Cellular and molecular processes in ovarian cancer metastasis

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ABSTRACT

Ovarian cancer is the most lethal gynecological malignancy. It is usually diagnosed at a late stage, with a 5-year survival rate of less than 30%. The majority of ovarian cancer cases are diagnosed after tumors have widely spread within the peritoneal cavity, limiting the effectiveness of debulking surgery and chemotherapy. Owing to a substantially lower survival rate at late stages of disease than at earlier stages, the major cause of ovarian cancer deaths is believed to be therapy-resistant metastasis. Although metastasis plays a crucial role in promoting ovarian tumor progression and decreasing patient survival rates, the underlying mechanisms of ovarian cancer spread have yet to be thoroughly explored. For many years, researchers have believed that ovarian cancer metastasizes via a passive mechanism by which ovarian cancer cells are shed from the primary tumor and carried by the physiological movement of peritoneal fluid to the peritoneum and omentum. However, the recent discovery of hematogenous metastasis of ovarian cancer to the omentum via circulating tumor cells instigated rethinking of the mode of ovarian cancer metastasis and importance of the “seed and soil” hypothesis for ovarian cancer metastasis. In this review, we discuss the possible mechanisms by which ovarian cancer cells metastasize from the primary tumor to the omentum, the cross-talk signaling events between ovarian cancer cells and various stromal cells that play crucial roles in ovarian cancer metastasis, and the possible clinical implications of these findings in the management of this deadly, highly metastatic disease.

Keywords: ovarian cancer, metastasis, stromal-tumoral interaction, passive dissemination, hematogenous metastasis
Ovarian cancer is the fifth leading cause of cancer deaths in women in the United States. Researchers estimated that approximately 21,290 new cases of epithelial ovarian cancer would be diagnosed in the United States in 2015 and that 14,180 deaths would occur (143). Despite ongoing efforts to develop effective surgery and treatment regimens for ovarian cancer, the overall survival rate remains a dismal 30%, which in large part results from diagnosis at a late stage. The late diagnosis of ovarian cancer is attributed to the fact that typical ovarian cancer symptoms, including abdominal discomfort, bloating, gas, nausea, and urinary urgency, are not explicit and are often mistaken for gastrointestinal problems(63, 144). When ovarian cancer is detected at an early stage when the tumor tissue is confined to one or both ovaries, the cure rate using conventional therapies such as cytoreductive surgery and chemotherapy is as high as 90%. However, after the disease has spread to other pelvic organs such as the uterus and bladder (stage II), the abdomen (stage III), or beyond the peritoneal cavity (stage IV), the cure rate decreases substantially owing to the limited efficacy of optimal debulking and tumor management(10, 31). Because more than 70% of ovarian cancer cases are diagnosed at a late stage at which cancer cells are actively metastasizing, understanding ovarian cancer pathogenesis and the mechanism of its metastasis therefore is crucial for the management of this deadly, highly metastatic disease.

Ovarian cancer often metastasizes throughout the peritoneal cavity, to the omentum, and even to the parenchyma of the liver or lung. However, its spread is widely believed to occur via the peritoneal circulation associated with ascites formation instead of the classic patterns of metastasis via the hematogenous route(91). This is because implantation of peritoneovenous shunts to palliate discomfort of ascites and the resulting infusion of billions of cancer cells into the patient’s circulation result in confinement of the disease within the abdomen(153).

However, does ovarian cancer metastasis occur exclusively via intraperitoneal seeding, or does it follow the “seed and soil” hypothesis, which plays an important role in the confinement of the disease within the abdomen? According to the seed and soil hypothesis proposed by the
English surgeon Stephen Paget in 1889, the pattern of metastasis is not a random outcome. Instead, certain tumor cells (seeds) have specific affinity for certain organs (soil)\(^{(119)}\). He stated that a plant’s seeds are carried in all directions but can only live and grow if they fall on congenial soil. Thus, he concluded that metastases form only when the tumor cells and organs are compatible. Indeed, after frequent revisiting of this theory in recent years, authors reported several new findings on the intrinsic properties of tumor cells surrounding stromal cells and the signaling crosstalk of cancer cells with the host, which contribute to the organ-specific pattern of metastasis. A clear understanding of these cellular and molecular mechanisms will definitely shed light on possible therapeutic strategies to increase survival rates in ovarian cancer patients. In this review, we discuss the possible mechanisms by which ovarian cancer cells metastasize from the primary tumor to the omentum, the cross-talk signaling events between ovarian cancer cells and various stromal cells that play crucial roles in ovarian cancer metastasis, and the possible clinical implications of these findings in the management of this deadly, highly metastatic disease.

PASSIVE DISSEMINATION OF OVARIAN TUMOR NODULES

Detachment of ovarian cancer cells from the primary tumor and physiological movement of peritoneal fluid to the peritoneum and omentum as well as direct extension of tumor lesions to adjacent organs are widely accepted as the most common routes of metastasis of epithelial ovarian cancer\(^{(91, 107)}\). The cells follow the circulatory path of peritoneal fluid along with the respiratory force from the pelvis up the paracolic gutters along the intestinal mesentery to the right hemidiaphragm, facilitating implantation at different peritoneal organs\(^{(28, 41, 101)}\). However, this transcoelomic route of dissemination only occurs when excess fluid is present in the peritoneum; otherwise, the motion of cancer cells is restricted to the primary site\(^{(28, 152)}\). Therefore, accumulation of ascites in the abdomen of an ovarian cancer patient, which contributes significantly to morbidity owing to gastrointestinal symptoms and abdominal
discomfort, is believed to play a key role in this passive mechanism of ovarian cancer cell spread.

