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Targeting the Tumor Microenvironment in Ovarian Cancer

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Abstract

The study of cancer initiation, growth and metastasis has traditionally been focused on cancer cells, and the view that they proliferate due to uncontrolled growth signaling owing to genetic derangements. However, uncontrolled growth in tumors cannot be explained solely by aberrations in cancer cells themselves. To fully understand the biological behavior of tumors, it is essential to understand the microenvironment in which cancer cells exist, and how they manipulate the surrounding stroma to promote the malignant phenotype.

Ovarian cancer is the leading cause of death from gynecologic cancer worldwide. The majority of patients will have objective responses to standard tumor debulking surgery and platinum-taxane doublet chemotherapy, but most will experience disease recurrence and chemotherapy resistance. As such, a great deal of effort has been put forth to develop therapies that target the tumor microenvironment in ovarian cancer. Herein, we review the key components of the tumor microenvironment as they pertain to this disease, outline targeting opportunities and supporting evidence thus far, and discuss resistance to therapy.

Keywords

Ovarian cancer; tumor microenvironment

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Introduction

Background

The study of cancer initiation, growth, and metastasis has traditionally been focused on cancer cells. This view postulates that cancer cells proliferate due to uncontrolled growth signaling pathways owing to derangements in both oncogenes and tumor suppressor genes[1]. However, despite the significant contributions of these pathways in the metastatic transformation of cells, the uncontrolled growth that occurs in tumors cannot be explained solely by aberrations in the cancer cells themselves. Tumors are complex tissues composed of tumor cells, as well as stroma consisting of blood and lymphoid vessels, nerves, fibroblasts and extracellular matrix proteins, endothelial cells, pericytes, and immune cells[1]. These collectively comprise the tumor microenvironment. To fully understand the biological behavior of tumors, it is essential to consider the context in which cancer cells exist, and how they manipulate and are manipulated by the surrounding stroma to promote the malignant phenotype[2].

Epidemiology

Ovarian cancer is the second most common gynecologic malignancy but is the most common cause of death from gynecologic cancer worldwide[3, 4]. Epidemiology, treatment and prognosis vary greatly by histopathologic subtype. Epithelial ovarian carcinoma (EOC) comprises approximately 85 percent of ovarian malignancies [5, 6], with high-grade serous (HGSC) being the most common histology.

While HGSC was historically thought to arise from the ovarian surface epithelium (OSE), contemporary paradigms suggest that other sources are more likely. Studies examining the distal, fimbriated end of the fallopian tubes in patients with serous carcinoma classified as either ovarian, fallopian tube or primary peritoneal in origin demonstrated that approximately 50% of patients had tubal intraepithelial carcinoma (TIC) present[7]. This suggests that TIC may be the precursor lesion and an important initiating factor in pelvic serous carcinoma[8]. Cells in the hilum of the ovary may be an alternative source of stem cells [9] and may have increased susceptibility to malignant transformation [9]. The primary mode of spread of HGSC was traditionally thought to be continuous exposure of the peritoneal surfaces to exfoliated tumor cells, however, there is evidence pointing to hematogenous mode of spread being an important component of the metastatic process [10] [11]. Ovarian cancer cells have tropism for the omentum, which is likely mediated by a variety of factors produced by omental adipocytes [12].

Herein, we review the key components of the tumor microenvironment as they pertain to ovarian cancer, discuss targeting opportunities for individual stromal cell types as well as their prognostic potential, and outline emerging areas of research. Emphasis will be placed on fibroblasts, endothelial cells, and the immune components of the tumor microenvironment.

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Cancer-Associated Fibroblasts

Background

Fibroblasts are the principal cellular component of connective tissue and are largely responsible for its maintenance and regeneration. The functions of fibroblasts include production and deposition of types I, III and V collagen and fibronectin, which are key components the fibrillar extracellular matrix[13], as well as synthesis of basement membrane proteins laminin and type IV collagen[14]. In addition, fibroblasts have an important role in the turnover and maintenance of the extracellular matrix by producing proteases such as matrix metalloproteinases [14]. Importantly, fibroblasts are crucial components in the process of wound healing, whereby they localize to wounds, generate extracellular matrix proteins, and aid in the contracture of the lesions that they occupy[13, 15]. Additionally, these fibroblasts gain contractile strength[16] by expressing characteristically increased levels of α-smooth muscle actin (α-SMA)[13]. This phenomenon is mediated by growth factors such as TGF-β[17, 18]. Once the wound has completed healing, activated fibroblasts undergo apoptosis [19, 20].

