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NON-THEMATIC REVIEW

Significance of vascular endothelial growth factor in growth and peritoneal dissemination of ovarian cancer

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Abstract Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis which drives endothelial cell survival, proliferation, and migration while increasing vascular permeability. Playing an important role in the physiology of normal ovaries, VEGF has also been implicated in the pathogenesis of ovarian cancer. Essentially by promoting tumor angiogenesis and enhancing vascular permeability, VEGF contributes to the development of peritoneal carcinomatosis associated with malignant ascites formation, the characteristic feature of advanced ovarian cancer at diagnosis. In both experimental and clinical studies, VEGF levels have been inversely correlated with survival. Moreover, VEGF inhibition has been shown to inhibit tumor growth and ascites production and to suppress tumor invasion and metastasis. These findings have laid the basis for the clinical evaluation of agents targeting VEGF signaling pathway in patients with ovarian cancer. In this review, we will focus on VEGF involvement in the pathophysiology of ovarian cancer and its contribution to the disease progression and dissemination.

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1 Introduction

Vascular endothelial growth factor (VEGF), a potent cytokine and a key regulator of physiological and pathological angiogenesis, has a major contribution to diverse pathological processes, in particular tumorigenesis [1]. In women, an exceptional role is played by VEGF through its involvement in ovarian biology. Closely linked to the normal function of ovaries, VEGF is also implicated in ovarian pathologies, including malignant neoplasms [2]. Ovarian cancer, the seventh leading cancer in women and the second cause of death from gynecologic malignancies worldwide [3], is a richly vascularized tumor that is known to be highly dependent on VEGF-mediated angiogenesis [4]. While representing a crucial, early event in ovarian carcinogenesis [5], VEGF expression is associated with tumor growth and aggression, as well as poor survival [6–8].

Peritoneal seeding is the most common pathway for the spread of ovarian cancer [9]. At diagnosis, about two thirds of the patients with ovarian cancer have already developed peritoneal carcinomatosis [10] and more than one third present with malignant ascites [11]. Both tumor burden and ascites are inversely associated with survival [12, 13]. Basically through promoting tumor angiogenesis and enhancing the vascular permeability, VEGF has been implicated in the peritoneal dissemination of ovarian cancer and the subsequent development of malignant ascites, further highlighting the key importance of VEGF in the pathophysiology of the disease. Here, we review different aspects of VEGF contribution to ovarian cancer, address VEGF-targeted strategies as a

therapeutic approach to the disease, and present the latest clinical data on the subject.

2 Vascular endothelial growth factor

2.1 Identification

In 1983, the identification of a polypeptide from the conditioned medium of a guinea pig tumor cell line was reported by Senger et al. [14]. To reflect its role in enhancing the permeability of tumor vasculature, they designated this polypeptide "vascular permeability factor (VPF)." Later in 1989, Ferrara and Henzel [15] isolated a potent endothelial mitogen from the conditioned media of bovine pituitary follicular cells and named it vascular endothelial growth factor to show its specificity for endothelial cells. In the same year and based on the work by Senger et al., the isolation and sequencing of human VPF from U937 cells were independently reported by Connolly et al. [16]. Following cDNA cloning of VEGF and VPF, it was eventually revealed that these molecules were the same [17, 18].

2.2 VEGF and its receptors

VEGF family include VEGF-A (hereafter VEGF), VEGF-B, VEGF-C, VEGF-D, placenta growth factor (PlGF), VEGF-E (viral VEGF homologs), and VEGF-F (snake venom VEGFs) [19]. VEGF is a 45-kDa heparin-binding homodimeric glycoprotein [15]. At least 14 various isoforms of VEGF result from alternative splicing [20]. $VEGF_{165}$ is the most abundant and mitogenic isoform which exists as both extracellular matrix-bound and freely diffusible protein. Plasmin and various metalloproteinases can split the bound fraction into bioactive fragments $VEGF_{110}$ or $VEGF_{113}$ [21]. $VEGF_{121}$ is a freely diffusible protein that does not bind to heparin. $VEGF_{189}$ and $VEGF_{206}$ are longer, heparin-binding isoforms which might also undergo extracellular proteolysis to generate bioactive, diffusible fragments [22]. Thus, VEGF proteins may become available to endothelial cells by at least two different mechanisms: alternative splicing and proteolytic cleavage. Besides, differential ability of VEGF isoforms in binding to the extracellular matrix generates an "angiogenic gradient" that is now considered of importance in a variety of pathophysiological conditions [23].

VEGF binding sites include three receptor tyrosine kinases (RTK) called vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3 (Fig. 1). VEGF family members show differential affinity for their receptors. VEGF, VEGF-B, and PlGF can bind and activate VEGFR-1, whereas VEGFR-2 is primarily activated by VEGF, and VEGFR-3 activation is only triggered by VEGF-C and VEGF-D [24].

Neuropilins (NRPs) have also been implicated in VEGFR activation [25]. In endothelial cells, NRP-1 is thought to act primarily as a co-receptor for $VEGF\text{-}A_{165}$ by forming complexes with VEGFR-2. VEGFRs were identified initially on the cell surface of vascular endothelial cells [26, 27] and, subsequently, on bone marrow-derived cells such as monocytes [28]. VEGFR-1 expression has been observed in vascular endothelial cells and a range of non-endothelial cells including hematopoietic stem cells, monocytes, and macrophages [29]. VEGFR-2 is expressed in vascular endothelial cells and, at lower levels, in neurons, osteoblasts, pancreatic duct cells, retinal progenitor cells, and megakaryocytes [30]. VEGFR-3 expression seems to be largely restricted to lymphatic endothelial cells [31]. VEGFRs, like other RTKs, become activated when ligand–receptor binding and resultant dimerization happen. Subsequent autophosphorylation on certain tyrosine residues triggers intracellular signaling cascade mediated by several effectors. Although VEGFR-1 affinity for VEGF is tenfold higher than that of VEGFR-2, VEGF activation of VEGFR-2 is much more significant. Thus, VEGFR-2 represents the major mediator of VEGF-driven responses in endothelial cells promoting angiogenesis and vascular permeability [21].