The etiology of ascites is not well understood, but preclinical and clinical observations demonstrated that the vascular permeability-enhancing factor vascular endothelial growth factor (VEGF) is responsible for ascites accumulation(87, 163), and what concerning ascites may be caused by blockage of lymphatic vessels(152). In most cases of ovarian cancer, ascitic fluid is composed of malignant cells, a large number of white blood cells, and a high level of lactate dehydrogenase(9, 51, 133). Detached cancer cells usually float in the ascites as multicellular spheroids(91), and implantation of the spheroids in peritoneal organs involves interaction between the cancer cells and mesothelium, which covers all organs within the peritoneal cavity, including the diaphragm, bowel serosa, omentum, and entire peritoneum(45, 85). The mesothelium is a single layer of mesothelial cells covering a basement membrane predominantly composed of collagen types I and IV, fibronectin, and laminin(83, 84, 162). Early adhesion and metastasis of ovarian cancer cells are mediated by the matrix metalloproteinase (MMP) family of proteins, whose expression is upregulated during ovarian cancer progression(49). In particular, MMP-2 enhances peritoneal adhesion of ovarian cancer cells via cleavage of fibronectin and vitronectin into small fragments, as cancer cells adhere more strongly to these fragments using fibronectin (α5β1-integrin) and vitronectin (αvβ3-integrin) receptors than to the whole extracellular matrix proteins(84). Another type IV collagenase, MMP-9, which is secreted by the tumor stroma, is also involved in the release of VEGF from tumor cells and stimulates ascites formation in ovarian cancer patients(13). Moreover, a recent study demonstrated that cancer-associated mesothelial cells facilitate initial metastatic colonization of ovarian cancer cells secreting fibronectin and providing access to the submesothelial extracellular matrix(82). In addition, upregulation of E-cadherin expression, a feature of metastatic epithelial ovarian cancer cells(149, 150), may increase adhesion of circulating ovarian cancer cells to peritoneal organs(76).
Once attached to the peritoneal surface, metastatic cancer cells proliferate and invade the mesothelium, which can be promoted by various ascitic constituents (152). For instance, lysophosphatidic acid, which is highly expressed in the ascites and plasma of ovarian cancer patients, promotes ovarian cancer motility and invasiveness (50) via induction of secretion of extracellular matrix-degrading proteinases, including MMPs (53) and urokinase plasminogen activator (126). In addition, lysophosphatidic acid activates mitogen-activated protein kinase 1, which facilitates the redistribution of focal adhesion kinase to focal adhesion complexes as well as cell motility and migration (16). On the other hand, CXCL12 in ascitic fluid binds to its receptor, CXCR4, on ovarian cancer cells, increasing the expression of β1 integrin and migratory potential of epithelial ovarian cancer cells (7). Furthermore, malignant ascites stimulates expression of CD44 by cancer cells. CD44 is the principal cell surface receptor for hyaluronic acid. Engagement of CD44 by hyaluronic acid facilitates epithelial ovarian cancer cell migration and binding to the peritoneal surface (29, 147).

Following ovarian cancer cell implantation, the resultant inflammation and injury stimulate the peritoneal cells and their associated immune and stromal cells to release cytokines such as interleukin (IL)-1, -6, and -8, which subsequently enhance tumor angiogenesis and further ascites formation via increased secretion of VEGF by cancer cells (57, 145). This creates a favorable microenvironment to support the growth of the implants. Indeed, high levels of VEGF expression in serum and ascites of ovarian cancer patients have been associated with ovarian tumor progression and poor prognosis (111). Studies also demonstrated that ovarian cancer cells preferentially metastasize to milky spots on the subdiaphragmatic surface and omentum and use the associated lymphatic vessels for spreading (12, 51). These milky spots are composed of aggregations of mesenchymal cells, mainly macrophages and lymphocytes. The spots are easily infiltrated by tumor cells and participate in intraperitoneal immune reactions (11, 140).
Lastly, microarray studies, comparative genomic hybridization, and high-resolution single-nucleotide polymorphism analysis demonstrated similar genetic alterations in primary ovarian tumors and their respective metastases, further supporting the passive mechanism of ovarian cancer metastasis described above. Therefore, transcoelomic metastasis is widely accepted as the most common route of metastasis of epithelial ovarian cancer.

HEMATOGENOUS METASTASIS OF OVARIAN CANCER

Although direct surface spread via intraperitoneal dissemination has long been thought to be the dominant mechanism of ovarian cancer metastasis, traces of evidence have prompted scientists to investigate an alternative route of metastasis. First, instead of a completely random distribution of metastases within the peritoneal cavity, investigators have consistently identified a predilection of metastases for implantation in the omentum. This prompted scientists to consider the existence of an active mechanism of metastasis of epithelial ovarian cancer to this large, apron-like fold of visceral tissue that hangs down from the stomach. In addition, at the initial diagnosis, some ovarian cancer patients have retroperitoneal or distant metastases and even submesothelial disease, which is not a common consequence of peritoneal dissemination. Finally, researchers found circulating tumor cells in blood samples obtained from ovarian cancer patients, suggesting a blood-borne route of ovarian cancer metastasis.

