
Serous	26	12 (46.2)	0.5234[b]	23 (88.5)	**0.0300**[b]
Mucinous	19	6 (31.6)		11 (57.9)	
Endometrioid	10	5 (50.0)		9 (90.0)	
Tumor grade					
G1 and G2	33	10 (30.3)	0.0511[a]	22 (66.7)	**0.0175**[a]
G3	22	13 (59.1)		21 (95.5)	
Debulking status					
≤1 cm residual disease	30	9 (30.0)	0.0613[a]	20 (66.7)	**0.0463**[a]
>1 cm residual disease	25	14 (56.0)		23 (92.0)	
Patients' response to chemotherapy					
CR	31	14 (45.2)	0.7306[a]	23 (74.2)	0.4032[a]
PR, SD, and PD	11	4 (36.4)		10 (90.9)	
Tumors' sensitivity to chemotherapy					
Platinum sensitive	32	13 (40.6)	0.7201[a]	24 (75.0)	0.4164[a]
Platinum resistant and refractory	10	5 (50.0)		9 (90.0)	

SI — staining index; a — P value, Fischer's exact test; b — P value, Pearson's χ^2 test; CR — complete response; PR — partial response; SD — stable disease; PD — progressive disease. Significant P values (P<0.05) were presented in bold fonts to distinguish them from nonsignificant P values.

Statistical analysis

The Fisher exact or Pearson's Chi^2 test was used for statistical analysis of the relationships between gene expression and clinicopathologic variables. Correlations between continuous variables were determined using Spearman's rank correlation test. The effect of clinicopathologic parameters and immunolabeling of cyclin I and VEGFR-2 on patients' survival was assessed by Kaplan–Meier method and log-rank test. A multivariate analysis was performed according to the Cox proportional

Table 2
Spearman correlation between cyclin I, VEGFR-2, cyclin A labeling index in cancer cells and ascites volume in 55 patients with epithelial ovarian cancer.

Variable	Cyclin I staining index (0–12)	VEGFR-2 staining index (0–12)
Cyclin A labeling index (0–100%)	**R = 0.3107, P = 0.0209**	**R = 0.4183, P = 0.0015**
VEGFR-2 staining index (0–12)	**R = 0.4587, P = 0.0004**	NA
Ascites volume (ml)	R = 0.0868, P = 0.5404	R = 0.1845, P = 0.1905

R — Spearman's rank correlation coefficient; P — P value, Spearman's test; NA — not applicable.
Significant P values (P<0.05) were presented in bold fonts to distinguish them from nonsignificant P values.

hazard model for statistically significant variables at the univariate level. The results with a 2-sided $P<0.05$ were considered statistically significant. All statistical analyses were done using SPSS software version 14.0 (SPSS Inc., Chicago, USA).

Results

Cyclin I and VEGFR-2 immunohistochemistry

Cyclin I staining was present in the cytoplasm and nuclei of cancer cells and fibroblasts (Figs. 1A–C). The immunolabeling of cyclin I in cancer cells was observed in 54 cases (98.2%), and 23 specimens (41.8%) with cyclin I SI≥6 were regarded as high expressers. VEGFR-2 staining was detected predominantly in the cytoplasm of cancer cells (Figs. 1D–F) in 54 EOCs (98.2%), and 43 cases (78.2%) with VEGFR-2 SI≥6 were indicated as high expressers. Additionally, we found membranous-cytoplasmic VEGFR-2 immunolabeling of cancer cells in 23 cases (41.8%, Figs. 1G–H). Furthermore, VEGFR-2 immunoreactivity was detected in the stromal compartment of all cases, and it was present in vascular endothelial cells (Fig. 1I), fibroblasts (Fig. 1J), and lymphocytes (Figs. 1K–L).