2.3 Biological effects of VEGF

VEGF biologically affects angiogenesis, hematopoiesis, hemodynamics, and vascular homeostasis. VEGF drives endothelial cell survival, proliferation, migration, and tube formation. Anti-apoptotic activity of VEGF is in part mediated through the activation of PI3K/Akt pathway [32]. VEGF also prevents endothelial apoptosis by inducing the expression of apoptosis-regulating proteins such as Bcl-2, A1 [33], XIAP, and survivin [34]. Acting as an endothelial cell mitogen [35], VEGF promotes sprouting of vascular endothelial cells during physiological and tumor angiogenesis. In addition, VEGF controls hematopoietic stem cells' survival during hematopoietic repopulation and plays an important role in regulating the differentiation of endothelial progenitor cells and their migration to regions of neoangiogenesis in adults [1]. Moreover, VEGF enhances vascular permeability to water and large molecular weight proteins [36], a process in which p38 MAPK pathway has been implicated as an essential mediator [37]. VEGF also stimulates nitric oxide-mediated vasodilatation [38]. VEGF, at low physiological levels, maintains vascular homeostasis in vascularized tissues, especially in fenestrated and sinusoidal vessels in endocrine and secretory organs as well as large blood vessels, skeletal muscle, and myocardium [39].

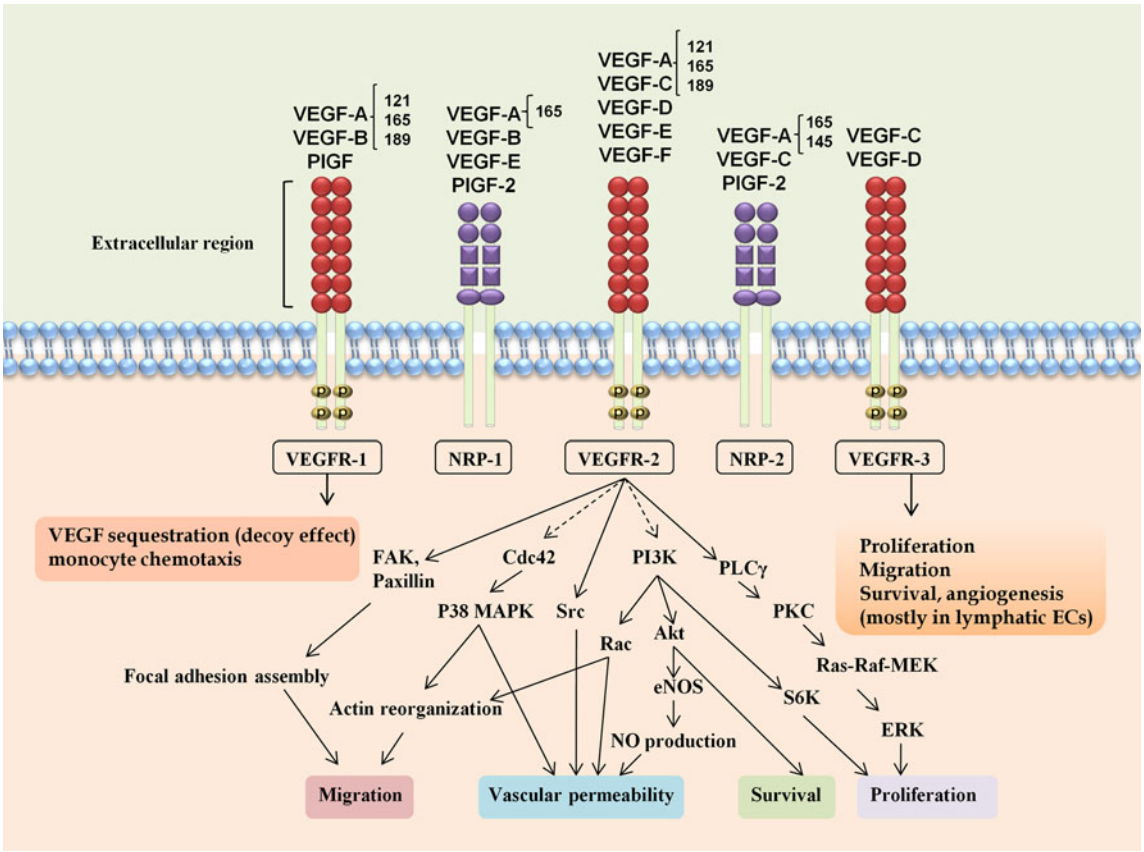

Fig. 1 Vascular endothelial growth factor (VEGF) family and their receptors. VEGF family members bind to specific receptor tyrosine kinases VEGFR-1, VEGFR-2, and VEGFR-3 and, through activating different cascades, exert their various biologic effects. VEGFR-2 represents the major mediator of VEGF-driven responses in endothelial cells responsible for most VEGF angiogenic activities. Neuropilins (NRP-1 and NRP-2) are known as co-receptors for VEGF

2.3.1 VEGF and physiological processes

VEGF promotes physiological angiogenesis activated in embryonic and postnatal development, skeletal growth, and endochondral bone formation [40]. Apart from its unique contribution to ovarian physiology described below, VEGF mediates other angiogenic processes in adults, such as wound healing [41, 42], tissue regeneration after injury and ischemia [43, 44], and exercise-induced angiogenesis in skeletal muscle [45].

The ovary is distinct from other endocrine organs in that it undergoes repetitive cycles of angiogenesis within its various glandular compartments [46]. VEGF expression and production within the ovary are critical for normal reproductive function [2]. Increased intrafollicular VEGF has been shown during the initial part of the ovulatory cycle, with peak concentrations just before the start of the luteal phase [47]. By driving thecal vascularization, VEGF supports the cyclic growth, development, and maturation of the follicle; ovulation; and associated endocrine changes. Consistently, VEGF inhibition in different stages of follicular phase can disrupt these normal events [46, 48–52]. As a permeability factor, VEGF is proposed to modulate differential vascular permeability leading to the preferential accumulation of gonadotropins within the dominant follicle, hence its likely involvement in the process of follicular selection [2]. In addition, VEGF might mediate the preovulatory increase in follicular vascular permeability and the resulting edema, a mechanism contributing to ovulation [53]. VEGF is also essential for corpus luteum angiogenesis [54] as inhibition of VEGF has a profound inhibitory effect upon luteal function [55]. Coexpression of VEGF and its receptors by granulosa cells has attributed another potentially important function to VEGF [56]. It has been suggested that VEGF may directly modulate the function of these cells by promoting cell survival [57], proliferation [56, 58], and/or migration [59].