Recently, using a parabiosis animal model, Pradeep and colleagues demonstrated that ovarian circulating tumor cells preferentially implant and grow in the omentum and subsequently spread to other peritoneal surfaces. Specifically, they excised skin of female mice from the shoulder to the hip joint and then surgically anastomosed pairs of mice. SKOV3ip1 ovarian cancer cells injected into the peritoneal cavity or ovaries of host mice were able to hematogenously metastasize to the omentum in the guest mice. Immunofluorescent staining of
sections of the anastomosed skin for blood (CD31) and lymphatic (LYVE1) vessel markers confirmed that the paired mice in the parabiosis model shared blood but not lymphatic vessels.

To delineate the mechanisms by which ovarian cancer cells preferentially metastasize to the omentum, Pradeep and colleagues also compared gene expression profiles for SKOV3ip1 and SKOV3-OM3, a cell line derived from omental tumors in the guest mice, in three repeated parabiosis cycles(124). In a network-based analysis of the gene expression data, the authors identified the epidermal growth factor receptor/ErbB2 network as having the greatest number of differentially expressed genes. Furthermore, they found that the expression of ERBB3 and NRG1, which encode for the ERBB3 ligand neuregulin 1 (NRG1), was markedly higher in SKOV3-OM3 cells than in SKOV3ip1 cells. In line with the fact that NRG1 is known to increase cell motility potential, the expression of several mesenchymal phenotype-related genes, such as vimentin, MMP3, COL1A, HMGA2, and HGF, was higher in SKOV3-OM3 cells than in SKOV3ip1 cells, whereas the expression of negative epithelial-mesenchymal transition regulators, such as E-cadherin, SFRP1, EFNA1, and SMAD7, was lower. Lastly, silencing of tumoral ErbB3 and omental NRG1 expression via systematic delivery of small interfering RNA using a nanoliposomal platform inhibited omental metastasis in mouse models of ovarian cancer, suggesting that the ErbB3/NRG1 axis is responsible for the hematogenous omental metastasis of circulating ovarian cancer cells.

**MOLECULAR MECHANISM OF OVARIAN CANCER METASTASIS**

The physical mechanisms by which ovarian cancer cells metastasize via passive dissemination and the hematogenous route appear to differ (Fig. 1). Whereas passive dissemination consists of relatively straightforward migration, adhesion, and invasion of disseminated cancer cells into the preferential soil (omentum), metastasis via the hematogenous route requires a series of sequential interrelated steps of invasion into surrounding tissue. In metastasis of ovarian cancer cells via the hematogenous route,
intravasation, which is the invasion of cancer cells through the basal membrane into blood or lymphatic vessels, is the first step. The cancer cells then transit in the blood or lymph and undergo extravasation, which is the exit of cells from the blood or lymph vessels. Subsequently, ovarian cancer cells establish secondary tumors at the metastatic site(108, 115). Despite the differences between these two mechanisms of ovarian cancer metastasis, both are primarily cell motility-driven, involving cycles of actin polymerization, cell adhesion, and actomyosin contraction(115).

Reorganization of the actin cytoskeleton is the primary mechanism of cell motility and is driven by polymerization of actin monomers into polarized filaments called F-actin(123, 127). This polymerization is regulated by members of the Rho family of small GTPases, such as Rho, Rac, and Cdc42(166). These GTPases act as molecular switches by cycling between a guanosine diphosphate-bound inactive state and a guanosine triphosphate-bound active state in response to stimuli, controlling the formation of filopodia, lamellipodia, and stress fibers for cell movement(128). Filopodia are F-actin–rich protrusions at the front of the cell, lamellipodia are sheets of F-actin that extend over the substrate, and stress fibers are thick actin cables in the cell body. In highly invasive cancer cells, proteolytically active plasma membrane protrusions called invadopodia form to induce focal degradation of extracellular matrix components. The potential of invadopodia formation by cancer cells is often correlated with their ability to enter the vasculature(165). These different actin filament structures are responsible for the diversity of actin organization in migrating cancer cells(35, 115).

To connect actin filaments to the plasma membrane and cell-matrix adhesions, the cytoskeletal protein α-actinin forms complexes with focal adhesion proteins such as vinculin and zyxin after being phosphorylated by focal adhesion kinase and cross-linking with actomyosin stress fibers and generates traction force to be transmitted to the substrate, driving cell motion(56, 66). The components of focal adhesion complexes are regulated by Ca²⁺ influx, which can occur in the form of waves, sparks, or flickers(125). Such increases in Ca²⁺
concentration can result from Ca\(^{2+}\) entry through membrane-bound Ca\(^{2+}\)-permeable channels and/or Ca\(^{2+}\) release from intracellular endoplasmic reticulum calcium stores through ryanodine and/or inositol triphosphate receptor channels. These signals stimulate downstream effectors such as Ca\(^{2+}\)-dependent myosin light-chain kinase, which mediates myosin light-chain phosphorylation and myosin II contraction to induce coordinated dynamic formation and disassembly of cell adhesions, known as focal adhesion turnover, which facilitates cell migration(20, 69).