The association between expression of cyclin I, VEGFR-2 and clinicopathologic characteristics

Table 1 shows the association between expression of cyclin I, VEGFR-2 and clinicopathological features. Immunostaining of cyclin I tended to be higher ($P=0.0511$), and immunolabeling of VEGFR-2 was significantly higher ($P=0.0175$) in poorly differentiated (G3) tumors compared to well and moderately differentiated (G1 and G2) cancers. Similarly, suboptimally resected EOCs had higher cyclin I ($P=0.0613$) and significantly higher VEGFR-2 immunolabeling ($P=0.0463$) than optimally resected cancers. High VEGFR-2 immunoreactivity appeared significantly more common in advanced (FIGO III and IV) tumors than in early (FIGO I and II) EOCs ($P=0.0200$), and mucinous EOCs had the lowest VEGFR-2 immunoreactivity compared with serous and endometrioid cancers ($P=0.0300$). Furthermore, immunolabeling of cyclin I and VEGFR-2 in cancer cells was not significantly associated with patients' response or EOCs sensitivity to platinum-based chemotherapy (Table 1, $P>0.05$). Table 2 shows Spearman correlation between immunostaining of cyclin I, VEGFR-2, cyclin A LI, and ascites volume. Expression of cyclin I and VEGFR-2 was directly and significantly correlated with cyclin A LI ($R=0.3107$, $P=0.0209$ and $R=0.4183$, $P=0.0015$, respectively). Moreover, cyclin I immunoreactivity was directly and significantly correlated with VEGFR-2 immunostaining ($R=0.4587$, $P=0.0004$). VEGFR-2 immunoreactivity in the membranes of cancer cells or in stromal compartment was not significantly associated with clinicopathologic parameters analyzed, patients' response or EOCs sensitivity to platinum-based chemotherapy ($P>0.05$, data not shown).

The association between expression of cyclin I, VEGFR-2, and patients' survival

Follow-up period ranged from 1 to 83 months (median 54 months). At the time of the final analysis (January 31, 2012) 24 (43.6%) women had died and 29 (52.7%) had tumor relapse or disease progression. In the univariate analysis, age≥60 years, the presence of residual disease >1 cm, poor tumor differentiation, ascites, the lack of complete response to chemotherapy, advanced clinical stage, and tumor resistance to chemotherapy significantly portended a decreased OS and DFS of patients with EOC (Table 3). Cyclin I did not influence OS (Fig. 2A, Table 3) or DFS (Fig. 2B, Table 3). There was a trend towards higher VEGFR-2 immunostaining in EOCs of patients with poorer OS (Fig. 2C, Table 3, $P=0.0500$), and higher VEGFR-2 immunolabeling was significantly associated with shorter DFS (Fig. 2D, Table 3, $P=0.0437$). In the multivariate analysis, patients' age and response to chemotherapy were independent risk factors for OS, and patients' response to chemotherapy was an independent risk factor for DFS (Table 3). VEGFR-2 immunoreactivity in the membranes of cancer cells or in stromal compartment did not significantly influence patients OS or DFS ($P>0.05$, data not shown).

Discussion

To our knowledge, we are the first to report that cyclin I is expressed in EOCs. Cyclin I immunoreactivity was detected in both the cytoplasm

Table 3
Univariate and multivariate survival analyses of the prognostic factors for overall and disease-free survival in 55 patients with epithelial ovarian cancer.