2.3.2 VEGF in nonmalignant disorders

VEGF has been implicated in various pathological conditions. Besides performing stabilizing functions in the developing retinal vasculature [60], VEGF has strikingly been correlated with intraocular neovascular syndromes

such as retinopathy of prematurity [61], diabetic retinopathy [62], central retinal vein occlusion [63], and neovascular age-related macular degeneration [64]. It has also been shown that VEGF may be an important mediator of such inflammatory disorders as psoriasis [65], rheumatoid arthritis [66], osteoarthritis [67], human renal [68] and cardiac [69] allograft rejection, as well as blood–brain barrier breakdown and the resulting edema in acute brain injury[70]. In addition, VEGF contribution to some cardiovascular diseases has been reported, including infantile hemangiomas [71], atherosclerosis [72], ischemic heart disease [73], peripheral vascular disease [74], and focal cerebral ischemia [75]. Moreover, different investigations have implicated VEGF in the pathology of female reproductive tract, such as polycystic ovary syndrome, ovarian hyperstimulation syndrome [76], endometriosis [77], and preeclampsia [78, 79]. Besides, VEGF insufficiency might contribute to neurodegeneration, respiratory distress and, possibly, cardiac failure [80].

2.3.3 Role of VEGF in malignancies

VEGF and its receptors are expressed in the vast majority of human solid tumors, including those of the lung [81], breast [82], gastrointestinal tract [83], kidney, bladder [84], ovary, endometrium [85], brain [86], and endocrine system [87]. In addition to tumor cells as the major source of VEGF, stromal cells such as monocytes, macrophages, and fibroblasts also produce VEGF [88, 89]. VEGF contributes to tumor vascularization, progression, and invasion. To grow beyond microscopic size, tumors need to undergo an "angiogenic switch" to enter a vascular phase in which blood perfusion provides a better delivery of oxygen and nutrients and an enhanced disposal of waste products [90]. VEGF is a key mediator of this early event in tumor angiogenesis [91]. Additionally, the endothelial cells of intratumoral vasculature are more dependent on VEGF as a survival factor and mitogen than those of normal vasculature elsewhere [92]. VEGF-induced enhancement of endothelial cell survival, proliferation, and migration together with the resulting synthesis of other pro-angiogenic factors, including basic fibroblast growth factor, heparin-binding epidermal growth factor (EGF)-like growth factor, granulocyte colony-stimulating factor, insulin-like growth factor-1, interleukin (IL)-6, and IL-8, provides a sustainable angiogenesis [93, 94].

Moreover, VEGF helps tumor stroma form and mature. By augmenting the permeability of the microvasculature, VEGF promotes extravasation of plasma proteins and water into the tumor milieu and the subsequent reshaping of extracellular matrix that makes it favorable to migration and proliferation of endothelial cells, monocytes, macrophages, and fibroblasts. This allows final degradation of the provisional matrix, which will be then replaced with fibroblast-produced proteins, proteoglycans, and glycosaminoglycans [95]. VEGF activation of matrix-degrading enzymes allows unhindered development of further blood vessels [96].

VEGF is a crucial factor in tumor invasion and metastasis as well. While tumor vascularization is a prerequisite for tumor cells to spread by shedding into the circulation, the newly formed, immature capillaries with fenestrated basement membrane allow greater accessibility for tumor cells [97]. VEGF has also been shown to enhance tumor invasion through a direct, autocrine effect on tumor cells [35, 98, 99]. Besides, VEGF helps the growing tumor evade host immune responses via inhibiting functional maturation of dendritic cells [100, 101], the most potent antigen-presenting cells with a central role in antitumor cell-mediated immunity. Additionally, VEGF stimulates hematopoietic progenitor cells to initiate the pre-metastatic niche, a regulatory event in tumor metastasis abolished by VEGF inhibition [102].

VEGF contribution to malignancies is not exclusive to solid tumors. VEGF is expressed in a wide variety of cell lines derived from hematological neoplasms, including T cell lymphoma, acute lymphoblastic leukemia, Burkitt's lymphoma, acute lymphocytic leukemia, histiocytic lymphoma, and promyelocytic leukemia [103]. Survival, proliferation, and migration of leukemia/lymphoma cells can be stimulated by autocrine and paracrine VEGF loops. VEGF/VEGFR-related pathways are thought to present a promising therapeutic target in some hematolymphoid malignancies [104].

3 VEGF and pathogenesis of ovarian cancer

The association of VEGF and ovarian cancer has been documented in several studies. Constitutive VEGF gene expression in normal and neoplastic human ovaries [105] as well as its differential expression in tumor specimens compared to benign ovarian tissue [106, 107] has been reported. Preclinical experiments have shown that overexpression of VEGF can transform normal, functional ovarian epithelium into ascites-producing, neoplastic tissue [4, 108]. Large amounts of VEGF are secreted in ovarian cancer *in vitro* and *in vivo* [109]. Overexpression of intratumoral VEGF, found to correlate with poorer prognosis [8, 110, 111] and enhanced odds of progression [112], has been suggested as an independent prognostic factor for overall survival [113]. VEGF expression within omental metastases appeared not only correlated with the extent of omental involvement but also as an independent prognostic indicator [114]. Elevated levels of VEGF were detected in fluid samples from malignant cysts generated during

ovarian cancer development which may represent a useful biomarker of angiogenesis and tumor progression [106, 107]. VEGF levels in ovarian cancer-induced malignant ascites are markedly elevated compared with those in ascitic fluids of nonmalignant origin [115] being reportedly of prognostic significance [116]. VEGF has been suggested as a serological biomarker for clinical diagnosis and a predictor of prognosis in patients with ovarian cancer [117–119]. In addition, overexpression of VEGF receptors [106] and co-receptors [120, 121] has been found in ovarian cancer. It has been reported that VEGF gene polymorphisms are an independent adverse prognosticator of overall survival [122].