Using traction force microscopy, McGrail and colleagues recently showed that the tropism of ovarian cancer metastasis to the soft omentum results from not only chemical signals but also mechanical cues(99). They reported an increase in both the magnitude of traction forces and degree of polarization in human ovarian cancer cells cultured on soft substrates. Moreover, ovarian cancer cells underwent epithelial-mesenchymal transition via the Rho/ROCK signaling pathway, suggesting that this transition is a critical step in both passive dissemination and the hematogenous route of ovarian cancer metastasis.

STROMAL CONTRIBUTIONS TO OVARIAN CANCER METASTASIS

Researchers have demonstrated that both intraperitoneal dissemination and hematogenous spread of circulating tumor cells are possible mechanisms of ovarian cancer metastasis. However, what are the factors and signaling pathways that cue the metastatic events? With increasing evidence demonstrating the importance of stromal involvement in cancer progression and the ability of stroma-derived factors to confer aggressive phenotypes to ovarian cancer(151, 155), we describe below recent findings on the cross-talk signaling events between ovarian cancer cells and various stromal cells that play crucial roles in ovarian cancer metastasis (Fig. 2).

CANCER-ASSOCIATED FIBROBLASTS
Stroma is a large component of many advanced ovarian tumors, with a median relative proportion of tumor tissue of 50% (range, 7-83%) (89). Although the tumor microenvironment is composed of a complex variety of mesenchymal cells, including fibroblasts, endothelial cells, pericytes, and diverse immune cells, cancer-associated fibroblasts (CAFs) often represent the majority of stromal cells in various types of human carcinoma (117). Common upregulated CAF markers include $\alpha$-smooth muscle actin (74), stromal-derived factor $1\alpha$ (CXCL12) (136), fibroblast activation protein-$1\alpha$ (117), vimentin (96), and fibroblast-specific protein-1 (148). CAFs possess very high contractile ability, promote angiogenesis, and stimulate epithelial cell growth via production of extracellular matrix and secretion of growth factors and cytokines (104, 139). Therefore, CAF-derived tumor-promoting factors may serve as both prognostic markers and therapeutic targets.

Authors have reported on multiple signaling pathways involved in the tumor-promoting roles of CAFs, such as the well-reported CXCL12/CXCR4 axis, which plays prominent roles in induction of angiogenesis at the tumor site and increasing tumor cell proliferation and migration in different cancer systems (32, 44, 98, 113, 114). However, the investigators performed most of these studies using in vitro co-culture systems; also, previous large-scale transcriptome profiling studies, including The Cancer Genome Atlas, which identified the prognostic gene signature for high-grade serous ovarian cancer (HGSOC), used mostly bulk ovarian tumor samples (14, 25, 90). As a result, the prognostic significance of CAF-specific gene expression in ovarian cancer cells remains largely unknown.

In 2014, Leung and colleagues presented large-scale transcriptome profiling data on microdissected tumor samples obtained from HGSOC patients and identified a specific gene signature for CAFs that was associated with survival (93). In addition, they functionally characterized microfibrillar-associated protein 5, a prognostic marker of poor survival of HGSOC, and demonstrated that the protein stimulates ovarian cancer motility and metastatic
potential via the calcium-dependent focal adhesion kinase/cAMP response element-binding protein/troponin C type 1 signaling pathway. In vivo animal studies demonstrated that targeting stromal microfibrillar-associated protein 5 using small interfering RNA encapsulated in nanoparticles markedly reduced ovarian tumor growth and metastasis(93), suggesting that targeting the CAF-derived factor microfibrillar-associated protein 5 is a new treatment modality for HGSOC.

Using the same transcriptome profiling data on microdissected ovarian tumor samples, Yeung and colleagues identified versican as a key upregulated gene in CAFs that promotes the motility and invasion of ovarian cancer cells by activating the nuclear factor-κB signaling pathway and upregulating CD44, MMP-9, and hyaluronan-mediated motility receptor expression in cancer cells(168). Interestingly, the authors found that versican expression in CAFs was modulated by the activation of transforming growth factor (TGF)-β signaling in CAFs induced by TGF-β ligands secreted by ovarian cancer cells. Although TGF-β ligands have limited direct effects on ovarian cancer cells(6, 164), the cross-talk between cancer cells and CAFs via versican plays a critical role in TGF-β–stimulated ovarian cancer progression. These findings provided important implications for the development and refinement of TGF-β–targeted therapy for ovarian cancer.

Whereas CAFs are normally regarded as transformed normal fibroblasts(103) or differentiated bone marrow-derived progenitor cells recruited to the tumor site(117), other efforts at identifying factors that maintain the “CAF state” also possess important therapeutic value in controlling ovarian cancer(2). Targeting CAFs for cancer treatment is believed to have two benefits: 1) the ongoing function of CAFs is critical to the growth of nearby neoplastic cells and 2) stromal cells are more genetically stable than carcinoma cells, which can accumulate adaptive mutations during the course of therapy to acquire drug resistance(79, 81, 102).
ENDOTHELIAL CELLS

Blood vessels are fundamentally lined at the luminal side by endothelial cells. Interconnected endothelial cells form tubes that direct and maintain blood flow, are an important getaway for hematogenous metastasis of ovarian cancer(70). Tumor-induced angiogenesis is a process by which growing solid tumors produce diffusible angiogenic factors that induce host capillary endothelial cells to proliferate, migrate, and form new vessels(18, 43, 54, 55). The most well-studied angiogenesis inducer is VEGF-A, which is secreted by tumor and stromal cells, including macrophages, endothelial cells, and fibroblasts, to facilitate vascular sprouting and increases vascular permeability(62). VEGF-A binds to the VEGF receptor tyrosine kinases VEGFR1 and VEGFR2 on endothelial cells, leading to the formation of VEGFR homodimers or heterodimers and the induction of downstream signaling events(116). Moreover, VEGFR signaling can be modulated by co-receptors such as neuropilins, which may increase the half-life of the receptor complex.