The importance of fibroblasts in tumor development is well established. Initial studies showed that injection of carcinogenic Rous sarcoma virus in chickens led to development of tumors [21]. Tumors have been described as “wounds that do not heal.”[22] Similarly, cancer cells have the ability to induce a reactive fibroblast phenotype, termed cancer-associated fibroblasts (CAF). CAFs are similar to activated fibroblasts in that they express α-SMA, but do not undergo apoptosis and do not lose their activated phenotype[23]. In addition, they express fibroblast activation protein (FAP)[15]. The interaction between cancer cells and fibroblasts in the tumor microenvironment is complex. CAFs can initially restrict tumor progression, similar to the relationship between cancer cells and immune components of the microenvironment[24]. However, CAFs eventually become activated by growth factors such as TGF-β1, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and interleukin-6 (IL-6). Vascular endothelial growth factor (VEGF), described in detail in the following section, is released by cancer cells and induces an influx of fibroblasts and thus, an increase in both the volume of tumor stroma[25]. CAFs contribute to vascular stabilization in ovarian and other cancers[26]. Lysophosphatidic acid produced by ovarian cancer cells has been shown to promote differentiation of adipose tissue-derived mesenchymal stem cells to CAFs[27], demonstrating not only the close interactions between CAFs and tumor cells, but also the ability of tumor cells to modify the surrounding microenvironment. Finally, the Hedgehog pathway, whose role is primarily developmental, has been implicated in the carcinogenesis of many cancer types [28, 29]. Hedgehog ligands produced by stromal cells provide essential growth signaling for tumor cells, emphasizing the interaction between tumor cells and their microenvironment. [30]. Overall, the result of the interaction between tumor cells and CAFs is a reciprocal, positive feedback mechanism in which cancer cells produce factors that activate and maintain CAFs, which in turn promote tumor progression by increasing cancer cell proliferation, angiogenesis, and remodeling of the extracellular matrix[23].

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Cancer-associated Fibroblasts as a Therapeutic Target

There are several factors that make CAFs an attractive target for therapy. They comprise a large portion of tumor mass of solid tumors, and there is constant two-way crosstalk between them and cancer cells. Furthermore, fibroblasts are relatively genetically stable compared to cancer cells, and conventional challenges of acquired resistance could potentially be avoided[23]. Recent studies have demonstrated that CAFs contribute to tumor growth and metastasis in ovarian cancer, and may be a clinically important target for diagnosis, treatment and surveillance [31, 32]. Infiltration of CAFs into ovarian carcinoma spheroids leads to vascular stabilization of the tumors via expression of angiopoietin-1 and angiopoietin-2[33]. Immunohistochemical analysis of benign, borderline and malignant ovarian specimens demonstrated lack of CAFs in benign ovarian tissue and abundant CAFs in ovarian carcinoma [31]. The quantity of CAFs was increased in ovarian carcinoma specimens with disease stage, and patients with lymph node and omental metastases had significantly higher α-SMA expression in their tumors as compared to those without metastatic disease. This suggests not only that CAFs may be necessary for metastases to occur, but that metastatic sites recruit stromal components to optimize cell survival and further metastasis. In addition, FAP expression level within tumors was found to correlate with platinum resistance and shortened interval to recurrence[34]. Furthermore, FAP silencing led to a decrease in ovarian cancer cell growth *in vivo*. Similarly, fibroblast growth factor receptor-3 (FGFR-3), whose ligand is FGF, has been shown to have significantly higher expression in clear cell ovarian cancer samples, as compared to normal ovarian tissue, and knockdown of FGFR-3 slowed cell migration and proliferation[35]. The reciprocal relationship between tumor cells and CAFs was further demonstrated when conditioned media from SKOV3 ovarian cancer cells led to differentiation of fibroblasts into CAFs, as evidenced by increased expression of α-SMA[36]. Although the latter two studies demonstrate the importance of fibroblasts in ovarian cancer progression, it is important to note that clear cell ovarian cancer is biologically and histologically distinct from high grade serous cancer. Finally, there is some evidence indicating that mutations in tumor suppressor genes in CAFs may contribute to the interaction between CAFs and cancer cells, leading to tumor progression. Of particular interest is p53, which is mutated in nearly all high grade serous tumors[37], but not in other histological subtypes. In an *in vivo* breast cancer model, there was significantly increased tumor size when tumor cells were injected into p53-null mice, as compared to tumor size in wild-type p53 mice[38]. This suggests a potential role for p53 in the surrounding tumor stroma, irrespective of p53 status of the tumor cells. Further, CAFs isolated from breast and colon cancer specimens have been shown to have aberrations in p53, including inactivation mutations[39] and intact, but non-functional protein[40]. Despite the frequency of p53 mutations in epithelial ovarian cancer, p53 in CAFs in ovarian cancer tumor stroma, and any potential role in tumorigenesis, has yet to be elucidated.