VEGF expression and/or production in ovarian cancer is induced not only by hypoxia [123–125] but also by different growth factors, mediators, and effectors, including insulin-like growth factor 1 [126], EGF [127], platelet-derived growth factor (PDGF) [128], transforming growth factor-β [129], tumor necrosis factor-α (TNF-α) [130], TNF-like weak inducer of apoptosis [131], IL-1β [132], IL-6 [133], endothelin-1 [134, 135], prostaglandine E2 [136], gonadotropins [137, 138], 4-hydroxy estradiol [139], matrix metalloproteinases (MMPs) [140], reactive oxygen species [141], and cyclooxygenase [142, 143]. Additionally, lysophosphatidic acid (LPA), a bioactive phospholipid present in high levels in the ascitic fluid and plasma from ovarian cancer patients, has proved to induce VEGF expression in ovarian cancer cells [144], a process in which NF-κB pathway has been recently implicated [145]. Moreover, oncogenes such as *PIK3CA* [146] and *Her-2/neu* [147] have been indicated to regulate VEGF production in ovarian cancer cells. Here, we review different aspects of VEGF implication in the pathogenesis of ovarian cancer.

3.1 VEGF, carcinogenesis, and tumor growth in ovarian cancer

The theory of incessant ovulation hypothesizes that repetitive wounding of the ovarian surface epithelium and cell proliferation in postovulatory repair result in a stepwise accumulation of genomic abnormalities. Ovarian epithelial inclusion cysts occur as a result and might increase risk of carcinogenesis by trapping cells in an environment of aberrant autocrine or paracrine stimulation by growth factors including VEGF which activate intracellular processes and signaling pathways [148].

Initial studies revealed that VEGF-driven angiogenesis is an early, crucial event in ovarian carcinogenesis [5, 106] and implicated VEGF-regulated angiogenesis as an important component of ovarian cancer growth [6, 149]. Schiffenbauer et al. attributed angiogenic potential of ovarian cancer to gonadotropin-induced expression of VEGF [137]. Later, Zhang et al. showed that VEGF derived from ovarian cancer cells upregulates angiopoietin 2 in host endothelial cells and induces in a paracrine manner the remodeling of host vasculature to support angiogenesis during tumor growth [150]. Besides, it has been indicated that Akt1 and Akt3, two downstream effectors of PI3K signaling pathway, have their important roles in ovarian tumorigenesis played via regulation of VEGF secretion and angiogenesis [151, 152]. Moreover, Kryczek et al. showed that tumor-derived VEGF and CXCL12 formed a synergistic angiogenesis axis critical for tumor neovascularization in human ovarian cancer [125].

Through locating VEGFR-2 on ovarian cancer cells coexpressed along with VEGF, Boocock et al. raised the possibility that an autocrine loop might directly enhance the tumor growth [153]. This has been further validated by other investigators. Mattern and colleagues showed the close correlation of VEGF expression with tumor cell proliferation [154]. Chen et al. indicated significant correlations between the expression levels of VEGF, VEGFR1, and VEGFR2 in ovarian cancer cells and the activation status of signal transducer and activator of transcription pathway (STAT3 and STAT5) in ovarian cancer cells [155]. Distinct VEGFR-2-mediated pathways promoting tumor growth by directly acting on ovarian cancer cells have been demonstrated [156–158].

3.2 VEGF and ovarian cancer dissemination

Primary tumor cell with its production of a unique array of growth factors, in particular VEGF, specifically dictates the pattern of tumor spread [159]. Kaplan et al. showed that the media conditioned by a tumor type, high in both PlGF and VEGF, was able to reprogram the metastatic profile of another tumor type, high in VEGF but low in PlGF [102]. They demonstrated that VEGF activation of VEGFR-1^{+} progenitor cells allows them to home to tumor-specific pre-metastatic sites before the arrival of tumor cells. VEGF also stimulates the migration of monocytes or macrophages that further support the tumor stromal microenvironment [160]. In addition, tumor angiogenesis leads to the formation of structurally abnormal blood vessels with fragmented, leaky basement membranes which are easily penetrated by tumor cells [161]. Moreover, Weis et al. demonstrated that Src family kinases, playing a specific and vital role in ovarian cancer [162], can be activated by VEGF that leads to a breakdown in the endothelial barrier function which in turn hastens tumor cell extravasation at metastatic sites [163]. They observed that lung colonization of ovarian tumor cells enhanced by VEGF was alleviated by VEGF inhibitors.

VEGF contributes to ovarian cancer progression through promoting angiogenesis as well as enhancing vascular permeability leading to malignant effusion, as described below. VEGF implication in ovarian cancer invasion and metastasis has been well established by different studies.

Through demonstrating VEGF expression in normal and neoplastic human ovaries and identifying the malignant ovarian epithelium as one source of ascitic VEGF, Olson et al. implicated VEGF as an important mediator of ascites formation and tumor metastasis in ovarian cancer [105]. They later showed that *in vivo* neutralization of VEGF inhibits ovarian cancer-associated ascites formation and tumor growth [164]. Mu and colleagues [6] indicated that growth/metastasis of ovarian cancer cells correlates with their VEGF-producing capacity and that angiogenesis inhibition hampers tumor growth and metastasis through mechanisms including the suppression of VEGF function *in vivo*. Ovarian cancer cells overexpressing VEGF hold a metastatic advantage over those lacking VEGF [163, 165]. Li et al. measured significantly higher levels of serum VEGF in patients with metastasis as compared to metastasis-free patients [118]. VEGF has also been implicated in ovarian cancer dissemination via interacting with tumor microenvironment. So et al. showed that LPA induction of ovarian cancer invasion and migration is mediated by VEGFR-2, leading to the secretion and activation of MMP-2 and urokinase type plasminogen activator [166]. VEGF regulation of ovarian cancer invasion and migration through VEGFR-mediated secretion and activation of MMP-2, MMP-7, and MMP-9 has been indicated *in vitro* [167, 168]. Matei et al. suggested a correlation between PDGF and VEGF networks in ovarian cancer cells and tumors in a way that PDGF blockade and the resultant inhibition of VEGF might affect the tumor microenvironment which is detrimental to tumor progression [128]. Belotti and colleagues implicated a complex cross talk between VEGF and MMPs in ovarian cancer progression and invasion [169]. Wang and others proposed a functionally significant role for VEGF in vasculogenic mimicry, the formation of a fluid-conducting, matrix-rich meshwork critical for a tumor blood supply associated with aggressive and metastatic features [170]. They concluded that this part is taken through VEGF upregulation of EphA2 and final activation of MMPs. VEGF might also contribute to ovarian cancer metastasis by directly stimulating proliferation, survival, and/or migration of tumor cells [171]. VEGF also allows ovarian cancer cells to evade immune response. It has been demonstrated that VEGF directly suppresses T cell activation [172] and that its levels in ascitic fluid are inversely correlated with immunologically important T cell subpopulations [116].