Other well-known inducers of angiogenesis include the fibroblast growth factors (FGFs) and their transmembrane tyrosine kinase receptors, FGFRs(19, 58). Birrer and colleagues showed that amplification of chromosomal region 5q31-5q35.3 and FGF1 in ovarian cancer tissues is a negative prognostic indicator for advanced-stage HGSOC, as this amplification promotes tumor angiogenesis and increases ovarian cancer cell motility and survival in an autocrine manner(17). In addition, Zaid and colleagues showed that overexpression of FGFR4, one of the key receptors for FGF1, in ovarian cancer cells was highly associated with decreased patient survival(169). In addition, silencing of FGFR4 in ovarian cancer cells markedly abrogated the FGF1-activated mitogen-activated protein kinase, nuclear factor-κB, and WNT signaling pathways. Silencing of FGFR4 with FGFR4-specific small interfering RNAs and blockage of FGFR4 activation by FGFR4 trap protein effectively attenuated ovarian tumor growth in vivo(169). In comparison, Wei and colleagues identified FGF18 as an independent predictive marker for poor survival in patients with advanced-stage HGSOC(160). Functional
studies demonstrated that FGF18 stimulates ovarian cancer cell migration, invasion, and
tumorigenicity via nuclear factor-κB activation and enhances tumor angiogenesis and M2
macrophage infiltration. Their observations of increased intratumoral microvessel density and
M2 macrophage infiltration in clinical ovarian tumor samples confirmed the in vitro data and
suggested that FGF18 is a therapeutic target for ovarian cancer(160). Taken together, these
findings demonstrate that FGFs and their receptors are important regulators of ovarian tumor
progression and angiogenesis. Enhanced tumor angiogenesis induced by the FGF/FGFR
signaling axis may facilitate hematogenous metastasis of ovarian cancer.

Expression of VEGF, VEGFRs, basic FGF, and neuropilin-1 is upregulated in ovarian
cancer cells(5, 34), and microvessel density is a prognostic marker for multiple tumor types(26,
33, 86, 131), including ovarian cancer(146). Therefore, investigators have developed multiple
therapeutic agents targeting angiogenesis inducers, such as bevacizumab, a VEGF-A–targeting
monoclonal antibody, and various FGF inhibitors(27). Most clinical trials of antiangiogenic
agents have included bevacizumab. In particular, two studies (GOG218 and ICON7)
demonstrated efficacy of using bevacizumab in addition to carboplatin and paclitaxel followed by
maintenance treatment in patients with International Federation of Gynecology and Obstetrics
stage III/IV ovarian cancer with residual tumors after primary surgery(22, 121).

MACROPHAGES

The omentum, which is the most common site of ovarian cancer metastasis via the
transcoelomic and hematogenous routes, is composed mainly of adipose tissue, immune
aggregates, and structural elements surrounding capillary beds(51, 140). Although researchers
have speculated that ovarian cancer cells preferentially localize to the omentum owing to 1) a
lack of a basement membrane and few mesothelial cells on milky spot surfaces which facilitate
cancer cell invasion, and 2) participation of the omentum in fluid drainage from the peritoneal
cavity, which may passively increase the chances of cancer cell attachment to the
omentum(140), rapid localization of ovarian cancer cells to the omentum within minutes after intraperitoneal injection of ovarian cancer cells(68, 88) suggested that an active component is involved in cancer cell homing and invasion(40, 68, 88, 157). In comparing lodging of a panel of ovarian cancer cells on milky spot-containing (omentum and splenoportal fat) and milky spot-deficient (uterine fat, gonadal fat, and mesentery) peritoneal adipose tissue, Clark and colleagues clearly demonstrated that the milky spots dramatically enhanced early cancer cell lodging on the omentum(40).

Milky spots are cellular aggregations of mesenchymal cells, composed mainly of macrophages and lymphocytes(140). To identify the key components involved in early cancer cell lodging, Clark and colleagues injected ovarian cancer cells into various immunodeficient mice, including athymic nude, Rag1, Igh6, and BN XID mice(40). The results demonstrated that ovarian cancer cell colonization was not affected by deficiency or absence of T, B, and/or natural killer cells in these mouse strains(40). Because macrophages are frequently observed in milky spots(11, 141, 142, 161) and stimulation of omental macrophages promotes tumor growth on the omentum(105), macrophages may contribute to the rapid and specific colonization of ovarian cancer cells on omental milky spots.

