Review

Molecular Cancer Research

Revisiting the Complexity of the Ovarian Cancer Microenvironment—Clinical Implications for Treatment Strategies

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Abstract

Epithelial ovarian cancer (EOC) is the leading cause of death among gynecological malignancies in North American women. Given that EOC encompasses a broad class of tumors consisting of a variety of different histologic and molecular subtypes, which generates genetically and etiologically distinct tumors, several challenges arise during treatment of patients with this disease. Overlaying this complexity is the contribution of supporting cells, particularly stromal components such as fibroblasts and immune infiltrates that collectively create a microenvironment that promotes and enhances cancer progression. A notable example is the induction of angiogenesis, which occurs through the secretion of pro-angiogenic factors by both tumor and tumor-associated cells. The recent development of angiogenic inhibitors targeting tumor vasculature, which have been shown to improve patient outcome when combined with standard therapy, has launched a paradigm shift on how cancer patients should be treated. It is evident that future clinical practices will focus on the incorporation of therapies that antagonize the protumoral effects of such microenvironment contributors. Herein, an overview of the varying tumor–host interactions that influence tumor behavior will be discussed, in addition to the recent efforts undertaken to target these interactions and their potential to revolutionize EOC patient care. *Mol Cancer Res; 10(10); 1254–64. ©2012 AACR.*

Introduction

Despite several routes of investigation over the past 3 decades, epithelial ovarian cancer (EOC) remains the most deadly of all gynecological malignancies among women, with the serous subtype being predominant to clear cell, endometrioid, and mucinous histologic subtypes. Inherent to a subset of EOCs is the local dissemination of tumor cells to the omentum and peritoneal surfaces, often accompanied by an accumulation of ascites fluid. Functionally, the role of ascites during cancer progression is to facilitate migration and spread of cancer cells to the omentum by providing a milieu rich in soluble growth factors secreted by neighboring cells. During cancer progression (Fig. 1), malignant cells derived from ovarian surface epithelium or extra-ovarian sites undergo rapid proliferation, leading to the establishment of a primary tumor. Cancer cells from the tumor may either invade underlying stroma or shed into the peritoneal cavity, where they form multicellular aggregates (MCA) that remain floating in ascites fluid. These MCAs may then form adhesions and implant on mesothelial cells of the peritoneum, followed by subsequent invasion and further metastasis.

For several years, it was believed that cancer progression was driven solely by cell-autonomous processes, however, there is now abundant evidence suggesting that tumor cells are vulnerable to contributing components present within their surrounding microenvironment (1). Thus far, many attempts to alter the behavior of individual cancer cells have been developed as key chemotherapeutic strategies, such as disrupting cell cycle processes and other vital molecular pathways. Although not entirely successful, these strategies are effective at reducing tumor burden. However, one main limitation of standard chemotherapy includes its ineffectiveness at treating patient relapse, as the majority of patients face cancer recurrence (2). As a result, these pitfalls have sparked interest in the investigation of the role of the cancer microenvironment in EOC pathogenesis, which may offer insights into the adaptive nature of these cells, as well as provide optional areas to target.

Traditionally, the microenvironment consists of any biologic component that interacts with tumor cells, which ranges from stromal cells, to extracellular matrix (ECM) molecules, to cytokines. One of the major difficulties within cancer research is dissecting the molecular mechanisms underlying heterogeneous tumor growth, as well as understanding the interplay between stromal components and tumor cells, and whether stromal cells participate

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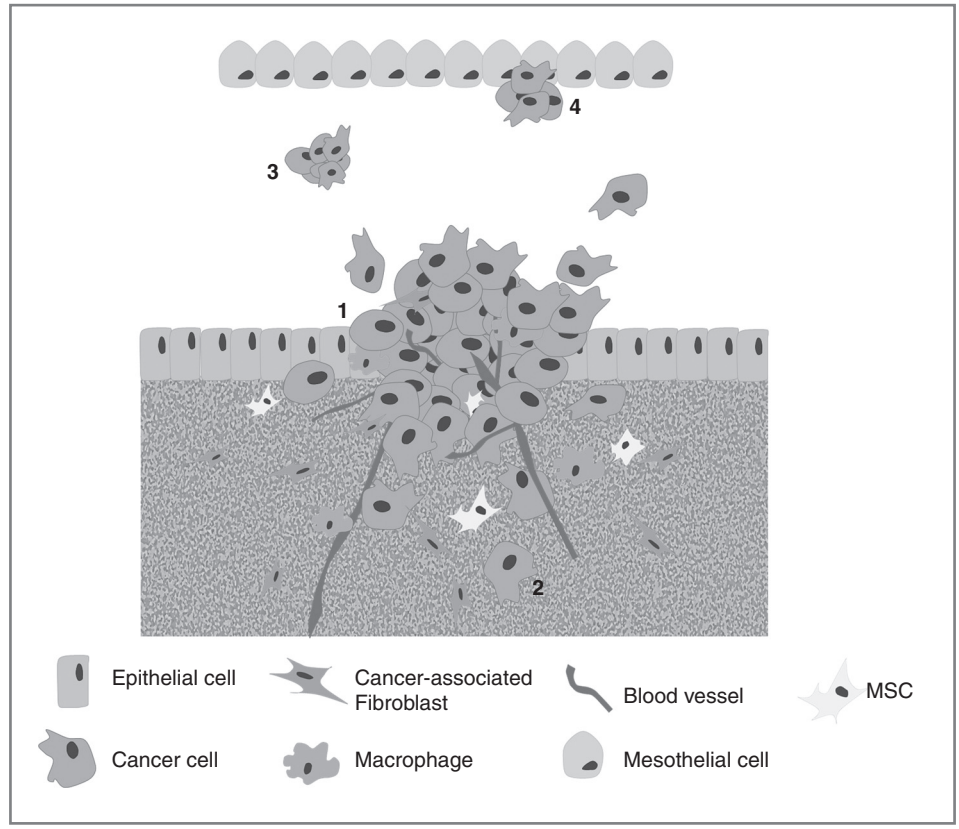