Variable	Overall survival				Disease-free survival			
	Univariate analysis	Multivariate analysis			Univariate analysis	Multivariate analysis		
	P^a	HR	95% CI for HR	P^b	P^a	HR	95% CI for HR	P^b
Cyclin I staining index in cancer cells (SI<6 vs SI≥6)	0.3162	NA	NA	NA	0.1228	NA	NA	NA
VEGFR-2 staining index in cancer cells								
SI<6	0.0500	NA	NA	NA	**0.0437**	1.000	Reference	0.4188
SI≥6						1.946	0.387–9.782	
Menopausal status (before vs after)	0.1896	NA	NA	NA	0.4517	NA	NA	NA
Histology (serous vs mucinous vs endometrioid)	0.8287	NA	NA	NA	0.9752	NA	NA	NA
Age								
<60 years	**0.0018**	1.000	Reference	**0.0020**	**0.0259**	1.000	Reference	0.0537
≥60 years		6.533	1.985–21.504			2.559	0.985–6.647	
FIGO stage								
I and II	<**0.0001**	1.000	Reference	0.3816	<**0.0001**	1.000	Reference	0.1929
III and IV		2.146	0.388–11.869			2.493	0.630–9.862	
Tumor grade								
G1 and G2	**0.0011**	1.000	Reference	0.6026	<**0.0001**	1.000	Reference	0.8718
G3		0.714	0.201–2.535			0.905	0.270–3.031	
Patients' response to chemotherapy								
CR	<**0.0001**	1.000	Reference	**0.0007**	<**0.0001**	1.000	Reference	**0.0005**
PR, SD and PD		12.166	2.890–51.213			19.769	3.721–105.084	
Debulking status (≤1 cm residual disease vs >1 cm residual disease)	<**0.0001**	Ex	Ex	Ex	<**0.0001**	Ex	Ex	Ex
Tumors' sensitivity to chemotherapy (Platinum sensitive vs platinum resistant and refractory)	<**0.0001**	Ex	Ex	Ex	<**0.0001**	Ex	Ex	Ex
Ascites (none vs present)	**0.0185**	Ex	Ex	Ex	**0.0017**	Ex	Ex	Ex

P^a — P value, log rank test; HR — hazard ratio; CI — confidence interval; P^b — P value, Cox regression; CR — complete response; PR — partial response; SD — stable disease; PD — progressive disease; NA — not applicable; Ex — debulking status, tumors' platinum sensitivity, and ascites presence were excluded from multivariate analysis due to high multicollinearity.
Significant P values ($P<0.05$) were presented in bold fonts to distinguish them from nonsignificant P values.