Fibroblast growth factor receptor (FGFR) isoforms have proven to be important targets in the treatment of solid tumors, based on preclinical and early clinical work. Lucitanib is a receptor tyrosine kinase inhibitor that acts on FGFR isoforms 1 through 3, vascular endothelial growth factor receptor (VEGFR) isoforms 1 through 3, and PDGF receptors (PDGFR) α and β, and is currently in phase II trials in patients with metastatic breast cancer

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and FGF amplifications (NCT02202746, NCT02053636). Dovitinib is a non-specific receptor tyrosine kinase inhibitor whose targets include FGFR3, and is currently undergoing phase I trials in solid tumors (NCT01497392) and phase II trials in urothelial cancer (NCT01732107) and prostate cancer (NCT01741116). Several other, similar receptor tyrosine kinase inhibitors targeting the FGF receptor are undergoing phase I evaluation. Finally, nintedanib is a non-specific receptor tyrosine kinase inhibitor of VEGFR 1-3, FGFR 1-3, and PDGFR α and β that is currently undergoing phase I, II, and III clinical testing as monotherapy and in combination with chemotherapy for first line and recurrent ovarian cancer (e.g., NCT01610869, NCT01669798, EudraCT 2013-002109-73). In an initial randomized, phase II, placebo-controlled trial, patients with ovarian cancer who had completed chemotherapy for recurrent disease were treated with nintedanib as maintenance therapy. Progression-free rates were 16.3% in the nintedanib and 5.0% in the placebo groups (HR=0.65, p=0.06)[41]. This prompted a phase III trial which demonstrated an improvement in progression-free survival (PFS) when nintedanib was used in the up-front setting in combination with carboplatin and paclitaxel, as compared to carboplatin and paclitaxel alone (27.1 versus 20.8 months, respectively, hazard ratio=0.84, 95% confidence interval=0.72–0.98, p=0.024)[42].

As evidenced above, the majority of fibroblast-directed therapies currently being tested in clinical trials are non-specific to FGFR. A notable exception is an FGFR-selective antibody drug conjugate currently in phase I trials (NCT02368951). Although results have been modest thus far, therapies that target multiple receptor types may prove to be helpful in circumventing common resistance mechanisms [43, 44].

Angiogenesis & Endothelial Cells

Background

The formation of new blood vessels is essential for tumor growth and metastasis [45]. Angiogenesis is a central hallmark of cancer and is crucial for solid tumor growth and metastasis[1]. Early studies demonstrated that tumor growth in isolated perfused organs was significantly decreased in the absence of tumor vascularization[46, 47], and that without adequate vascularization, tumor cells undergo necrosis or apoptosis[48, 49]. An “angiogenic switch” becomes activated during the early stages of tumor development and is a key step in tumorigenesis[45]. This can be activated by conditions that require increased oxygen and nutrient delivery to the tumor, including hypoxia, hypoglycemia, mechanical stress, and inflammation[50]. There are other mechanisms by which tumors produce a microcirculation to acquire oxygen and nutrients. In contrast to angiogenesis, several tumor types display vascular cooption, a process by which a tumor mass coopts already-established host vessels, allowing the tumor to be initially well-vascularized [51]. Moreover, aggressive tumors are capable of directly contributing to vasculature, a process described as vasculogenic mimicry [52, 53]. In addition, mutations in tumor suppressor genes and oncogenes can alter the balance between pro-angiogenic and anti-angiogenic factors to promote tumor growth [1, 54]. Well-recognized promoters of angiogenesis include VEGF [55, 56], FGF1 and 2, and their associated receptors. In addition to these pathways, PDGF, epidermal growth factor (EGF), angiopoietins, and hepatocyte growth factor (HGF) are growth factors known to