3.2.1 Role of VEGF in intraperitoneal dissemination of ovarian cancer

At peritoneal metastasis, primary tumors—generally confined to the ovaries—shed tumor cells into the peritoneum. Malignant cells, often forming cellular aggregates or spheroids, are then transported throughout the peritoneal cavity and subsequently implant on the peritoneal wall, gastrointestinal tract, omentum, and diaphragm. Figure 2 illustrates a proposed model for intraperitoneal dissemination of ovarian cancer [173, 174]. It has been suggested that intraperitoneal fluid and peritoneal surface motion (peristalsis) are prominent mechanisms controlling the patterns of spread [175]. This "seeding" of the peritoneum and the resultant peritoneal carcinomatosis are the most widely recognized behaviors of ovarian cancer that is frequently associated with ascites formation [4, 171]. Peritoneal carcinomatosis is a frequent cause of death in patients with primary advanced or recurrent ovarian cancers [176, 177], the extent of which is a predictor of tumor resectability and the disease prognosis [178].

Malignant ascites is a manifestation of end-stage disease in a variety of cancers, among which ovarian cancer has reportedly been the predominant cause [179–182]. Malignant ascites arises from both tumor surface and tumor-free peritoneum [183]. This metastatic pattern is dependent on establishing new blood vessels at the newly seeded site [4]. VEGF contributes to intraperitoneal dissemination of ovarian cancer by promoting neovascularization and enhancing vascular permeability leading to the subsequent growth of intraperitoneal tumors, development of peritoneal carcinomatosis, and formation of malignant ascites [184, 185]. Ovarian cancer cells [105] along with peritoneal mesothelial cells have been identified as possible sources of ascitic VEGF [132]. The role of VEGF in the peritoneal metastasis of ovarian cancer has been explored by different investigators. Senger et al. initially described VEGF as a tumor-secreted vascular permeability factor which promotes accumulation of ascites fluid [14, 186]. Yeo et al. later showed that high VEGF concentrations correlate with tumor cell growth and ascites fluid accumulation in experimental guinea pig tumors [187]. Using animal models of peritoneal carcinomatosis, it has been demonstrated that overexpression of VEGF leads to an increase in tumor and peritoneal-associated neovasculature and increased peritoneal vascular permeability [188, 189]. Thus, ascites accumulation is increased not only indirectly by allowing tumor growth through stimulation of angiogenesis but also directly through its ability to increase the peritoneal vasculature permeability [190]. Byrne et al. [184] reported that enforced expression of VEGF by ovarian cancer cells dramatically reduced the time to onset of ascites formation and that even tumor-free peritoneal overexpression of VEGF was sufficient to cause ascites to accumulate. Mesiano and colleagues [149] indicated that tumor-derived VEGF played a pivotal role in the development of malignant ascites from ovarian cancer. However, they raise the possibility that intraperitoneal carcinomatosis has an angiogenesis-dependent component represented by the larger solid intraperitoneal tumors in need of neovascular-