Figure 1. Pivotal events during ovarian cancer progression. In the early stages of EOC pathogenesis, malignant cells undergo neoplasia, and establish a primary tumor (1). Cancer cells may become migratory and invade the stroma (2), or shed into the peritoneal cavity and form multicellular aggregates (3). These aggregates may then generate cell adhesions to the peritoneal wall (4), breach this layer, and further metastasize.

cooperatively with other cell types to influence cancer behavior. Most studies typically delineate cancer–host interactions with the perturbation of a single molecule or cell type, however, communication of stromal cells with other stromal components should also be considered. It is clear that 2 hallmarks of cancer, angiogenesis and the ability of cancer cells to invade and metastasize, are dependent on the tumor microenvironment, which highlights the potential of disrupting the tumor–host interface for therapeutic intervention (1). It is predicted that these interventions will contribute to a new era of personalized medicine as targeted therapies will be combined with standard clinical treatments, which are anticipated to improve patient survival.

In this review, an overview of the different roles of various microenvironment components and how they influence tumor progression will be discussed, in addition to the emerging therapies that target these interactions. Moreover, current conventional treatments will be re-examined, particularly, the effect that microenvironment-targeting agents have on existing therapies, as well as their ability to improve patient outcome when used in combination.

Current EOC Treatment Strategies Leave Opportunities for Microenvironment-Targeting Agents

The lack of effective early detection screening methods is a major contributing factor toward the high mortality rates of EOC. Although several early detection serum biomarkers have been proposed, some of which are clinically used, no single marker or panel of markers can predict early-stage disease with high specificity and sensitivity. Generally, the unavailability of early screening tools results in initial EOC diagnosis during advanced stages, therefore, patient survival is highly dependent on existing treatment options. Because of the limitations of current strategies, future efforts should focus on the development of therapies that target tumor–host interactions, as the microenvironment has a substantial influence on tumor progression.

Currently, surgical debulking in combination with either platinum/taxane-based chemotherapy (carboplatin and paclitaxel) continues to be the first-line treatment of patients with ovarian cancer (2). Several clinical features are examined to determine which available therapies would be most effective in yielding a favorable outcome. For instance, assessing histologic subtype, tumor grade, stage, age, and the size of the tumor after primary cytoreduction allows physicians to make informed decisions regarding the implementation of anticancer strategies (2). However, one of the major challenges faced by patients with this disease is cancer recurrence, as tumor cells acquire a chemoresistant phenotype making them insusceptible to anticancer agents (2).

It is becoming increasingly clear that cancer progression is a multistep process that requires the recruitment of host cells to grow and metastasize. For this reason, the tumor microenvironment has become an attractive target for the development of therapies that will complement and improve

current standard treatments. This has been exemplified by the success of ongoing clinical trials that assess the efficacy of inhibitors against pro-angiogenic molecules that promote tumor vasculature and angiogenesis (3). Further development of these strategies will transform the way EOC patients are treated in the future.

Inflammatory Cytokine and Chemokine Networks in Ovarian Cancer

Numerous studies have highlighted the association between inflammation and cancer, particularly in the context of tumor progression (4). Consequently, this phenomenon posed by inflammatory cells and cytokine networks presents challenges for cancer treatment, rendering alternative strategies to complement existing therapies or to treat ovarian cancer recurrence with single administration. Before considering the impact of inflammatory cells to cancer development, it is important to identify the cytokine population within the ovarian cancer microenvironment, which forms an intricate network of soluble mediators that have a profound impact on tumor growth, angiogenesis, and more importantly, infiltration of leukocytes.