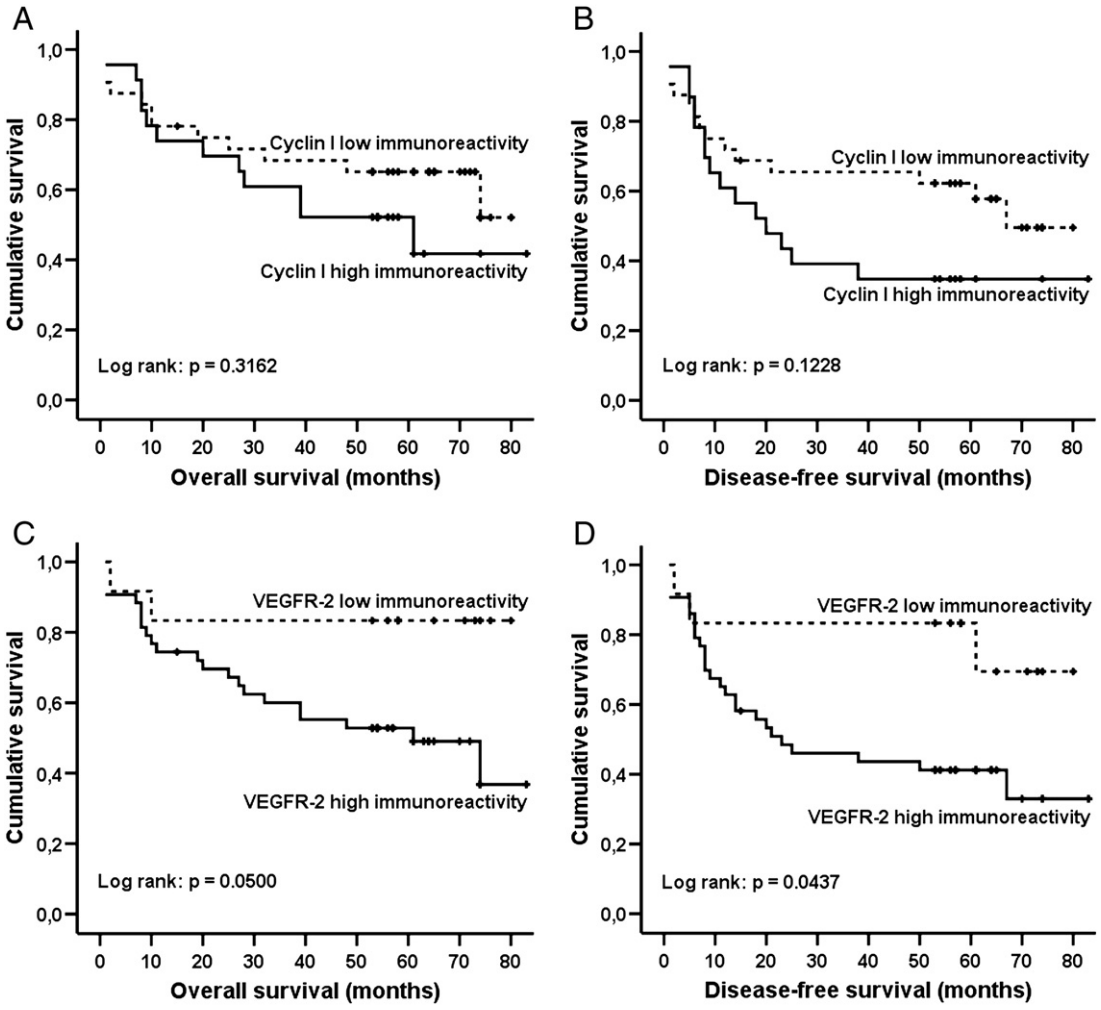

Fig. 2. Kaplan–Meier plots for the overall survival and disease-free survival of ovarian carcinoma patients according to expression of cyclin I (A–B) and VEGFR-2 (C–D) in cancer cells. The survival curves were analyzed by the log rank test. +, censored data; low immunoreactivity — staining index<6; high immunoreactivity — staining index≥6. As can be seen from the plot higher VEGFR-2 protein expression was significantly associated with reduced disease-free survival (D, P = 0.0437).

and nuclei of cancer cells. Similarly, cyclin I immunolabeling was discovered in the cytoplasm and nuclei of human breast [8] and pancreatic cancer cells [7]. In the current study, there was a trend suggesting higher cyclin I immunostaining in G3 tumors and in EOCs of patients with residual tumors > 1 cm. Moreover, cyclin I immunolabeling was significantly correlated with cancer cells proliferative activity evaluated using cyclin A LI as a marker. Cyclin I is expressed at high levels in post-mitotic tissues and its level does not fluctuate during the cell cycle, that's why it is believed that cyclin I has no role in regulating cell proliferation [4–6]. Landberg et al. [8] discovered no significant association between cyclin I immunostaining and tumor grade or proliferation of human breast cancer cells using Ki-67 LI as a marker. However, it was reported that cyclin I may be post-transcriptionally regulated by ubiquitination [16], and dysregulation of ubiquitin-mediated degradation of cyclin I may be related to aberrant cell cycle regulation and/or resistance to apoptosis, which are characteristic of invasive breast and other tumors [8]. Indeed, experimental evidence has been provided that cyclin I protects cultured mouse podocytes from injury-induced apoptosis by increasing the levels of prosurvival proteins Bcl-2, Bcl-X_L [17], and p21(Cip1/Waf1) [4]. Additionally, cyclin I immunostaining in breast cancer was significantly correlated with the expression of tumor suppressor p53, a regulator of cell division and apoptosis [8]. In the present study, cyclin I expression was directly and significantly correlated with VEGFR-2 immunostaining. Previously, Landberg et al. [8] discovered such relationship in human breast cancer. VEGF/VEGFR-2 signaling system enhances ovarian cancer cell proliferation and survival [10–12]. Moreover, experiments on cancer cell lines proved that suppression of VEGFR-2 expression with siRNA inhibits proliferation of human breast cancer cells, and suppresses tumor growth in mice xenograft model [18]. Furthermore, recent studies showed that therapies with anti-VEGF antibody bevacizumab [19] or oral tyrosine kinase inhibitors targeting receptors for pro-angiogenic growth factors, including VEGFR-2 [20–22], reduce tumor growth, angiogenesis, ascites production, and improve survival in murine models of EOC. Our data indicate that cyclin I is correlated with VEGFR-2, proliferation of EOC cells, and may be involved in angiogenesis through VEGF/VEGFR-2 signaling pathway. Further investigation of cyclin I in ovarian cancer is needed to evaluate if cyclin I may become a novel target for an anticancer therapy.