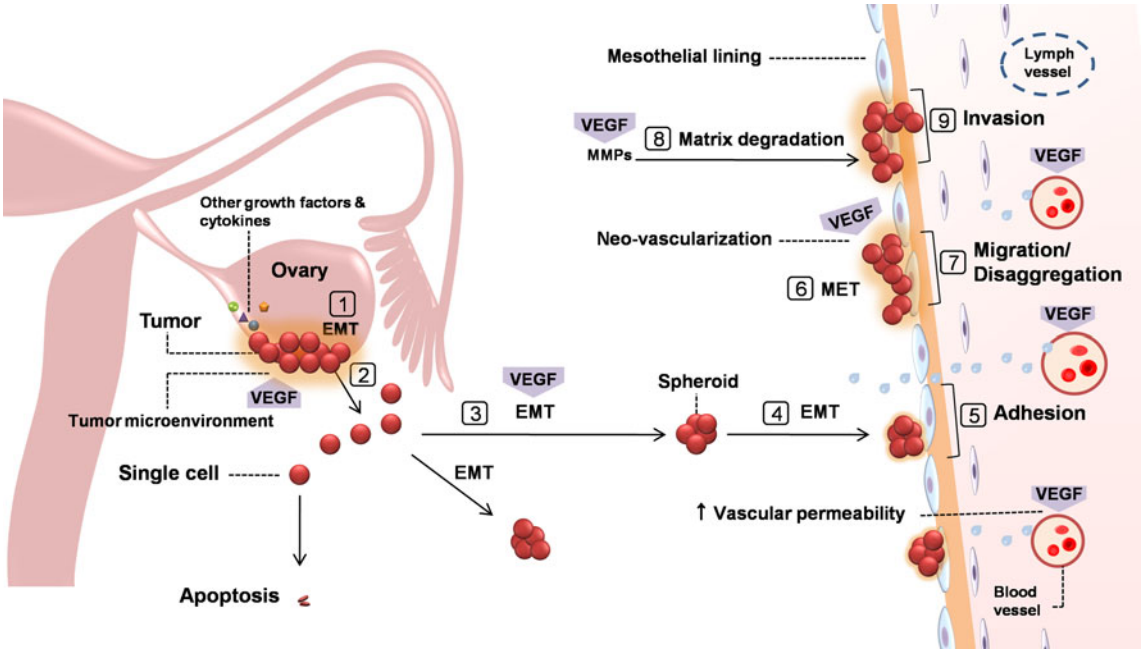

Fig. 2 A proposed model for intraperitoneal dissemination of ovarian cancer [173, 174] with focus on the role of VEGF. Primary tumors are generally confined to the ovaries. To metastasize, tumor cells undergo epithelial–mesenchymal transition (*EMT*) to attain motility *1* Subsequently, ruptured tumor sheds malignant cells into the peritoneum *2* where they often form spheroids to survive. *3* Spheroids undergo changes into invasive mesenchymal phenotype to maintain survival and motility. *4* These cellular aggregates are transported throughout the peritoneal cavity by normal peritoneal fluid and then adhere to and implant on the peritoneum and mesothelial linings of pelvic and abdominal organs *5*, where they undergo a reverse transition (*MET*) *6* and disaggregation *7* to initiate metastatic growth. Through the activity of MMPs, matrix degradation occurs *8* and invasive tumor cells infiltrate the mesothelial lining and the extracellular matrix *9*. Cytokines and growth factors, such as VEGF, TNF-α, IL-6, IL-8, and bFGF, contribute to EMT and invasiveness of spheroids via autocrine and paracrine loops. VEGF is also significantly involved in different steps of the process, including primary tumor angiogenesis, neovascularization at newly seeded sites, MMP-mediated matrix degradation, and malignant ascites formation

zation for continued growth and an angiogenesis-independent component including the thin layers of tumor and some of the smaller solid buds surviving by passive diffusion of nutrients from the underlying host vasculature and the surrounding peritoneal fluid. Moreover, VEGF inhibition hampered the formation of malignant ascites and the progressive growth of peritoneal tumors in ovarian cancer-bearing mice [191, 192]. Similarly, we have shown in a murine model of ovarian cancer with peritoneal carcinomatosis that plasma and, in particular, ascitic VEGF levels correlate well with malignant ascites formation and that VEGF inhibition dramatically halts ascites production [185]. Liao et al. have recently reported that TGF-b blockade in an experimental model of ovarian cancer inhibited ascites production via inhibition of VEGF production [129]. Dong and others proposed that elevated levels of VEGF in ascites might be useful in differentiating benign from malignant ascites [193]. In addition, an anti-apoptotic role for VEGF in protection of the ovarian cancer cells shed into ascetic fluid has been suggested by Sher et al. [157]. They implicated an autocrine VEGF/VEGFR-2 loop in protecting ovarian cancer cells from apoptosis under anchorage free growth conditions (anoikis).

Moreover, interplay between VEGF and MMPs in the peritoneal spread of ovarian cancer has been studied in different investigations. Yabushita et al. reported an association between the VEGF levels and the expression and activation of MMP-2 which they implicated in the progression of the peritoneal involvement [194]. Belotti and others indicated that MMPs, mainly MMP9, contributed to the release of biologically active VEGF and consequently to the formation of ascites [140]. Conversely, VEGF stimulated host expression of MMP-9 in an organ-specific manner, and VEGF inhibition reduced VEGF-induced MMP9 expression while halting ascitic liquid formation and intraperitoneal tumor burden [169].

3.2.2 Extraperitoneal metastasis of ovarian cancer

Upon diagnosis, metastases outside of the peritoneal cavity are rare in ovarian cancer, and the disease is usually confined to the peritoneal cavity at presentation. However, extraperitoneal dissemination through lymphatics and blood stream is increasingly recognized during treatment [195]. The pleural cavity is the most common extraperitoneal site to which stage IV epithelial ovarian cancer

preferentially metastasizes [196]. The liver and lung are other common sites of metastasis. VEGF contribution to ovarian metastasis of the lymph nodes, lung, and liver has been reported [6, 163]. VEGF-C, another member of the VEGF family, has been implicated in lymphatic spread of ovarian cancer [197].

4 VEGF/VEGFR-targeted therapy in ovarian cancer

Based on the major role played by VEGF in the pathogenesis of ovarian cancer along with the promising preclinical results, agents targeting VEGF signaling pathways have been under clinical investigation for activity in ovarian cancer (Table 1).

4.1 VEGF neutralization

Known as the first anti-VEGF agent approved by Food and Drug Administration in 2004 for clinical use in colorectal cancer, bevacizumab has been the first and most studied anti-VEGF agent in clinical evaluation for ovarian cancer. Bevacizumab is a recombinant humanized VEGF monoclonal antibody derived from its murine equivalent A4.6.1 that targets all active isoforms of VEGF, preventing them from binding to VEGFRs [198]. In initial reports, Mesiano et al. demonstrated that A4.6.1 inhibited subcutaneous and intraperitoneal tumor growth and completely prevented ascites production in an athymic mouse model of ovarian cancer [149]. The same group later reported additive or synergistic effects of this antibody in combination therapy as enhanced sensitivity to paclitaxel and marked reduction of tumor growth and ascites formation [199]. Using a murine ovarian cancer model, Mabuchi et al. reported significant antitumor activity of bevacizumab as a single agent or in combination with cisplatin and concluded that it might also prolong survival when used as maintenance therapy after a complete response to cisplatin-based chemotherapy [200]. Preclinical studies employing VEGF immunoneutralization laid the basis for clinical evaluation of bevacizumab in ovarian cancer. Table 2 summarizes the accessible results from some of these investigations.

Aflibercept (VEGF trap) [201] is a soluble decoy receptor that binds and inactivates some members of the VEGF family, including VEGF, VEGF-B, and PlGF. Byrne et al. found that systemic administration of aflibercept prevented ascites accumulation, inhibited the growth of disseminated cancer, and resulted in dramatic remodeling of the blood vessels in an experimental model of ovarian cancer [184]. Hu et al. later showed that aflibercept plus paclitaxel strikingly reduced tumor burden and inhibited ascites while prolonging survival [202]. Aflibercept has further been investigated in some clinical studies (Table 3).