One of the most prominent cytokine members implicated in inflammation includes TNF-α, which is constitutively expressed in ovarian cancer tumors and stromal cells, such as macrophages (5). Interestingly, only tumors of the serous subtype displayed a positive association between TNF gene expression and tumor grade (5). TNF-α has a multifaceted role in cancer spread through its autocrine and paracrine actions (6), which are exerted through the stimulation of other cytokines and angiogenic factors (7). For instance, stable knockdowns of TNF-α in ovarian cancer cell lines resulted in reduced production of these factors, including chemokine (C-C motif) ligand 2 (CCL2), interleukin-6 (IL-6), chemokine (C-X-C motif) ligand 12 (CXCL12), vascular endothelial growth factor (VEGF), and migration inhibitory factor (MIF; ref. 7). More importantly, reduced tumor growth and vascularization was observed in mice injected with TNF-α-deficient cancer cells, when compared with mock-transfected cells (7). In a similar study, Charles and colleagues used an ovarian cancer mouse model to study the regulatory properties of TNF-α on various cytokines in the tumor microenvironment (8). The authors showed that TNF-α/TNFR1 signaling in CD4+ cells is fundamental for tumor growth, which was also associated with increased IL-17 levels in malignant ascites (8). Treatment with a TNF-α neutralizing antibody resulted in lower ascitic levels of IL-17 and plasma levels of IL-6, in addition to decreased tumor burden and leukocyte infiltrate after 8 weeks, compared with control mice (8). To explore the applicability of these findings in humans, the authors examined IL-17 ascitic levels and TNF-α serum levels in ovarian cancer patients, and found a decrease in patients treated with the TNF-α antibody, Infliximab (8). A phase I clinical trial in patients displaying advanced cancer also resulted in lower IL-17 serum levels after Infliximab treatment (Table 1; refs. 8, 9). Another TNF-α antagonist, Etanercept, which is a soluble p75 TNF receptor that inactivates TNF-α by competitive binding, was also assessed in phase I trials to evaluate its efficacy in treating recurrent ovarian cancer. In this study, 2 cohorts of 17 and 13 patients, respectively, were treated with dosages of 25 mg, 2 (cohort 1) and 3 (cohort 2) times a week (Table 1; ref. 10). Six of 18 patients who received a minimum of twelve weeks of therapy reached disease stabilization; 11 and 13 patients (cohort 1) displayed a drop in IL-6 and CCL2, respectively (10). Taken together, these findings reveal TNF-α as a viable target for EOC treatment.

In addition to TNF-α, high levels of several other pro-inflammatory cytokines have been identified in ascites fluid from ovarian cancer patients, including IL-6, IL-8, CCL2, and macrophage inflammatory protein-1β (MIP-1β; refs. 11, 12). To evaluate the prognostic significance of IL-6 and IL-8 levels in ascites, Lane and colleagues correlated levels to a number of clinical measures, including progression-free survival (12). Using multivariate analyses, the authors concluded that IL-6 could serve as a predictor of shorter progression-free survival (Table 2; ref. 12). Similarly, in an alternate study, high serum IL-6 levels were also correlated with poor prognosis (Table 2; ref. 13). A monoclonal IL-6 antibody, Siltuximab, was shown to effectively abrogate IL-6 signaling pathways by suppressing Stat3 phosphorylation, leading to a decrease of downstream anti-apoptotic factors (14). Interestingly, Siltuximab combined with paclitaxel enhanced sensitivity and cytotoxicity in a paclitaxel-resistant cell line, SKOV-3_{TR}; however, these observations could not be recapitulated *in vivo* (14). Therapeutic efficacy of this agent was assessed in a phase II clinical trial with 20 patients displaying advanced platinum-resistant ovarian cancer (Table 2; ref. 15). Of these, 1 patient had a partial response, whereas 7 patients reached disease stabilization, in addition to exhibiting decreased plasma levels of several cytokines including CCL2, CXCL12, and VEGF, suggesting that they are regulated by IL-6 (15).

Emerging evidence has showed that pro-inflammatory cytokines and chemokines form complex networks with each other, which collectively influence events that drive metastasis. Recently, Kulbe and colleagues delineated a link between 3 mediators of inflammation, TNF, CXCL12, and IL-6, and their paracrine actions on tumor angiogenesis and leukocyte infiltration (16). As expected, treatment with Infliximab led to decreased levels of CXCL12 and IL-6, thus, illustrating their interdependency, and also resulted in reduced tumor growth, vascularization, and infiltration of myeloid cells (16). These seminal studies illustrate the ability of cytokines to form complexes that promote cancer pathogenesis, which may be disrupted by direct targeting of 1 molecule.

Tumor-Associated Stromal Cells Contribute to Ovarian Cancer Pathogenesis

The EOC microenvironment encompasses a diverse subset of host cells that acquire an altered behavior when recruited to the tumor site. Here, we discuss the roles of these various tumor-associated cells including macrophages,

Table 1. Microenvironment-targeting agents used in various cancer clinical trials

Drug/agent	Type	Target/mechanism of action	Impact on microenvironment regulation	Stage in clinical development	Results of study	Reference
Infliximab	Monoclonal antibody	Binds to TNF-α with high affinity	Decreases levels of pro-inflammatory cytokines	Phase I	Decreased levels of IL-17, CXCL12, TNF-α, and IL-6	(8, 9, 16)
Etanercept	p75 TNF receptor fusion protein	TNF-α blocker	Inhibits actions of TNF-α	Phase I	Lowers IL-6 and CCL2 levels; 6 of 30 patients reaches prolonged disease stabalization	(10)
Siltuximab	Monoclonal antibody	Neutralizes IL-6	Inhibits functional activity of IL-6	Phase II	Decreased levels of pro-angiogenic factors; 7 of 20 patients reached disease stabilization	(15)
Trabectedin	Tetrahydro-isoquinoline alkaloid	Binds minor groove of DNA, preventing cell cycle completion; causes apoptosis	Inhibits monocyte-to-macrophage differentiation; decreases production of pro-tumoral cytokines	Phase III	Along with PLD, improved progression-free survival and overall response in platinum-free interval >6 months patients	(35, 36)
Sibrotuzumab	Monoclonal antibody	Binds to FAP	Targets major constituents of tumor stroma	Phase I	1 of 20 colorectal cancer patients had stable disease for 2 yrs	(42)
Volociximab	Monoclonal antibody	Binds α5β1 integrins	Blocks attachment of cancer cells to the mesothelium	Phase II	No complete and partial responses	(65)
Bevacizumab	Monoclonal antibody	Binds all isoforms of VEGF-A	Angiogenesis	Phase III	Prolonged progression-free survival	(72, 73)