In this study, for the first time, we discovered significantly higher VEGFR-2 immunostaining in advanced EOCs in comparison to early tumors. Our results are in the line with those obtained by Nishida et al. [23], where it was shown that VEGFR-2 immunolabeling in EOCs was rising with increasing FIGO clinical stage, but this association was not significant (P = 0.18). Additionally, in previous studies, VEGFR-2 expression was increasing with disease stage in patients with bladder [24], prostate [25], and lung cancer [26]. In our study, VEGFR-2 immunostaining was significantly higher in G3 and suboptimally resected EOCs. Similarly, VEGFR-2 immunostaining in EOC cells was rising with increasing tumor grade (P = 0.17), and it was correlated with ovarian cancer extension, including peritoneal metastases outside the pelvic cavity, lymph node metastases, and positive ascitic cytology in early stage in previous study [23]. Higher VEGFR-2 levels in breast cancer

tissues were detected in larger, G3 tumors, and in distant metastases [27]. Furthermore, higher VEGFR-2 expression was associated with the presence of metastases in patients with renal cancer [28], thyroid carcinomas [29], and melanomas [30], supporting the idea that VEGFR-2 participate in the progression of several human cancers. In this study, VEGFR-2 immunolabeling was significantly lower in mucinous EOCs, which may be explained by lower frequency of advanced (FIGO III and IV) and G3 tumors in mucinous EOCs compared to other histological subtypes (42.1% vs 52.8% and 21.1% vs 50.0%, respectively).

In our study, we discovered that expression of cyclin I and VEGFR-2 is not associated with patients' response or EOCs' sensitivity to platinum-based chemotherapy. Similarly, VEGFR-2 protein level evaluated in ovarian cancer tissues by Western-blot was not related to the response to paclitaxel–platinum chemotherapy [31].

In the present study, cyclin I was not significantly related to OS or DFS of patients with EOC. Similarly, immunostaining of cyclin I did not influence breast cancer specific survival [8]. In our study, higher VEGFR-2 immunolabeling in EOCs was significantly associated with shorter DFS in univariate analysis only, which may be explained by higher expression of VEGRF-2 in more advanced disease. Our results are in line with those obtained by Nishida et al. [23] who reported significantly shorter DFS and disease-specific survival of patients with higher VEGFR-2 immunostaining in ovarian carcinomas.

VEGFR-2 immunoreactivity in the stromal compartment of EOCs was not significantly associated with clinicopathologic parameters analyzed, patients' response or EOCs sensitivity to platinum-based chemotherapy, or patients' survival. There are no studies analyzing prognostic value of stromal VEGFR-2 immunoreactivity in EOC. Andersen et al. [32] discovered that VEGFR-2 immunostaining in stromal compartment of non-small cell lung cancers was not associated with patients' survival. Similarly, VEGFR-2 immunostaining in vascular endothelial cells was not associated with DFS of patients with clear cell renal carcinomas [33].

In conclusion, our results indicate that elevated expression of cyclin I and VEGFR-2 is likely to provide a proliferative advantage to the EOC cells, and cyclin I may be linked with angiogenesis in EOC. Higher expression of VEGFR-2 is associated with more advanced disease. Further investigation of cyclin I is needed to evaluate if it may become a new target for an anticancer therapy, as at this moment toxicity of anti-VEGF/VEGFR-2 treatment in human ovarian cancer is worrisome [2,34,35].

Conflict of interest statement

We declare no conflicts of interest.

Acknowledgements

This study was supported by the grant from the Ministry of Science and Higher Education of Poland (No. N407 092 32/3452).

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