4.2 Receptor tyrosine kinase inhibitors

4.2.1 VEGFR antagonists

Agents targeting VEGF receptors have been evaluated for the use in the treatment of ovarian cancer. Ramucirumab, a fully humanized monoclonal antibody specifically blocking VEGFR-2, resulted in reduced tumor growth, increased

Table 1 Therapeutic agents targeting VEGF/VEGFR in clinical development for ovarian cancer

Type		Drug	Target(s)
VEGF binders		Bevacizumab	VEGF (all isoforms)
		Aflibercept	VEGF, VEGF-B, PlGF
Receptor tyrosine kinase inhibitors	VEGFR inhibitors	Ramucirumab	VEGFR2
		Cediranib	VEGFR1-3, c-Kit, PDGFR-β
		Semaxanib	VEGFR2
	Multiple RTK inhibitors	Sunitinib	VEGFR1-3, Flt-3, PDGFR-α, PDGFR-β, c-Kit, CSF-1R, RET
		Sorafenib	VEGFR1-3, PDGFR-β, Flt-3, c-Kit, Raf-1
		Vatalanib	VEGFR1-3, PDGFR-β, c-Kit, c-Fms
		Intedanib	VEGFR1-3, PDGFR-α, PDGFR-β, FGFR1-3
		Pazopanib	VEGFR1-2, PDGFR-β, c-Kit
		Motesanib	VEGFR1-3, PDGFR, c-Kit
		Vandetanib	VEGFR2-3, EGFR
		AEE788	VEGFR, EGFR

CSF-1 R colony-stimulating factor 1 receptor, *EGFR* epidermal growth factor receptor, *FGFR* fibroblast growth factor receptor, *PDGFR* platelet-derived growth factor receptor, *PlGF* placenta growth factor, *RTK* receptor tyrosine kinase, *VEGF* vascular endothelial growth factor, *VEGFR* vascular endothelial growth factor receptor

Table 2 Released data of some clinical investigations evaluating bevacizumab in ovarian cancer

Investigators	Phase	Target	No. of patients		Outcome	
Single-agent therapy						
Burger et al. [237]	II	Persistent or recurrent EOC, PPC	62		CR, 3%; PR, 18%; MPFS, 4.7; MOS, 17	
Cannistra et al. [238]	II	Recurrent EOC or PSC	44		CR, 0; PR, 15.9%; MPFS, 4.4; MOS, 10.7	
Combined with chemotherapy						
Micha et al. [239]	II	Newly diagnosed stage III/IV	20		CR, 30%; PR, 50%	
Garcia et al. [240]	II	Recurrent platinum-sensitive	70		CR, 0; PR, 24%; MPFS, 7.2; MOS, 16.9	
Richardson et al. [241]	II	Recurrent platinum-sensitive	33		CR, 48%; PR, 30%; MPFS, 12	
Penson et al. [242]	II	Newly diagnosed stage ≥IC	62		*CT* CR, 56%; PR, 22%; *CA-125* CR, 89%; PR, 7%	
Rose et al. [243]	II	Newly diagnosed stage IB-IV	132		CR, 32.8%; PR, 29.1%; SD, 32.7%	
Brown et al. [244]	II	Newly diagnosed stage III/IV	13		CR, 30.8%; PR, 30.8%; SD, 30.7%; MPFS, 5.8	
Tillmanns et al. [245]	II	Recurrent platinum-resistant EOC, PPC	48		MPFS, 8.3; MOS, 16.5; In 39 patients, PR, 46.1%; SD, 30.8%	
Burger et al. [246]	III	Newly diagnosed EOC, PPC, FTC	1,873		CP	CP+Bev and maintenance Bev
					10.3	14.1
McGonigle et al. [247]	II	Recurrent platinum-resistant EOC, PPC, FTC	40		PR, 25%; SD, 35%; MPFS, 7.8; MOS, 16.6	
del Carmen et al. [248]	II	Recurrent platinum-sensitive	54		ORR, 72.2%; MTTP, 14.1; MPFS, 14.1	
Horowitz et al. [249]	II	Recurrent platinum-sensitive	19		CR, 5.26%; PR, 63.15%; MPFS, 8.61; MOS, 21.1	
Wenham et al. [250]	II	Recurrent platinum-resistant	37		CR, 3%; PR, 54%; MPFS, 5.8	
Aghajanian et al. [251]	III	Recurrent platinum-resistant EOC, PPC, FTC	484		CG	Bev+CG
					ORR, 57.4; MPFS, 8.4	ORR, 78.5; MPFS, 12.4
Kristensen et al. [252]	III	Newly diagnosed EOC, PPC, FTC	1,528		Maintenance CP	CP+Bev and maintenance Bev
					MPFS, 16	MPFS, 18.3
					Overall trend: OS improvement	
Kudoh et al. [253]	N/A	Heavily pretreated ROC	30		ORR, 33%; CR+PD+SD, 73%; MPFS, 6	
O'Malley et al. [254]	N/A	Heavily pretreated ROC	70		P	P+Bev
					MPFS, 6.2	MPFS, 13.2
Ojeda et al. [255]	N/A	Highly pretreated, relapsed EOC	RECIST 66	CA-125 76	Response rate was higher for the combination group. However, a similar OS was observed.	

CR complete response, *PR* partial response, *SD* stable disease, *MPFS* median progression-free survival (months), *MOS* median overall survival (months), *MTTP* median time to tumor progression (months), *ORR* overall response rate, *OS* overall survival, *PD* progressive disease, *EOC* epithelial ovarian cancer, *PPC* primary peritoneal cancer, *FTC* fallopian tube cancer, *PSC* peritoneal serous carcinoma, *ROC* recurrent ovarian cancer, *RECIST* Response Evaluation Criteria in Solid Tumors, *Bev* bevacizumab, *CG* carboplatin+gemcitabine, *CP* carboplatin+paclitaxel, *P* paclitaxel

Table 3 Released data of three phase II clinical trials evaluating aflibercept (VEGF trap) in ovarian cancer

Investigators	Phase	Target	Results
Tew et al. [256]	II	Platinum-resistant and topotecan and/or liposomal doxorubicin-resistant advanced ovarian cancer	Preliminary results showed a partial response of 11%.
Colombo et al. [257]	II	Platinum-resistant and topotecan and/or liposomal doxorubicin-resistant advanced ovarian cancer with recurrent symptomatic malignant ascites	First results demonstrated the efficacy of two weekly intravenous aflibercept in prolonging the time to repeat paracentesis in 8 out of 10 evaluable patients.
Coleman et al. [258]	II	Recurrent EOC, PPC, FTC	ORR, 54%; CR, 21.7; MPFS, 6.2; MOS, 24.3

