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Explorative investigation of vascular endothelial growth factor receptor expression in primary ovarian cancer and its clinical relevance

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HIGHLIGHTS

- We systematically analyzed expression pattern and compartmental distribution of VEGF-receptor family in ovarian cancer.
- Total VEGF-receptor expression correlated with tumor cell dissemination to the bone marrow and VEGF-R1 positivity indicated decreased progression-free survival.
- We suggest VEGF-receptor status as a molecular biomarker for ovarian cancer, paralleling tumor cell spread and recurrence risk.

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ABSTRACT

Objectives. The identification of novel molecular biomarkers, predicting outcome of ovarian cancer, is highly desirable. Considering that angiogenesis is a critical factor for ascites development and peritoneal dissemination in ovarian cancer and given that the vascular endothelial growth factor (VEGF) receptor signaling axis is a major driver of angiogenesis, we sought to analyze expression and compartmental distribution of VEGF-receptor family in ovarian cancer and to assess its clinical relevance with regard to established clinicopathological parameters, tumor cell dissemination to the bone marrow (BM) and the patient's survival.

Methods. A total of 73 patients with primary ovarian cancer were enrolled into this study. Primary tumor tissue was analyzed for the expression of VEGF-R1, VEGF-R2 and VEGF-R3 by immunohistochemistry. The presence of disseminated tumor cells (DTC) in the BM was analyzed by immunocytochemistry using the pancytokeratin antibody A45B/B3 and subsequent automatic detection based on staining and cytomorphology.

Results. In primary ovarian cancer tissue, VEGF-receptor expression, detected with an overall frequency of 44%, was mostly located in the vascular wall and across the stroma; positivity rates for VEGF-R1, VEGF-R2 and VEGF-R3 were 34%, 18% and 26%, respectively. Total VEGF-receptor expression correlated with residual tumor after primary debulking surgery and the presence of DTC at primary diagnosis ($p = 0.035$, $p = 0.023$, respectively). Interestingly, VEGF-R1 positivity significantly correlated with decreased progression-free survival ($p = 0.026$).

Conclusions. This is the first report, suggesting total VEGF-receptor status as a molecular biomarker for monitoring tumor cell spread to the BM and, particularly, revealing prognostic significance of VEGF-R1.

Introduction

Ovarian cancer is the leading cause of death among women with gynecologic malignancies [1]. Standard treatment of ovarian cancer constitutes primary radical surgery, aiming at macroscopically complete tumor resection, and subsequent platinum- and paclitaxel-based chemotherapy [2]. So far, residual tumor burden after primary surgery is believed to be one of the most relevant prognostic factors for ovarian malignancies [3,4]. Notably, advanced ovarian cancer is a comparatively chemo-sensitive tumor with overall clinical response rate of 70–80% [5]. However, despite this profound sensitivity to platinum-based chemotherapy and despite continuous attempts to implement maintenance therapies into the present treatment regime, more than half of all patients still experience recurrence, resulting in poor overall prognosis

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[5,6]. Innovative therapy approaches are therefore needed to improve the patient's outcome. In this regard, the discovery of predictive molecular biomarkers is of high clinical interest, in order to individualize prognosis and therapy decision for each patient.

The formation of new vessels from pre-existing vasculature, referred to as angiogenesis, is usually regulated through a dynamic balance of pro- and anti-angiogenic factors, in order to maintain physiological homeostasis. However, angiogenesis is also critically involved in ascites development and peritoneal dissemination of ovarian cancer [7]. In this context, it has been shown that vascular endothelial growth factor (VEGF) receptor signaling is one of the major regulators of angiogenesis [8,9] and that VEGF-ligands (comprising VEGFA, VEGFB, VEGFC, VEGFD and VEGFE) are capable of interacting with three different tyrosine kinase VEGF-receptors (VEGF-R1, VEGF-R2, and VEGF-R3). This interaction, in turn, triggers downstream signaling pathways and promotes angiogenesis, by regulating e.g. migration, proliferation and survival of endothelial cells [10]. Given that VEGF-overexpression has been observed in tumor tissue and in the circulation [11,12], the addition of the monoclonal antibody bevacizumab, directed against VEGF, combined with platinum- and taxane-based chemotherapy, followed by bevacizumab monotherapy, significantly prolonged PFS in first-line therapy [13,14]. In addition, in patients with response to adjuvant platinum- and taxane-based chemotherapy, maintenance monotherapy with pazopanib, a tyrosine kinase inhibitor against VEGF-receptor, PDGF-receptor and c-kit, showed significantly prolonged PFS, too [15]. Moreover, in platinum-sensitive and platinum-resistant relapse, the addition of bevacizumab to chemotherapy also significantly improved PFS [16,17].