Authors have reported robust local and systemic host inflammatory responses accompanying ovarian tumor progression, particularly during tumor dissemination(71, 132, 158). To determine the cell types responsible for the ovarian tumor-promoting effect of enhanced inflammatory responses, Robinson-Smith and colleagues depleted specific innate immune cell populations from the peritoneal cavity in animal models(132). The results demonstrated that neither neutrophil nor natural killer cell depletion significantly affected either ovarian tumor growth or peritoneal metastasis. In contrast, macrophage depletion had a profound suppressive effect on both primary tumor development and tumor progression(132). They reported that the reduced tumor dissemination in macrophage-depleted mice was not solely a result of decreased primary tumor growth, as they also observed this effect in an experimental metastasis
model(132). Possible tumor-promoting mechanisms of macrophages include increasing the vascularity of tumors, destruction of the extracellular matrix, and release of tumor-stimulating factors(132). Indeed, the vasculature in milky spots of the omentum is CD105-positive and contains vascular sprouts, indicating active angiogenesis(61). Also, researchers observed reduced VEGF expression in macrophage-depleted mice(132). Because VEGF production induces ascites production, tumor angiogenesis, and metastasis(64, 100, 106, 170), macrophages and VEGF are logical therapeutic targets for ovarian cancer. Regarding extracellular matrix remodeling, investigators showed that macrophage-derived MMP-9 contributed to ovarian tumor angiogenesis and human ovarian cancer cell growth(52, 75). Furthermore, Hagemann and colleagues demonstrated that macrophages induce invasiveness of epithelial cancer cells via nuclear factor-κB and c-Jun N-terminal kinase signaling(67). Authors also have reported on the relationship between ovarian tumor progression and other ovarian tumor macrophage-derived proteins, including survival factors such as IL-6, IL-8, and VEGF-C, which promote lymphatic metastasis(137, 158).

**ADIPOCYTES**

Ovarian cancer metastasis is not a random event, as 80% of patients with serous ovarian carcinoma present with omental metastases(109). Because the omentum is a fat pad primarily composed of adipocytes, these cells may contribute to the predilection of the metastatic cascade for the omentum. In fact, researchers introduced the term cancer-associated adipocytes (CAAs) in 2010 for adipocytes in close vicinity to cancer cells(47). Studies demonstrated that CAAs promote the growth of cancer cells(46, 97, 156). Nowicka and colleagues showed that the origin of CAAs(42)—adipose-derived mesenchymal stem cells in the omentum—promote proliferation, migration, and chemoresistance of ovarian cancer cells(112). In addition, several adipokines secreted by adipose tissue, including tumor necrosis factor-α, IL-6, IL-8, and monocyte chemoattractant protein-1, are implicated to play a role in
tumor progression(65, 77, 134). Furthermore, several reports suggested that cancer cells induce metabolic changes in adipocytes and stimulate delipidation of mature differentiated adipocytes to release their lipids and promote tumor progression(4, 46, 109).

In a study using adipocyte-conditioned medium and primary human omental adipocytes, Nieman and colleagues showed that adipocytes promote homing, migration, and invasion of ovarian cancer cells, possibly by soluble factors(109). A cytokine array and antibody-mediated inhibition identified that adipocyte-secreted IL-6 and IL-8 promoted the early steps of ovarian cancer metastasis to the omentum, including homing and adhesion. Furthermore, in co-culturing adipocytes loaded with fluorescently labeled lipids and SKOV3ip1 ovarian cancer cells, the authors demonstrated direct transfer of fatty acids from adipocytes to ovarian cancer cells. In addition, co-cultivation of ovarian cancer cells with adipocytes activated hormone-sensitive lipase-mediated lipolysis in adipocytes and β-oxidation in cancer cells, suggesting that adipocytes support ovarian cancer cell growth by providing energy-dense lipids to and stimulating mitochondrion metabolism in cancer cells. Consistent with the role of lipids as an energy source for ovarian cancer cell growth, Clark and colleagues observed an inverse relationship between the metastatic burden and adipocyte content in the omentum in in vivo time-course studies(40). Finally, upregulation of fatty acid-binding protein 4 expression in omental metastases and a drastically reduced tumor burden in a fatty acid-binding protein 4-deficient mouse model indicated that this protein plays a key role in ovarian cancer metastasis(109).

However, because obesity is not significantly associated with ovarian cancer incidence and mortality(23, 129), the tumor-promoting effect of adipocytes is believed to be a local effect mediated by direct contact of cancer-associated adipocytes with ovarian cancer cells and/or paracrine signaling, leading to remodeling of the tumor microenvironment(110). Recent findings demonstrated that treatment with metformin inhibited adipocyte-mediated ovarian cancer cell proliferation, migration, and adipogenesis via regulation of transcription factors (CCAAT-
enhancer binding protein alpha, CCAAT-enhancer binding protein beta, and sterol regulatory element-binding protein-1) and activation of 5' AMP-activated protein kinase(154). These findings demonstrated that metformin is a potential therapeutic agent for ovarian cancer via targeting the interaction between adipocytes and ovarian cancer cells.