EOC epithelial ovarian cancer, *PPC* primary peritoneal cancer, *FTC* fallopian tube cancer, *VEGF* vascular endothelial growth factor

apoptosis, and decreased tumor microvessel proliferation and density *in vivo* [203]. Following a phase I evaluation [204], it is currently being assessed in a phase II trial (NCT00721162) as monotherapy in patients with persistent or recurrent epithelial ovarian cancer. Cediranib, a tyrosine kinase inhibitor selectively blocking VEGFR1-3, platelet-derived growth factor receptor-β (PDGFR-β), and c-Kit, has been shown to inhibit the growth of human tumor xenografts, including ovarian cancer, in a dose-dependent manner [205]. Single-agent activity of cediranib in phase II trials has been reported [206, 207]. A number of the clinical studies testing cediranib in ovarian cancer are ongoing, including a three-arm randomized placebo-controlled phase III trial set to investigate cediranib in combination with platinum-based chemotherapy and as a single agent maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer (ICON6). In a study by Holtz et al. [208], semaxanib reduced tumor growth and microvessel density in ovarian cancer tumors with high VEGF expression. A phase I study of semaxanib in combination with carboplatin in patients with platinum-refractory ovarian cancer (NCT00006155) has been completed (results awaited).

4.2.2 Agents targeting multiple receptor tyrosine kinases

As a single agent, sunitinib has demonstrated efficacy in advanced ovarian cancer in both preclinical [209] and clinical [210, 211] studies. Sorafenib, in a study by Matsumura et al., was shown to have an antitumor effect against ovarian clear cell carcinoma [212]. It has shown some efficacy in phase I/II trials [213–215] and is now under further clinical investigations. In an ovarian cancer mouse model, single-agent vatalanib reduced ascites and tumor growth and yielded increased survival [191]. In a phase I study of vatalanib combined with carboplatin and paclitaxel in advanced ovarian cancer, Shroder et al. showed that vatalanib was feasible and well tolerated [216]. Monotherapy with vandetanib showed a significant antitumor effect in an ovarian cancer nude mice model [217]. However, no significant clinical benefit was made by vandetanib monotherapy in patients with recurrent ovarian cancer in a phase II clinical trial [218]. Its efficacy in combination with docetaxel in persistent or recurrent ovarian cancer will be assessed in a phase II clinical trial (NCT00872989). In a study by Hilberg et al. [219], intedanib demonstrated high activity at a well-tolerated dose as decreased vessel density and vessel integrity, and profound growth inhibition in all tested tumor models. In a phase II trial, maintenance intedanib delayed disease progression in previously treated ovarian cancer patients [220]. To investigate its efficacy and safety, intedanib combined with carboplatin and paclitaxel is currently being examined in a randomized, double-blind phase III trial in patients with advanced ovarian cancer (NCT01015118). In an animal model of ovarian cancer, treatment with AEE788 plus paclitaxel significantly reduced tumor weight and increased survival of nude mice implanted with paclitaxel-sensitive cell lines compared with those treated with AEE788 alone or paclitaxel alone [221]. Also, metronomic docetaxel chemotherapy plus AEE788 was effective even in the taxane-resistant model [222]. A phase I/II randomized study of AEE788 in adult patients with advanced cancer (histologically confirmed solid tumors) has been completed (NCT00118456) and results are awaited. Pazopanib, alone or in combination with metronomic topotecan, showed anti-angiogenic and antitumor activity in a preclinical model of ovarian cancer [223]. Friedlander et al. reported pazopanib activity in a phase II trial in women with advanced epithelial ovarian cancer [224]. Other trials evaluating pazopanib efficacy as mono- or combination therapy are underway. Motesanib, that indicated antitumor activity in animal models [225], is now under clinical investigation in a phase II clinical trial (NCT00574951).

4.3 VEGF-targeted therapy: current challenges

Adverse effects such as hypertension, proteinuria, bowel perforation, impaired wound healing, hemorrhage, and arterial thrombotic events represent the most common toxicities associated with VEGF-targeted therapy [226, 227]. Inherent [228] and/or acquired resistance/refractoriness [226, 229] might complicate the treatment as well. Divergent effect of receptor tyrosine kinase blockade, including VEGFR inhibition, on primary tumor growth

and metastasis leading to enhanced invasion and accelerated metastasis has been reported in preclinical studies [230, 231]. However, the clinical relevance of these findings is unclear and limited to small studies or case reports [232].

As improving overall survival while maintaining the quality of life remains the goal of all cancer treatment [233], efficacy of extended adjuvant bevacizumab in unselected populations may not be justified by the clinical benefits reported thus far [234]. Consistently, a recent analysis of GOG218 study has raised doubts about cost-effectiveness of utilizing bevacizumab in the adjuvant management and maintenance therapy of patients with advanced ovarian cancer [235, 236]. This might be in part attributed to limitations of predictive preclinical models and thus lack of appropriate preclinical testing that precedes clinical settings [232]. Besides, accurate predictors of prognosis and ideal surrogate biomarkers required for individualization of VEGF-targeted therapy are to be identified and clinically validated [227].

5 Conclusion

The role of VEGF in ovarian cancer growth and progression is well established. Through induction of tumor angiogenesis and vascular permeability, VEGF has a major contribution to the pathophysiology of ovarian cancer. VEGF promotes tumor growth and facilitates malignant cell invasion and dissemination. Peritoneal carcinomatosis with malignant ascites formation is the prominent pattern of ovarian cancer spread in which VEGF is significantly involved, further highlighting the crucial part played by VEGF in the progressive course of the disease. Preclinical data obtained over the last 15 years or so clearly demonstrate a central role of VEGF inhibitors in the management of ovarian cancer. On the other hand, the complexity of VEGF signaling cascade and the interacting pathways as well as the failure of preclinical studies in closely mimicking actual clinical settings represent major restrictions and barriers in translating preclinical promises to the clinic. However, this has not hindered the extensive evaluation of bevacizumab and other VEGF-targeted agents in phase II and III clinical trials, and VEGF still remains a target in the treatment of ovarian cancer.

Conflict of interest The authors declare that they have no conflict of interest.

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