Table 2. Factors that serve as prognostic predicators for ovarian cancer patients

Factor	Prognostic outcome	Implications to ovarian cancer patients	Reference
IL-6	Poor	Increased levels of IL-6 in ascites corresponded with shorter progression-free survival; elevated serum levels in primary EOC patients are associated with poor prognosis	(12, 13)
CD20, FoxP3, TIA-1	Positive	Immunohistochemistry staining coupled with Kaplan-Meier survival analysis revealed high expression of CD20, FoxP3, and TIA-1 was correlated with longer patient survival	(19)
CSF-1/CSF-1 receptor	Poor	Immunohistochemical analysis showed that strong co-expression of CSF-1 and CSF-1 receptor in metastatic ovarian cancer correlated with shorter disease-free survival	(30)

fibroblasts, adipocytes, and mesenchymal stem cells (MSC) in EOC progression, along with agents that have been developed to disrupt the tumor–host interface. Figure 2 depicts a subset of the interactions that occur between malignant tumor cells and various microenvironment components.

Tumor-associated macrophages

The recruitment of tumor-infiltrating leukocytes (TIL) during cancer was naturally perceived to be the body's immune response to a solid tumor; however, numerous studies have revealed that distinct leukocyte populations, with the exception of lymphocytes, are in fact, tumor promoting rather than tumor inhibiting. On the other hand, lymphocytic infiltrates are associated with favorable prognosis and have been correlated with improved rates of progression-free and overall survival of EOC patients (17, 18). For example, a series of immunohistochemical studies revealed that the presence of lymphocyte markers, T cell intracellular antigen-1 (TIA-1), FoxP3, and CD20, could be indicators of positive prognosis for patients displaying high grade serous EOC (Table 2; ref. 19). This suggests that distinct populations of T cells are recruited to the tumor site and impose cytotoxic effects; however, many cancer cells

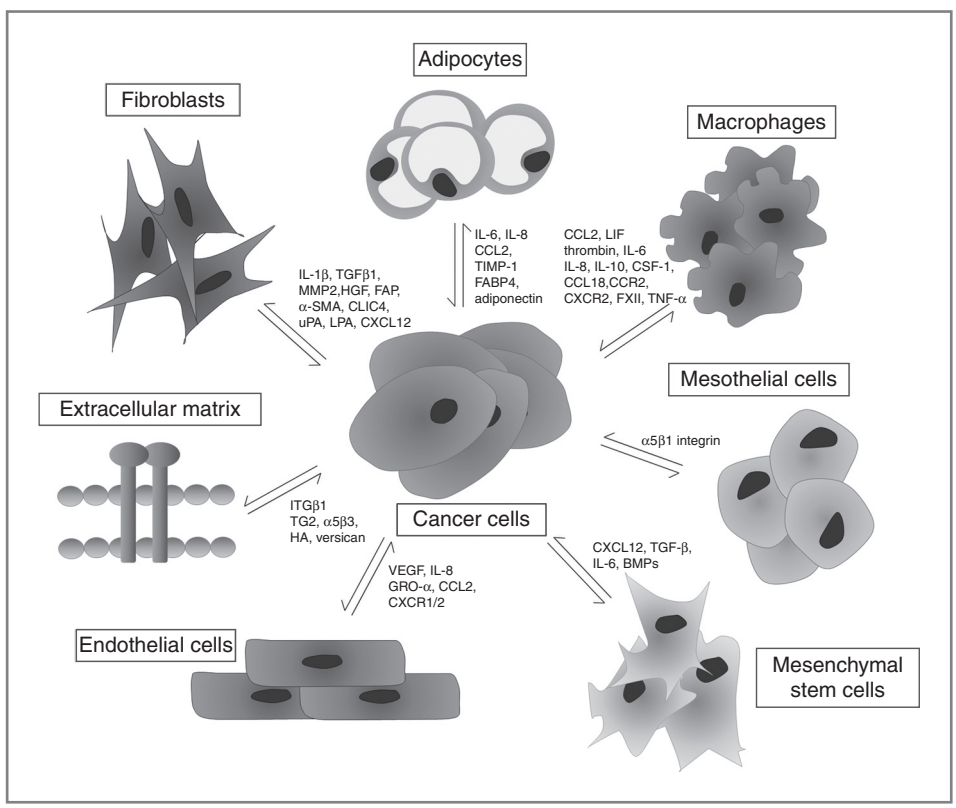

Figure 2. Interactions between tumor cells and various microenvironment components. Several of these interactions are mediated through the exchange of soluble factors, or via cell adhesion mechanisms.

escape detection by the immune system. Although it is not entirely known how tumor cells evade immune surveillance, Martinet and colleagues showed that this escape may be mediated through the immunosuppression of T cells by stromal cells in ascites, referred to as Hospicells (20). Hospicells produce an abundant supply of nitrous oxide, which suppresses CD4+ T cell proliferation and cytokine production, whereas conferring chemoresistance in cancer cells (20).