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contribute to tumor angiogenesis [57]. These growth factors bind to receptor tyrosine kinases, leading to the initiation of intracellular signaling. While the mechanisms regulating angiogenesis in tumors are complex and multi-factorial, VEGF has emerged as a dominant pathway. The VEGF family of molecules includes VEGF-A, -B, -C, -D and placental growth factor (PIGF) [58]. VEGF is constitutively expressed in most human cancers[58], and is mediated via hypoxia-inducible transcription factors 1α and 2α [59]. Additionally, VEGFR-3 plays an important role in sustaining angiogenesis, even in the presence of VEGFR-2 inhibitors [60]. Traditionally, the relationship between the VEGF ligands and their receptors has been described as paracrine; however, there is evidence that VEGF can be produced by stromal cells in the tumor microenvironment[25] as well as by hematopoietic stem cells[61] and VEGFRs can be expressed directly on cancer cells[62].

Targeting the VEGF Pathway

Despite a modest increase in survival in women with ovarian cancer over the past several decades, a significant proportion of women will experience disease recurrence[5], as the median progression free survival in these patients is approximately 18 months[4]. While patients with platinum-sensitive relapsed disease can be re-treated with platinum-based therapy, the options for those with platinum-resistant or -refractory disease are limited [63, 64]. Among the various options, anti-angiogenesis strategies are attractive. Bevacizumab (a monoclonal antibody to VEGF-A) is the only anti-angiogenic therapy that is approved by the Food and Drug Administration (FDA) in combination with chemotherapy in patients with platinum-resistant recurrent ovarian cancer treated with 1 or 2 prior regimens. Approval was based on the results of the AURELIA trial, which compared bevacizumab plus conventional chemotherapy (paclitaxel, pegylated liposomal doxorubicin, or topotecan), to chemotherapy alone in patients with platinum-resistant recurrent epithelial ovarian cancer [65]. Adding bevacizumab to chemotherapy resulted in statistically improved progression free survival (median PFS 3.4 months with chemotherapy alone versus 6.7 months when bevacizumab was added) and overall response rate, however, did not improve overall survival[65]. In addition, bevacizumab is approved for frontline therapy for ovarian cancer in many countries outside the United States, based on findings from the GOG-218 and ICON7 trials [66, 67]. To date, there have been five positive phase III trials with bevacizumab in combination with chemotherapy for patients with newly diagnosed or relapsed ovarian cancer (Table 1).

Other Anti-Angiogenic Therapeutic Targets

Despite FDA approval, improvements in overall survival are modest in patients using bevacizumab, and resistance is common, emphasizing the need for development of alternative anti-angiogenic therapies [43].

PDGF has four isoforms (A-D) that bind to specific receptors, PDGFR-α and –β. PDGF is secreted by endothelial cells at the site of angiogenesis and attracts pericytes to the region in order to stabilize newly formed blood vessels[68]. Inhibition of PDGFR prevents pericyte coverage of new blood vessels, leading to vessel destabilization and subsequently preventing oxygen and nutrient flow to tumor cells[69]. As detailed above in the discussion about fibroblasts, therapies targeting PDGF also target VEGFR and FGFR isoforms, as well as

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other receptor types. Blockade of the PDGF pathway may enhance the effectiveness of VEGF pathway blockade [70, 71]. Targeted agents with completed trials which have shown effectiveness in ovarian cancer are highlighted below.

Cediranib is a receptor tyrosine kinase inhibitor that inhibits VEGFR 1-3, PDGFR-α, and c-kit. In an initial phase II trial, cediranib yielded a PFS of 5.2 months, with partial responses in 17% of enrolled patients with recurrent ovarian cancer [72]. The follow-up phase III trial (ICON6) demonstrated a prolonged PFS and OS in the group of patients who received chemotherapy plus cediranib followed by 18 months of cediranib for maintenance, as compared to chemotherapy plus cediranib with placebo maintenance or chemotherapy plus placebo with placebo maintenance (median PFS 11.1 versus 8.7 months, hazard ratio=0.57, 95% confidence interval=0.45–0.74)[73, 74]. There are additional ongoing trials with cediranib alone (NCT00278343) and in combination with other targeted therapies for ovarian cancer (e.g., temsirolimus; NCT01065662). A phase II trial with olaparib plus cediranib demonstrated an improvement in PFS when the two agents were used in combination as compared to the use of olaparib alone (median PFS 17.7 versus 9 months, hazard ratio=0.42, 95% confidence interval=0.23–0.76, p=0.005)[75].