However, despite recent efforts to reveal components of the VEGF-axis as novel biomarkers for response to anti-angiogenic treatment and despite the well described prognostic relevance of intratumoral and circulating VEGF [18,19], only little is known about expression pattern and clinical relevance of VEGF-receptors in ovarian cancer. Therefore, the present study aimed at systematically analyzing VEGF-R1, VEGF-R2 and VEGF-R3 protein expressions and its compartmental distribution in primary ovarian cancer tissue and at assessing clinical relevance of the VEGF-receptor family with regard to established clinicopathological parameters, the presence of disseminated tumor cells (DTC) in the bone marrow (BM) and survival (PFS, OS).

Patients and methods

Characterization of study patients

The present study was conducted at the Department of Gynecology and Obstetrics at the University Hospital of Essen, Germany. In total, 73 patients with primary epithelial ovarian cancer were studied from January 2006 until November 2010. Survival data of these patients were obtained from the local municipal registry. The median follow-up time was 2.6 years, ranging from 0.08 to 6.58 years. Informed written consent was obtained from all patients, and the study was approved by the local Essen Research Ethics Committee (05/2856). Clinical data of the patients are summarized in Table 1. The whole study population received primary radical surgery aiming at macroscopically complete tumor resection. Individual patients, relapsing within the first six months after the end of chemotherapy, were defined to be clinically platinum resistant.

Table 1
Patient characteristics at primary diagnosis of ovarian cancer.

Total 73	
Age	Mean: 61 years (range 21–89 years)
Tumor stage	
FIGO I–II	11 (15%)
FIGO III	49 (67%)
FIGO IV	13 (18%)
Lymph node metastasis	
N_0	23 (32%)
N_1	28 (38%)
N_x	22 (30%)
Tumor grading	
I–II	37 (51%)
III	36 (49%)
Histologic subtype	
Serous histology	60 (82%)
Mucinous histology	3 (4%)
Any other histology	10 (14%)
Residual tumor	
Macroscopic complete resection	38 (52%)
Any residual tumor	35 (48%)
Platinum resistance	
Platinum sensitive	49 (67%)
Platinum resistant	13 (18%)
DTC in the bone marrow	
DTC[a]-negative	43 (59%)
DTC-positive	25 (34%)
No status available	5 (7%)
Survival	
PFS[b]	28 months (range 2–77 months)
No relapse	20 (27%)
Relapse	48 (66%)
OS[c]	36 months (range 1–79 months)
Alive	29 (40%)
Dead	44 (60%)

The present table recapitulates the patient's characteristics at primary diagnosis of ovarian cancer and comprises clinicopathological variables, platinum-sensitivity status, information on the presence of disseminated tumor cells in the bone marrow and survival data.
[a] DTC, disseminated tumor cells in the bone marrow.
[b] PFS, progression-free survival.
[c] OS, overall survival.

Immunohistochemical VEGF-receptor staining

VEGF-receptor analysis was performed in formalin fixed paraffin embedded (FFPE) primary ovarian cancer tissue, obtained during debulking surgery. Immediately after resection, specimens were incubated in buffered paraformaldehyde (4%, 24 h) and subsequently embedded in paraffin. For immunohistochemical investigation, 4 consecutive sections of 5 μm thickness were processed from each FFPE-block, deparaffinised with xylene and subsequently rehydrated with descending alcohol concentrations. After rehydration, sections were incubated with primary antibodies for 12 h at RT, detecting VEGF-R1, VEGF-R2 or VEGF-R3 (IgG rabbit polyclonal, Santa Cruz, Dallas, Texas, 1:50, 1:50, 1:100, respectively). Subsequent staining was performed by the streptavidin–biotin–peroxidase method [20,21]. Briefly, sections were incubated with a secondary antibody (anti-rabbit polyclonal, 1:200, 60 min, RT), followed by incubation with alkaline-phosphatase-complex (PAP, 1:200, 30 min, RT) and avidin–biotin-complex (ABC, 1:250 30 min, RT). Specific immunostaining was visualized by a modified nickel-enhanced glucose oxidase method [20,22]. In order to analyze the underlying tissue structure, all sections were counterstained with calcium red and separate consecutive Hematoxylin & Eosin stained sections were prepared for each patient.

Detection of disseminated tumor cells in the bone marrow

Between 5 and 10 ml blood of BM per site was aspirated from the anterior iliac crests (local anesthesia with mepivacain) and processed within 24 h. Tumor cell detection was performed according to recommendations by the German Consensus group of Senology [23]. Briefly, mononuclear cells (MNC) were isolated from heparinized BM (5000 U/ml BM) by Ficoll-Hypaque density gradient centrifugation (density 1.077 g/mol; Pharmacia, Freiburg, Germany) at 400 *g* for 30 min. In total, 8×10^6 MNC per patient were analyzed for the presence of cytokeratin (CK)-positive cells using the murine monoclonal antibody A45-B/B3, directed against a common epitope of CK-polypeptides, including the CK-heterodimers 8/18 and 8/19 (Micromet,