Immune infiltrates also include a rich supply of macrophages, which are recruited by tumor cells through their secretion of chemokines, particularly, CCL2 (also referred to as monocyte chemotactic protein-1; ref. 21). It is well established that tumor cells and macrophages engage in a bidirectional interaction through the exchange of soluble mediators, which influence cell behavior and phenotype. For instance, after being recruited to the tumor site, tumor cells induce changes in macrophage secretion of cytokines, chemokines, as well as matrix metalloproteinases (MMP), such as MMP9, that enhance tumor growth in mice (22). Alterations in cytokine production activate a tumor-associated macrophage (TAM) M2 immunosuppressive phenotype, which is representative of those found in ovarian tumors (23). Polarization of monocytes and macrophages toward an M2 phenotype, which is marked by an increased expression of CD163, IL-10, CCL18, IL-8, chemokine (C-C motif) receptor 2 (CCR2), and chemokine (C-X-C motif) receptor 2 (CXCR2), can be stimulated by coagulation factor XII (FXII) or thrombin (24, 25). Interestingly, when treated with either FXII or thrombin, conditioned medium (CM) from TAMs increased ovarian cancer cell invasiveness, with IL-8 being identified as the major chemoattractant mediating this invasion (24, 25). Several lines of evidence indicate that activation of the M2 phenotype can also be induced by CM from EOC cells (26), or more specifically, leukemia inhibitory factor (LIF), IL-6, and colony stimulating factor-1 (CSF-1; ref. 27). Consequently, CSF-1 is elevated in tumor cells, displaying higher expression in malignant tumors compared with benign neoplasms, and has also been associated with poor prognosis (28–30). Another study revealed that immunosuppressive TAMs could be converted back to immunostimulatory macrophages upon treatment with interferon-γ (IFNγ; ref. 31). IFNγ-stimulated TAMs secreted less tumor-promoting mediators, inhibited the production of TAMs from monocyte precursors, and, more importantly, enhanced the proliferation of $CD4^+$ T cells (31). As such, local administration of IFNγ at the tumor site may synergize with other antitumor immunotherapies, and enhance T cell-mediated destruction of tumor cells (31).

One attractive method for direct targeting of both tumor cells and TAMs with chemotherapeutic agents involves the folate receptor, which is expressed on both cell populations and has been used for the uptake of folate-linked drugs via receptor mediated endocytosis (32). Turk and colleagues used this strategy to construct folate-conjugated liposomes and measured its uptake ability by cancer cells and TAMs using an *in vivo* mouse model that recapitulates advanced staged EOC (32). Overall, liposomes linked to folate showed a greater targeting capacity toward TAMs than tumor cells, which highlights the utility of liposome-linked to folate for the delivery of drugs to TAMs (32).

In another study, Geller and colleagues (2010) assessed the implications of paclitaxel and carboplatin-based chemotherapy on CCL2 expression in an ovarian cancer cell line, MA-148 (33). Following administration of either drug, mRNA expression of CCL2 increased in MA-148 cells, which were further confirmed *in vivo* through mRNA validation of mouse tumors following mouse exposure to the same chemotherapeutic regimen (33). Further studies will need to address the impact of CCL2 on ovarian tumorigenesis, as it may be a marker of poor prognosis because it is an indicator of TAM recruitment and could possibly facilitate tumor recurrence (33).

Currently, few treatments developed against tumor-associated macrophages have shown promising potential, the best known being Trabectedin (34). Trabectedin binds minor grooves in DNA and prevents cell cycle, and has been shown to inhibit the differentiation of monocytes into macrophages (Table 1; ref. 34). *In vitro* production of protumoral mediators such as CCL2 and IL-6, potent stimulators of cancer progression, was decreased in TAMs and ovarian tumor cells when treated with Trabectedin (34). Previously, a randomized phase III clinical trial, OVA-301, assessed the efficacy of Trabectedin in combination with pegylated lysosomal doxorubicin (PLD) compared with PLD alone, in patients with recurrent EOC following platinum-based chemotherapy failure (35). The authors concluded that Trabectedin/PLD combination improved progression-free survival and overall response rate in patients displaying a platinum-free interval of more than 6 months, compared with patients receiving PLD alone (35, 36). In a more recent preclinical study, it was shown that Trabectedin inhibited tumor growth in xenograft models of clear cell carcinoma of the ovary, which further shows its potential as either a first-line or second-line therapy for certain subgroups of ovarian neoplasms (37). As such, patients exhibiting high amounts of immune infiltration in tumors may benefit from agents targeting activated TAMs.