Sorafenib acts on VEGFR 1-3, PDGFR-β, and Raf-1, and is FDA approved for use in advanced renal cell and hepatocellular carcinoma. In an initial phase II study, 24% of patients had stable disease for 6 months, and 3.4% of patients had partial responses when patients with recurrent ovarian cancer were given sorafenib alone[76]. However, completed trials have demonstrated a high rate of toxicity leading to increased frequency of dose reductions and treatment discontinuation [76, 77]. Trials testing sorafenib in combination with bevacizumab (NCT00436215) and carboplatin and paclitaxel (NCT003900611) are ongoing. Pazopanib, an inhibitor of VEGFR 1-3, PDGFR α and β, and c-kit, is FDA approved for use in advanced sarcoma and renal cell carcinoma. A phase II of patients with recurrent ovarian cancer demonstrated 3 partial responses and a CA-125 response rate of 31%[78]. A phase III trial of pazopanib maintenance therapy after first-line chemotherapy showed prolonged PFS with pazopanib as compared to placebo (17.9 versus 12.3 months, hazard ratio=0.77, 95% confidence interval =0.64–0.91, p=0.002), but substantially more toxicity, particularly among the Asian cohort[79]. None of the receptor tyrosine kinase inhibitors have been FDA approved for use in ovarian cancer so far.

Aflibercept is a soluble decoy VEGF receptor that binds to circulating VEGF-A and –B molecules. This acts as a “VEGF trap,” binding VEGF at very high affinity, thereby decreasing the amount of circulating VEGF available to act on its receptors [80]. It is FDA approved for the treatment of neovascular (wet) age-related macular degeneration, however, it has shown promise in recurrent ovarian cancer. In a phase II trial in which aflibercept was given in combination with docetaxel, there was an overall response rate of 54% (25 of 46 patients), with 11 patients with a complete response and 14 with a partial response [81]. A subsequent phase II study showed that aflibercept was effective at reducing symptomatic malignant ascites in this patient population, however, frequency of fatal intestinal perforations was higher in the aflibercept group than placebo (three events versus one)[82].

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Resistance to Anti-VEGF therapy

Despite the success of anti-VEGF therapies, the duration of effect is often short as tumors quickly become resistant to therapy[43] via a variety of mechanisms. While the modes of resistance are varied, a common contributing factor is that patients are treated with anti-VEGF therapies irrespective of the characteristics of their tumors. The stromal components of the tumor microenvironment may offer important means of resistance to VEGF blockade. The Notch signaling pathway has been shown to have a central role in anti-VEGF resistance and is closely related to the VEGF pathway[83]. Tumors with increased activity of the Notch pathway via the DLL4 ligand, produced by endothelial cells, had increased formation of large vessels, thereby decreasing sensitivity to anti-VEGF therapy [84]. This finding emphasizes the need for molecular testing and tumor evaluation prior to initiating targeted therapy. Tumor-associated macrophages (TAMs), which are discussed in the next section in the context of their immune properties, have also been implicated in resistance to anti-VEGF therapies. As a major component of the tumor microenvironment, TAMs contribute to tumor growth and metastasis via several mechanisms, including promoting angiogenesis[85]. Sub-populations of TAMs that produce Tie2 lead to vasculogenic mimicry in the tumor via the production of primitive capillary-like structures[86]. Given their ability to induce pro-angiogenic pathways, TAMs in the tumor microenvironment likely play an important role in resistance to anti-VEGF therapy.

Immune Components of the Tumor Microenvironment

Background

Immune cells are present not only in the tumor microenvironment, where they interact closely with fibroblasts and endothelial cells, but also in areas of the tumor predominated by cancer cells [87]. The importance of the interaction between cancer cells and immune cells was first described in 1863 by Virchow, who observed that cell proliferation was enhanced at sites of tissue injury and resultant inflammation[88, 89]. This concept is demonstrated by the fact that approximately 15% of cancers globally can be attributed to infectious etiology [90]. For example, infection with human papillomavirus is instrumental in the pathogenesis of cervical dysplasia and progression to squamous cell carcinoma of the cervix. Chronic inflammatory conditions not related to infections are also known to predispose to cancer, exemplified by the role of ulcerative colitis in the development of colorectal cancer[91]. The development of colitis-associated colorectal cancer is driven by IL-6 produced by immune cells in the intestinal microenvironment, which protects premalignant cells from apoptosis[92]. Tumors have hence been described as a “Darwinian microenvironment,” which adapt and select for the level of inflammation that maximally promotes their growth and metastasis [93].