CLINICAL IMPLICATIONS OF STROMAL INVOLVEMENT IN OVARIAN CANCER METASTASIS

Researchers have demonstrated the involvement of four major stromal cell types—CAFs, endothelial cells, cancer-associated macrophages, and CAAs—in ovarian cancer progression and metastasis in multiple studies. Despite increasing understanding of stromal involvement in ovarian cancer metastasis, therapeutic regimens that target the ovarian cancer stromal components are still relatively underdeveloped. Because therapeutic approaches that target both cancer cells and the tumor-supporting microenvironment may be more effective than targeting cancer cells alone, investigators are actively pursuing approaches that can suppress tumor metastasis and progression by targeting tumor stroma. To that end, researchers have begun to evaluate the potential of inhibiting tumor progression via stromal ablation. However, multiple studies demonstrated that ablation of CAFs in tumors actually promoted tumor metastasis and progression and reduced survival durations in mice(118, 130). One of the possible reasons for this is that global depletion of CAFs may drastically change the architecture of tumor tissue as well as the cell-cell interactions in the tumor microenvironment; these changes may actually promote tumor progression and reduce survival durations. Therefore, instead of global stromal ablation, researchers have proposed that CAF reprogramming by targeting specific CAF-derived tumor-promoting factors may be a more effective therapeutic approach for cancer(167). Identification and validation of metastasis-promoting genes are crucial for achieving the goal of suppressing tumor metastasis by targeting stroma-derived factors. However, identification of these genes and evaluation of their functional roles in a
systemic approach remains challenging because of complex interactions among multiple cell
types in tumor tissues. Because bulk tumor samples often contain various numbers of stromal
components, normalization and identification of stromal-specific tumor-promoting factors may be
problematic. To address this, researchers have performed transcriptome profiling and next-
generation sequencing using microdissected tumors. For example, large-scale expression
profiling of ovarian CAFs(93, 168) and endothelial cells(21) has identified stromal-specific
factors that are highly correlated with clinical outcomes and disease progression in ovarian
cancer patients. In addition to generation of highly cell-type-specific expression profiles using
laser microdissection, computational scientists have used these expression profiles to identify
cross-talk networks of cancer and stromal cells and potential signaling pathways involved in
disease progression(37). A combination of these approaches would allow for identification of
stroma-derived metastasis-promoting factors for the development of targeted therapies for
ovarian cancer.

Despite the challenges faced by researchers and clinicians during the development of
stroma-targeting therapies, great research effort has been put into uncovering the mechanisms
involved in stroma-mediated tumor growth, metastasis and drug resistance. With increasing
understanding in stroma-tumor interactions, new therapeutic targets from the tumor stroma are
being identified. For example, recently Chi and colleagues reported that tumor associated
macrophages and fibroblasts response to cisplatin and 5-fluorouracil treatment by activating the
CCAAT/enhancer binding protein delta (CEBPD) which subsequently transcriptionally
upregulates pentraxin 3 (PTX3). Using a PTX3 peptide inhibitor, RI37, researchers were able to
suppress cancer progression and metastasis(36). Also targeting tumor associated
macrophages, Cieslewicz and colleagues described the use of proapoptotic peptides that
targets M2 macrophages in mouse. Peptide sequence that preferentially binds to murine M2
cells was fused with a proapoptotic peptide and then delivered into colon cancer bearing mice
via tail vein injection. Animals treated with M2 macrophage targeting fusion peptide had
significantly delayed mortality and selective reduction in M2-like tumor associate macrophage population(39). Endothelial cell has long been the target for antiangiogenic therapies. In addition to bevacizumab, a VEGF-targeting monoclonal antibody we mentioned above, the therapeutic efficacy on ovarian cancer of multiple antiangiogenic small-molecule tyrosine kinase inhibitors (TKIs) have been evaluated in phase II and III clinical trials(60). While Sunitinib, which targets VEGFR, PDGFR, RET, FLT3, c-kit and colony-stimulating factor 1(38), demonstrated modest activity in ovarian cancer(15, 24, 92), Pazopanib, which targets VEGFR1/2/3, PDGFR and c-kit (138) demonstrated significant improvement in patient progression-free survival (PFS) when compared to placebo (HR=0.77, p=0.0021) in the AGO-OVAR 16 trial, which 940 FIGO stage II-IV ovarian cancer patients were enrolled(48). Cediranid, a TKI that binds VEGFR, PDGFR and c-kit(159), has been tested in a recent phase II study in combination with olaparib, a PARP inhibitor. 90 ovarian cancer patients were randomized to receive either PARP inhibitor alone or PARP inhibitor in combination with cediranid. Clinical trial results showed that median PFS is significantly longer for the combination arm when compared to the PARP inhibitor single agent arm (PFS 17.7 months vs 9.0 months, p=0.005)(95). These research findings suggested that in addition to single agent therapies that target the stromal microenvironment, stroma-targeting agents could be used in combination with small molecule drugs (e.g. PARP inhibitors) or cytotoxic chemoagents (e.g. cisplatin and paclitaxel) to achieve a higher therapeutic efficacy.

With increasing understandings in the molecular mechanisms by which stromal cells support ovarian tumor progression and metastasis, as well as chemoresistance, new therapeutic targets are being discovered and validated. While the implementation of new therapeutic agents that target newly discovered proteins or signaling pathways could be costly and time consuming, drug repositioning by placing noncancer therapies into new therapeutic niches offers an alternative approach for the discovery of targeted therapeutic agents(135). With the aid of modern bioinformatics tools and analysis platforms, FDA approved drugs with favorable toxicity profile can be efficiently prioritized and integrated into existing drug screening
pipelines (59, 80). Though further clinical and epidemiological studies will be required, several repositioned drugs including PPAR ligands and ritonavir have demonstrated tumor suppressive effect in ovarian cancer (8). While current drug repositioning efforts have mostly focused on targeting cancer cells, we believe that repositioned drugs can also be used to target stromal cells and their crosstalks with cancer cells. Taking tumor-stroma interaction into account when identifying repositioned drugs would facilitate the identification of effective therapeutic agents.