Cancer-associated fibroblasts

Fibroblasts are essential components of connective tissue that are normally recruited and "activated" during wound repair. In addition to their role in wound healing, fibroblasts have also been extensively linked to tumor progression, and are well recognized as one of the major constituents of tumor stroma (38). Contrary to their behavior in wound repair, rather than regressing to their "inactive" form, fibroblasts associated with cancers remain activated, similar to wounds that never heal (38). These fibroblasts undergo a desmoplastic reaction by forming reactive stroma, thus deemed cancer-associated fibroblasts (CAFs) or myofibroblasts (38). During carcinogenesis, CAFs are vastly characterized by their increased deposition of ECM components such as collagens and are often distinguished from normal fibroblasts (NF) by their altered phenotype and expression of 2 myofibroblastic markers, α-smooth muscle

actin (α-SMA) and fibroblast activation protein (FAP; refs. 38, 39).

Several studies have revealed the ability of CAFs to control the differentiation of epithelial cells through the secretion of cytokines and soluble factors (38). The resulting paracrine signaling between cancer cells and CAFs results in the release of growth and migratory signals that enhance the invasion of malignant cells and promote tumor progression (38). As such, further investigation of the paracrine signaling axis between cancer cells and fibroblasts may yield novel candidates for therapeutic targeting. Although the contribution of fibroblasts to the oncogenic microenvironment was previously elucidated, few studies have examined the connection and impact of CAFs to EOC progression.

Evidence of fibroblast-to-myofibroblast transdifferentiation via cancer and fibroblast mediated crosstalk has been illustrated in a number of *in vitro* studies using well-established ovarian cancer cell lines. For instance, activation of normal ovarian primary fibroblasts and their conversion to a myofibroblast phenotype was observed upon their stimulation with TGF-β1 or conditioned medium from SKOV3 cells, a human ovarian carcinoma cell line (40). This stimulation also resulted in increased cellular reactive oxygen species (ROS) levels, causing an upregulation of chloride intracellular channel 4 (CLIC4). Higher CLIC4 levels correlated with increased expression of α-SMA, which provided a solid indication of a myofibroblast phenotype (40). In a similar study, CM from a highly metastatic ovarian cancer cell line, HO-8910PM, induced FAP-1α expression *in vitro*, and identified TGF-β1 and IL-1β as putative paracrine signals that mediated this fibroblast activation (41). Interestingly, elevation of cell surface FAP-1α was found to promote proliferation, adhesion, and migration of HO-8910PM cells, and hence, may serve as a promising molecule for targeting CAFs (41). Thus far, few efforts have been made to target FAP, including a phase I study with the monoclonal antibody, Sibrotuzumab, which was used in 20 and 6 patients with metastatic colorectal carcinoma or non–small cell lung cancer, respectively (Table 1; ref. 42). In this study, 1 colorectal cancer patient displayed stable disease for 2 years, however, no other tumor responses were observed in the remaining patients (42). More recently, a DNA vaccine generated against FAP was shown to supress tumor growth and increase lifespan in a mouse model exhibiting colon cancer (43). Vaccinated mice showed a 1.5-fold increase in lifespan, which reveals FAP as a candidate target for immunotherapy-based treatment (43).

In addition to myofibroblast differentiation from NFs, human adipose tissue-derived mesenchymal stem cells (hADSCs) induced by lysophosphatidic acid (LPA) have also been shown to achieve CAF phenotypic status (44). Congruent with previous studies, treating hADSCs with conditioned medium from cancer cells or ovarian cancer patient ascites fluid resulted in the upregulation of α-SMA expression in these cells (44). Moreover, stimulation by LPA also resulted in increased production of CXCL12 via TGF-β1 autocrine signaling in hADSCs, which was abrogated when pretreated with an LPA receptor antagonist (44).

As alluded to earlier, in ovarian cancer, tumor cells and CAFs also participate in a reciprocal exchange of soluble components, which leads to the activation of particular signaling networks (38). For example, it was showed that cytokines present within medium conditioned by an ovarian clear cell carcinoma cell line, ES-2, induced urokinase-type plasminogen activator (uPA) mRNA transcription in fibroblast cells, which is a protease implicated in cancer invasion and migration (45). Moreover, a recent study suggested that a premetastatic niche is created in the omentum through the activation and proliferation of NFs that are stimulated by the release of TGF-β1 from cancer cells (46). The establishment of the premetastatic niche would provide an altered microenvironment that enhances tumor invasion and implantation on peritoneal surfaces, through the secretion of hepatocyte growth factor (HGF) and MMP-2 (MMP2; ref. 46). An inhibitor of the TGF-β type I receptor, A83-01, abrogated TGF-β1 signaling and reduced the proliferation and activation of NFs, as well as reduced α-SMA and MMP-2 expression in SKOV3/NF tumors (46). Such interventions should be considered for further development, as targeting elements of signal transduction pathways between cancer and stromal cells will lead to reduced levels of EOC metastasis. In a parallel study assessing the contribution of CAFs to metastasis, using myofibroblast-specific markers, Zhang and colleagues concluded that CAFs were more abundant in disease during advanced stages and were associated with increased lymphatic vessel and microvessel density in addition to lymph node and omentum metastases (47). Interestingly, cancer-associated fibroblasts isolated from ovarian cancer patients induced more cancer cell migration than fibroblasts extracted from normal ovarian tissues (47).