When tissue is injured, platelets accumulate at the site of injury. In this setting, platelets serve a dual purpose: initiating both coagulation and the host inflammatory response. Platelets secrete plasma proteins, coagulation factors, and cellular growth factors including platelet-derived growth factor (PDGF), transforming growth factor-α and –β (TGF-α and TGF–β), and basic fibroblast growth factor (bFGF) [88], all of which potentiate the inflammatory response. In addition to promoting formation of the extracellular matrix and

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new vasculature, platelets are instrumental in neutrophil chemotaxis. Not all tumors are characterized by a classical inflammatory response, but tumor infiltrating immune cells can be present in smaller quantities and still have influential effects on tumor growth and metastasis[94]. Given the depth and breadth of the involvement of the immune system in cancer, emphasis will be placed on T-lymphocytes and macrophages, and their role in the treatment of ovarian cancer.

Lymphocytes and Associated Therapies

In 1984, Rosenberg and colleagues used an infusion of interleukin-2 (IL-2), a potent cytokine that induces proliferation of lymphocytes, to treat a patient with progressive metastatic melanoma. This patient had a complete response to treatment [95]. IL-2 is primarily secreted by antigen-stimulated CD4+ T cells, but can also be secreted by CD8+ T cells, natural killer cells, and activated dendritic cells [95]. Since its initial use, recombinant IL-2 has been FDA approved for the treatment of metastatic melanoma and renal cell carcinoma. Administration of tumor-infiltrating lymphocytes isolated from tumors, and propagated in IL-2, has also shown to be effective in metastatic melanoma [96–98].

Ovarian cancer was traditionally not believed to be an immunogenic tumor type, but there is now ample evidence suggesting the opposite[99]. The presence of intratumoral T cells was found to correlate with improved clinical outcome in advanced ovarian cancer[100], as had been previously demonstrated in patients with melanoma[101], colorectal[102], breast[103], prostate[104], renal cell[105], and esophageal cancers[106]. This finding has been confirmed by other investigators, who have also shown that intratumoral T-cells, despite predicting improved survival, were more prevalent in tumors with increased proliferation[107]. The evidence has led to an increase in the use of immune therapies in ovarian cancer. A phase II trial in which patients with platinum-resistant or –refractory ovarian cancer were administered weekly intraperitoneal recombinant IL-2 had a 17% complete response rate. This study also found a significant association between changes in peripheral lymphocytes and overall survival [108]. In addition to the use of IL-2, improved survival rates have been observed in patients who underwent adoptive transfer of tumor-infiltrating lymphocytes [109], as well as after treatment with CTLA-4 antibody [110, 111].

Programmed death 1 (PD-1) is an inhibitory immune checkpoint receptor expressed by activated T cells. PD-1 interacts with its ligands, programmed death-ligand 1 and 2 (PD-L1 and 2), present on tumor and stromal cells [112–114]. Blocking the interaction of PD-1 with its ligands has been shown to mediate antitumor activity in preclinical models [115–117]. PD-L1 is highly expressed in ovarian cancer cell lines and high expression is associated with poorer survival in patients [118]. Silencing PD-L1 in animal models has been shown to decrease peritoneal dissemination of ovarian tumors [119]. Pembrolizumab and nivolumab, humanized antibodies against PD-1, and avelumab, a humanized antibody against PD-L1 have shown response rates of 10–20% in patients with recurrent or refractory ovarian cancer [120–122]. In ovarian carcinosarcoma, PD-L1, PD-L2 and CD8+ tumor infiltrating lymphocytes are highly expressed, suggesting that PD-1/PD-L1 targeting may also be beneficial in this disease.[123]