CONCLUSIONS

Ovarian cancer metastasis is a highly regulated process that involves interaction between cancer cells that either disseminate from the primary tumor into the peritoneal cavity or migrate via the bloodstream and the omentum, which is the preferred metastatic site. In this review, we described the two major routes of ovarian cancer metastasis and the molecular mechanisms involved in conferring motility to metastatic ovarian cancer cells. We also highlighted the involvement and roles of different stromal cell types, including CAFs, endothelial cells, cancer-associated macrophages, and CAAs, in metastasis. With increasing knowledge of the overall ovarian cancer pathogenesis and mechanisms of ovarian cancer metastasis, new approaches to the management of metastatic disease may be developed. More importantly, with researchers further delineating the roles of different stromal components in the tumor microenvironment in ovarian cancer progression and metastasis, an increasingly comprehensive landscape of the disease is being generated. With authors reporting on newly identified and validated prognostic markers and therapeutic targets, novel effective treatment regimens are being developed.

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Figure 1: **Two mechanisms of ovarian cancer metastasis.** As ovarian cancer progress, cancer metastasis could occur via passive dissemination of cancer cells and hematogenous metastasis. In this figure, the two mechanisms are illustrated and the protein factors involved are listed. For the passive dissemination model, ovarian cancer cells detached from the primary tumor are carried by peritoneal fluid and ascites. At the metastatic site, cancer cells adhere to the surface of the organ, establishing tumor nodules. For hematogenous metastasis, ovarian cancer cells at the primary tumor site invade through the basal membrane of blood vessels and enter the circulation via intravasation. Subsequently, cancer cells leave the circulation at the metastatic site via extravasation establishing metastatic tumor nodules.

Figure 2: **Cellular crosstalk and signaling events in ovarian cancer metastasis.** Ovarian cancer progression and metastasis from the primary site to the omental metastatic tumor site is facilitated by the interaction between cancer cells and various stromal components. At the primary tumor site (ovary), ovarian cancer cell motility enhanced by cancer associated fibroblasts (CAFs)-derived secretory proteins. Versican (VCAN) expression is upregulated in CAFs via activation of TGFβ signaling. VCAN then activates NF-κB signaling in ovarian cancer cells and promotes cancer cell motility and invasion potential via upregulation of CD44, hyaluronan-mediated motility receptor (HMMR) and matrix metallopeptidase 9 (MMP9). At the same time, CAFs-derived microfibrillar associated protein 5 (MFAP5) binds to the αvβ3 integrin receptors on ovarian cancer cell surface, activating the calcium-dependent FAK/CREB/TNNC1 signaling pathways. Activation of such signaling stimulates the reorganization of F-actin cytoskeleton and enhances the generation of cell traction force, thereby increasing the migration potential of ovarian cancer cells. In addition, chemokine (C-X-C motif) ligand 12 (CXCL12) is a well reported tumor-promoting factor from CAFs. CXCL12 binds to its receptor, chemokine (C-
X-C motif) receptor type 4 (CXCR4) on cancer cells to increase tumor cell proliferation and migration and CXCR4 on endothelial cells to induce tumor angiogenesis. On the other hand, cancer cell-derived fibroblast growth factor 1 (FGF1) and fibroblast growth factor 18 (FGF18), in addition to promoting tumor progression via an autocrine mechanism by binding to its receptor, fibroblast growth factor receptor 4 (FGFR4), on cancer cells, also promotes tumor angiogenesis through a paracrine mechanism by binding to FGFR4 on endothelial cells. Together with cancer cell-derived vascular endothelial growth factor (VEGF), these pro-angiogenic factors promote the establishment of tumor microvessels which could subsequently facilitate hematogenous metastasis of ovarian cancer cells. In the omentum, a common metastatic site for ovarian cancer, secretory factors from adipocytes and macrophages contribute to a favorable microenvironment for tumor development. Interactions between adipocytes and cancer cells in the omentum promote tumor progression. In addition to providing energy source to cancer cells, adipocytes also secrete multiple cytokines including TNF-α, IL-6 and IL-8 which act on ovarian cancer cells and accelerate tumor progression. Macrophages are highly abundant within the omentum, especially at the milky spots. Macrophages actively produce and secrete TNF-α and VEGF into the tumor microenvironment, which promotes tumor progression and tumor angiogenesis respectively.
Passive Dissemination

Accumulation of ascites within the peritoneal cavity

Dissemination of cancer cells from the primary tumor into the peritoneal cavity

Adhesion of cancer cells to the surface of metastatic sites

Enhanced cancer cell motility and invasiveness facilitates the establishment of tumor at the metastatic sites

Metastatic tumors generate new supportive microenvironment

Facilitated by-
- VEGF
- Blocking of lymphatic vessels
- MMP2
- MMP9
- Fibronectin
- Vimentin
- E-Cadherin
- MMPs
- β1 Integrin
- UPA
- LPA
- CD44
- FAK
- Ca²⁺
- CXCL12
- MLCK
- IL-1
- IL-8
- IL-6
- VEGF

Hematogenous metastasis

E-Cadherin

NRG1
Vimentin
MMP3
Collagen type I
HMGA2
HGF

SFRP1
EFNA1
SMAaT

Epithelial-mesenchymal transition (EMT) of cancer cells

Cancer cells enter circulation via intravasation at primary site

Cancer cells leave circulation via extravasation at metastatic site

Establishment of metastatic tumors