Targeting CAFs or their associated autocrine–paracrine signaling loops appears promising for the development of future ovarian cancer therapies. More recently, an approach using MRI and optical imaging tracked the recruitment of prelabeled fibroblasts to the ovarian cancer stroma, which lined the outer rim of the tumor and colocalized with angiogenic vessels (48). Consequently, this imaging technique provides a noninvasive approach to target stromal cells for cellular therapy in the future (48).

Omentum-derived adipocytes

Adipocytes have often been classified as energy storing residents of adipose tissue; however, recent studies suggest that these fat-storing cells may serve other functions as well. Their heterotypic interactions with malignant tumor cells have been documented in breast, ovarian, colon, and gastric cancers (49). Tumor-promoting effects of adipocytes have been linked to their secretion of adipokines, hormones, and growth factors into the cancer microenvironment, which enhance cancer cell migration and invasion (50). A study conducted by Walter and colleagues revealed that normal adipose cells stimulated the migration and invasion of estrogen receptor (ER)-negative breast cancer cells (51). This effect was mediated via a cytoskeletal element, cofilin-1, and it's regulation of IL-6 secretion in adipocytes (51). Another study revealed that during coculture with breast

cancer cell lines, adipocytes acquired an activated phenotype, characterized by increased production of proteases and cytokines, IL-6 and IL-1β, as well as delipidation and a loss of adipocyte-associated markers (50).

In a comprehensive study, Nieman and colleagues used fluorescent tracking of cancer cells in murine models to show the preferential migration of ovarian cancer cells to the omentum, an organ enriched in adipose cells (49). Similar to previous studies, this migratory behavior was mediated by adipokines (IL-6, IL-8, CCL2, tissue inhibitor of metalloproteinase 1 [TIMP-1], and adiponectin) secreted by adipocytes derived from the omentum (49). Interestingly, coculture of adipocytes and ovarian cancer cells induced lipolysis in adipocytes, resulting in the transfer of free fatty acids to cancer cells, which in turn, stimulated tumor cell proliferation through the generation of energy via β-oxidation. Fatty acid binding protein 4 (FABP4) was identified as a putative mediator in the transfer of lipids to cancer cells (49). As such, emerging therapies for personalized medicine will include those targeting mechanisms that enhance tumor metabolism, as in this case, lipid metabolism (49). In addition, hormones derived from adipose cells such as leptin, have been associated with an increase in ER-positive ovarian cancer cell growth, as ERα can be transcriptionally activated through the signal transducer and activator of transcription-3 (STAT-3) signaling pathway (52). These findings suggest that consideration of ER status, as well as the growth promoting effects of adipocytes on cancer cells should be taken into account in ovarian cancer patients who are also obese (52).

Mesenchymal stem cells

MSCs have recently been recognized as active cellular components that are recruited to the tumor microenvironment, and their multipotency permits their differentiation into a variety of different cell types. Interestingly, Coffelt and colleagues have showed that this recruitment may be partly stimulated by LL-37 (leucine, leucine-37), which is a proinflammatory peptide of human cationic antimicrobial protein 18, in addition to other migratory signals (53). In an EOC xenograft model, human bone marrow-derived MSCs differentiated into CAFs, which was confirmed by the elevation of markers specific to fibroblast activation (54). In addition, MSC-derived CAFs produced soluble protumorigenic factors, including IL-6, which enhanced tumor growth and proliferation (54). Combining cancer-associated MSCs with tumor cells *in vivo* and *in vitro* has been shown to result in increased expression of the bone morphogenetic protein (BMP) signaling network, which has several pathologic roles in cancer progression (55). To determine the phenotypic changes that are orchestrated during MSC-to-myofibroblast differentiation, Cho and colleagues treated adipose tissue-derived MSCs with exosomes isolated from 2 ovarian cancer cell lines, OVCAR-3 and SKOV-3 (56). Exosome-treated MSCs displayed an elevation of α-SMA expression, which, again, is indicative of an activated fibroblast phenotype, and also resulted in increased production of protumoral cytokines, CXCL12, and TGF-β (56).

Chemoresistance and unresponsiveness following application of standard therapies is common in most cancer patients, and usually results in cancer cells acquiring a "cancer stem cell (CSC)-like" phenotype. This phenotypic change is often associated with epithelial–mesenchymal transition (EMT), a key biologic event implicated in cancer metastasis. As such, a metastatic cell line, OVA433 treated with cisplatin, expressed higher levels of EMT and stem cells markers, and enhanced activation of ERK2 signaling (57). Blockage of ERK2 signaling using a MEK inhibitor repressed EMT and CSC markers, suggesting that targeting this pathway may minimize tumor recurrence, by reducing mesenchymal characteristics that enhance tumor migration (57).

Human MSCs have been recently evaluated for their potential use as vehicles in cancer therapy, by exploiting their ability to preferentially migrate to and proliferate at tumor sites (58). For example, such efforts have been undertaken by using MSCs transduced with recombinant adenoviruses encoding endostatin, an inhibitor of angiogenesis (59). As a result, SKOV3 cells were able to induce migration of transduced MSCs, which, in turn, conferred antiproliferative effects on cancer through secretion of endostatin (59). Similarly, Hu and colleagues used human umbilical blood mononuclear cell-derived MSCs as delivery vehicles for administration of interleukin-21 to ovarian tumors in mice, which has been shown to boost antitumor immunity in murine models (60). As such, this treatment hindered tumor growth in addition to prolonging survival (60). These data, taken together, provide supporting evidence for the application of MSCs as gene delivery vehicles as a feasible therapeutic strategy.