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Macrophages

Macrophages are the most abundant immune cell population in the tumor microenvironment [88, 124], and are termed tumor-associated macrophages (TAM) when present in association with tumors. TAM are derived from monocyte precursors[125] and are recruited to the tumor microenvironment by chemokines CCL2, CCL5, CXCL1, and others[126]. Once monocytes are recruited to tumor areas, the chemokines TGF-β, IL-10 and IL-4 promote their differentiation into the M2 macrophage phenotype [127, 128]. This phenotype has poor antigen presenting capacity, and promotes wound healing, tissue remodeling and angiogenesis[129]. As such, TAM predominantly accumulate in hypoxic areas of tumor due to HIF-1 dependent upregulation of CXCR4[130]. TAM survival is promoted in the tumor microenvironment by macrophage colony-stimulating factor (M-CSF) and VEGF, both produced by tumor cells[93]. TAM generally have pro-tumorigenic functions and as a result, high levels of TAMs in tumors are associated with poor prognosis[85, 93, 131]. Macrophages produce VEGF[132], PDGF[128] and other pro-angiogenic factors. There is also concurrent dissolution and remodeling of the extracellular matrix by MMPs, urokinase-type plasminogen activator (uPA) and its receptor, and plasmin produced by TAMs can enable tumor cell migration[133, 134]. TAMs also act as suppressors of anti-tumor immune responses by producing immunosuppressive chemokines including IL-10, TGF-β, and prostaglandin E2 (prostaglandin E2)[93, 127, 128], and producing chemokines such as CCL17, CCL18 and CCL22 that recruit only immune cell populations that lack cytotoxic activity. Finally, TAMs can directly stimulate growth of cancer cells via production of EGF, IL-6 and tumor necrosis factor[93, 135]. The multi-factorial nature by which TAMs promote tumor progression make them an appealing therapeutic target. Zoledronic acid, a bisphosphonate, has been shown to suppress MMP-9 production by TAMs, and could be a potential therapeutic approach [136].

Myeloid-Derived Suppressor Cells and Associated Therapies

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of myeloid-derived cell types including myeloid progenitor cells, and immature macrophages, granulocytes and dendritic cells[137]. They differ from TAMs in that they have granulocytic morphology and upregulation of both arginase 1 and inducible nitric oxide synthase, resulting in increased production of immunosuppressive nitric oxide and reactive oxygen species [138]. Thus, the presence of MDSCs in tumors leads to suppression of the tumor-directed immune response. This suppression must be abrogated in order for immune therapies to be successful. One such strategy is to promote the differentiation of MDSCs into immunocompetent mature myeloid cells. This has been accomplished by the use of all-*trans* retinoic acid, which has been shown to decrease MDSCs, increase antigen-specific T cell response, and prolong vaccine effect when used in combination with anti-tumor vaccines [139, 140]. While a variety of other approaches are under investigation, the role for these therapies in ovarian cancer has yet to be determined.

Resistance to Immune Therapy

While immune therapy has shown considerable promise in the treatment of ovarian and other cancers, immune suppression is an important mechanism of resistance. This is

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accomplished in a variety of ways, which often operate simultaneously. Tumor cells downregulate MHC class I molecules in order to avoid detection by T cells, and upregulate factors that are inhibitory to T cell signaling, such as PD-L1[141]. Ovarian cancer cells and TAMs produce CCL22, a chemokine that recruits T regulatory cells to the tumor. This particular T cell population inhibits tumor-specific T cell immunity and is associated with reduced patient survival [142]. This represents a mechanism by which tumors actively promote their own immune privilege. MDSCs appear to have multiple roles in decreasing the immune response to tumors. First, they are recruited to areas of hypoxia in tumors to stimulate angiogenesis [143, 144]. They also inhibit the activity of T cells and natural killer cells via TGF-β, IL-10, and reactive oxygen species, thereby dampening the tumor-directed immune response [138]. Finally, CAFs can inhibit recruitment of effector T cells to the tumor by overexpression of TGF-β, yielding an immunosuppressive effect[141]. These mechanisms of resistance highlight the importance of the interaction between immune cells and other components of the microenvironment, and provide important therapeutic opportunities. Indeed, initial reports of PD-1/PD-L1 blockade have yielded objective responses in patients with measurable recurrent ovarian cancer. While the range of response is in line with salvage chemotherapy, some of the responses were complete and occurred in chemotherapy refractory settings[120–122].

Finally, immune cell recruitment in the presence of induced hypoxia (e.g. anti-angiogenic therapy) may define angiogenic escape. Therapeutic targets, for example CSF-1R, are now entering clinical investigation as an opportunity to reverse this phenotype. VEGF produced by tumor and endothelial cells can also contribute to resistance to immune therapies, highlighting the interplay between tumor microenvironment components. VEGF can serve as a chemoattractant for immature myeloid cells from the bone marrow [145] to tumor sites. Exogenously administered VEGF was shown to decrease the number of mature CD4+/CD8+ thymocytes in animal models and inhibited dendritic cell maturation[146]. VEGF can also induce expression of Fas ligand, a known regulator of T cell apoptosis[147, 148], on human tumor endothelial cells [149, 150], resulting in the preferential apoptosis of tumor-infiltrating CD8+ T cells[149]. Overall, VEGF appears to promote tumor growth *via* diminishing the microenvironment immune cell population.

Conclusions

The treatment of epithelial ovarian cancer, particularly in the setting of platinum-resistant or - refractory disease, remains a challenge. Theoretically, targeting the tumor microenvironment is advantageous because stromal components do not develop mutations or genetic aberrations as frequently as do tumor cells. However, the intricate signals between components of the tumor microenvironment can ultimately lead to adaptive resistance and treatment failure. Many of the strategies outlined in this article hold hope for improving the efficacy of microenvironment-targeted therapies and enhancing patient outcomes.

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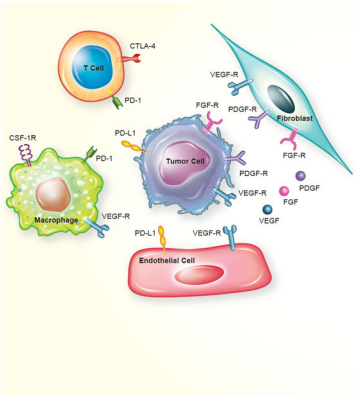
Table 1

Summary of therapies targeting the tumor microenvironment in ovarian cancer. The agents listed below have demonstrated safety/efficacy in phase I/II trials, and/or improved survival in phase III trials.



Molecular Target	Therapeutic Agent	Mechanism of Action	Hazard ratio for PFS and OS (95% CI)	Reference (Study)
VEGF	Bevacizumab	Monoclonal antibody to VEGF-A	HR-PFS: 0.72 (0.63–0.82) HR-OS: 0.89 (0.75–1.04)	Burger RA, et al. *NEJM*. 2011;365:2473–2483. [66] (GOG-218)
			HR-PFS: 0.81 (0.70–0.94) HR-OS: 0.99 (0.85–1.14)	Perren TJ, et al. *NEJM*. 2011;365:2484–2496. [67] (ICON7)
			HR-PFS: 0.48 (0.38–0.60) HR-OS: 0.85 (0.66–1.08)	Pujade-Lauraine E, et al. *J Clin Oncol*. 2012;30 (18suppl): LBA5002. (AURELIA)
			HR-PFS: 0.53 (0.41–0.70) HR-OS: 0.96 (0.76–1.21)	Aghajanian C, et al. *J Clin Oncol*. 2012;30:2039–2045. [152] (OCEANS)
			HR-PFS: 0.61 (0.52–0.72) HR-OS: 0.83 (0.68–1.005)	Coleman RL. SGO 2015. (GOG-213)
	Aflibercept	Soluble VEGFR	Not available (ongoing)	Coleman RL, et al. *Lancet Oncol*. 2011;12:1109–1117. [81]
FGFR, VEGFR, PDGFR	Nintedanib	Receptor tyrosine kinase inhibitor for FGFR1–3, VEGFR1–3, PDGFR α,β	HR-PFS: 0.84 (0.72–0.98) HR OS: Not available	Du Bois A, et al. *J Clin Oncol*. 2013;31 (18suppl): LBA5503. (AGO-OVAR12)
VEGFR, PDGFR, c-kit	Cediranib	Receptor tyrosine kinase inhibitor for VEGFR 1–3, PDGFR-α, c-kit	HR-PFS: 0.57 (0.44–0.74) HR-OS: 0.70 (0.51–0.99)	Ledermann JA, et al. *Eur J Cancer*. 2013;49 (suppl): LBA (ICON6)
	Pazopanib	VEGFR 1–3, PDGFR α,β, c-kit	HR-PFS: 0.77 (0.64–0.91) HR-OS: 0.99 (0.75–1.32)	Du Bois A, et al. (AGO-OVAR16)
PD-1	Nivolumab	Anti-PD1 antibody	Not available (ongoing)	Hamanishi J, et al. *J Clin Oncol*. 2014;32 (suppl): 5511.
	Pembrolizumab	Anti-PD1 antibody	Not available (ongoing)	Varga A, et al. *J Clin Oncol*. 2015:33 (suppl): 5510.
PD-L1	Avelumab	Anti-PD-L1 antibody	Not available (ongoing)	Disis ML, et al. *J Clin Oncol*. 2015;33 (suppl): 5509.

VEGF(R): Vascular endothelial growth factor (receptor)
FGFR: Fibroblast growth factor receptor
PDGFR: Platelet-derived growth factor receptor
PD-1: Programmed cell death protein 1
PD-L1: Programmed death-ligand 1
PFS: Progression free survival
OS: Overall survival