ECM Components and Cell Adhesion Molecules Assist Cancer Migration

Alterations in ECM components have been well described in the context of tumor adhesion and invasion. Malignant cells are constantly changing cell adhesion surface molecules in response to signals in their surroundings, and this will enhance their ability to disseminate locally. For these reasons, targeting the interactions between certain cell adhesion apparatuses and the ECM has been proposed, including cell membrane integrins (61, 62). Integrin-related mechanisms have been shown to be essential at different stages of EOC progression. For example, multicellular aggregated formation is mediated via β1-integrins and their subsequent attachment to the mesothelium is partially dependent on α5β1-integrins (63, 64). Efforts to antagonize α5β1-integrin interactions were implemented in phase II clinical trials, using a chimeric antibody, Volociximab (Table 1), in patients with platinum-resistant advanced EOC, although clinical efficacy was not accomplished (65). Moreover, targeting of $\alpha_v\beta_3$ with antibodies and radiolabeled nucleotides in xenografts has opened up new avenues and opportunities for therapeutic intervention (66–68).

Another efficient approach for targeting integrin-related attachment to ECM components involves the perturbation

of a protein cross-linker, tissue transglutaminase (TG2; ref. 69). TG2 facilitates the construction of integrin and fibronectin networks; therefore, inhibition would result in decreased cellular adhesion (69). In a recent study, Khanna and colleagues sought to block its transpeptidase activity by using a high-throughput screen of small molecule inhibitors and overall, 7 compounds were able to inhibit cancer cell adhesion by at least 50% (69). Further development and initiation of clinical trials to test these small molecule inhibitors may render alternative strategies that will minimize cancer spread.

Apart from integrins, other ECM adhesion molecules that contribute to cancer migration and invasion involve interactions between hyaluronan (HA) and versican, which can be blocked by small HA oligosaccharides (70). More importantly, there have been several documented studies showing the pivotal role of HA in the adhesion of cancer cells to the peritoneum, which is one of the early events of EOC progression (71). As a result, inhibition of this early step in metastasis may lead to improved patient outcomes.

Angiogenic Vasculature is Required for Tumor Growth

To sustain growth and development, tumors need a constant supply of oxygen and nutrients, often achieved through the formation of new blood vessels. These angiogenic mechanisms are induced by several pro-angiogenic factors in the microenvironment, the most notable being VEGF. As the contribution of angiogenesis to tumor spread is indispensable, anti-angiogenic therapies targeting tumor vasculature have presented a practical approach when used in combination with other conventional treatments in targeting ovarian cancer.

Rigorous phase III clinical trials have investigated the potential of a VEGF inhibitor, Bevacizumab (Avastin), as a complement to existing chemotherapies for improving progression-free survival of EOC patients (Table 1; ref. 72). Overall, Bevacizumab improved progression-free survival in patients who displayed high risk for cancer progression (72). In a comparable study, Burger and colleagues established that treatment with Bevacizumab along with carboplatin and paclitaxel therapy prolonged survival by approximately 4 months (73).

Like many other biologic processes, chemokine signalling has also had a profound influence on promoting angiogenic mechanisms, and has been identified as an attractive alternative to inhibiting neovascularization. Activation of the G-protein coupled receptor protease-activated receptor-1 (PAR1) by MMP1 prompted ovarian cancer cells to release IL-8, growth regulated oncogene-α (GRO-α), and CCL2, potent inducers of endothelial cell proliferation in mice via CXCR1/2 (74). As expected, treatment with Bevacizumab was not able to prevent the effects of these angiogenic factors on endothelial proliferation; however, it was able to reduce angiogenesis through VEGF inhibition (74). Nevertheless, treatment with a peptide that interferes with G-protein coupled receptors, X1/2pal-i3 pepducin, hindered PAR1 activation by MMP1 (74). Recent findings suggest that specific modulators that impede angiogenesis, such as tumor necrosis factor superfamily-15 (TNFSF15), are downregulated by VEGF and CCL2, which are not only released by cancer cells, and therefore, emphasize the importance of contributing cell populations to neoangiogenesis (75).

Conclusions

The efforts formerly discussed are aimed toward the development of microenvironment-targeting agents that are intended to perturb tumor–host interactions. Thus far, these agents have showed potential to improve patient survival when combined with other cytotoxic chemotherapies. However, major limitations for the ongoing development of these therapies lie in the fact that ovarian cancer tumors are molecularly heterogeneous, and therefore, these treatments will elicit a varying degree of responses among different subgroups of patients.

Tremendous progress in the past decade has been made on the discovery and development of novel therapeutic strategies. Future studies should focus on improving existing therapies, identifying possible drug combinations for optimal effectiveness, and developing a system to stratify patients into subgroups, which will allow physicians to make informed decisions on which therapeutic regimen should be administered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: N. Musrap
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Musrap
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Musrap
Writing, review, and/or revision of the manuscript: N. Musrap, E.P. Diamandis
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Musrap

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