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Influence of Aberrant Epigenetic Changes and the Tumor Microenvironment in Ovarian Cancer Metastasis

Diego Aviles[1] • David Warshal[1] • Michelle Buchbinder[2] • Olga Ostrovsky[3]

[1]Department of Gynecologic Oncology, MD Anderson Cancer Center at Cooper, Cooper University Health Case, Camden, NJ, USA; [2]Cooper Medical School of Rowan University, Camden, NJ, USA; [3]Department of Surgery, Division of Surgical Research, Cooper University Health Care, Camden, NJ, USA

Author for correspondence: Olga Ostrovsky, Department of Surgery, Division of Surgical Research, Cooper University Health Care, Camden, NJ, USA. Email: ostrovsky-olga@CooperHealth.edu

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Abstract: Metastasis in ovarian cancer is a primary driver of poor outcomes for patients because of its association with chemoresistance and low five-year survival rates. Epigenetic changes to gene expression in cancer cells are key factors that contribute to the high rates of metastasis and chemoresistance. However, ovarian cancer cells do not act alone. Once the cancer spreads to the omentum and peritoneum, it hijacks intercellular communication systems to transform neighboring cells within the tumor microenvironment into potent engines that produce critical growth factors that facilitate metastasis, chemoresistance, immune evasion,

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and invasion. By reversing these aberrant epigenetic modifications in cancer cells and the tumor microenvironment, novel epigenetic therapies can specifically target cancer cells while sparing healthy cells, minimizing toxicity to normal tissues. When combining these pharmaceutical agents with standard chemotherapy, metastasis and chemoresistance can be suppressed, making ovarian cancer cells newly susceptible to current cytotoxic treatments, and providing patients with hope for a cure.

Keywords: ascites in ovarian cancer metastasis; epigenetics in ovarian cancer; ovarian cancer metastasis; site of origin of ovarian cancer; tumor microenvironment in ovarian cancer

INTRODUCTION

Ovarian cancer is a dangerous disease that affects approximately 1.3% of women in their lifetime and remains the deadliest cancer that originates in the female reproductive tract (1). According to the Surveillance, Epidemiology, and End Results database (2), 2021 saw about 21,410 new cases of ovarian cancer with 13,770 deaths (2). While ovarian cancer is initially responsive to first-line treatments such as surgery and chemotherapy, it is particularly lethal because it frequently relapses within the first three years (3). The National Cancer Institute defines metastasis as the spread of cancer cells from the place where they first formed to another part of the body (4). Since this malignancy is so mobile and resistant to therapies, there is a critical need for the development of new treatments that specifically target this cancer's metastatic potential so that more patients can be cured. In this chapter we focus on the pathophysiology of ovarian cancer metastasis while exploring how ovarian cancer cells modify the omental microenvironment to support its own growth and spread. We also discuss novel treatment strategies that target this malignancy's metastatic properties.

When diagnosing ovarian cancer, a four-stage system classifies the extent of metastatic disease (Figure 1), with stage I referring to a cancer that is confined to the ovary and stage IV, the most advanced, characterizing cancer that has spread to distant organs such as the lung or liver. Currently, clinicians are unable to reliably diagnose ovarian cancer at its earliest stages, causing patients to present once the cancer has spread and therefore missing a precious chance to potentially cure. Patients with ovarian cancer confined to the ovary, or stage I disease, have a five-year survival that is nearly 90% (1). While the prognosis is favorable for early disease, also known as stage I and II, survival rates plummet to under 30% once the cancer has spread to become stage III or IV (Figure 1) (1). Numerous studies have tried to develop screening programs for ovarian cancer with only minimal success. In the United Kingdom, researchers conducted a randomized controlled study in which they deployed a screening program for ovarian cancer that consisted of ultrasound and blood serum markers (5). Unfortunately, the study identified no survival benefit for women who participated in the screening program when compared to women who received no screening (5). Therefore, ovarian cancer screening is currently not recommended (5). However, the development of new screening and diagnostic tests provides tantalizing hope that ovarian cancer

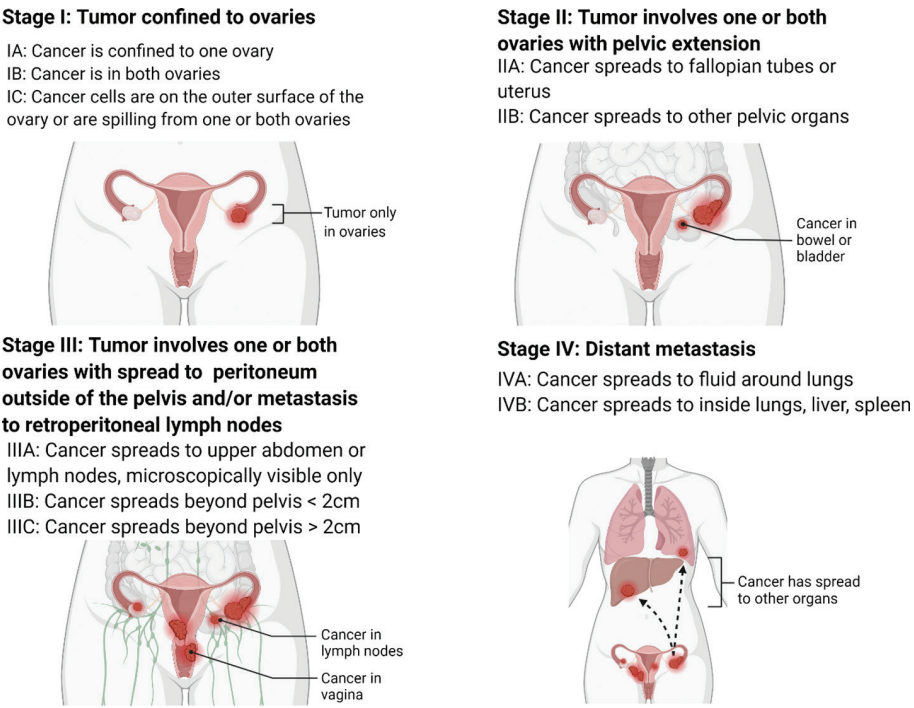

Figure 1. Stages of ovarian cancer. Adapted from "Ovarian Cancer Staging," by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates. Created with BioRender.com. Used under BioRender's Academic License Terms.

can be caught at less metastatic and chemoresistant stages, giving patients a greater opportunity for a cure.

The current standard treatment for ovarian cancer is cytoreductive surgery, a heroic attempt at complete removal of the disease, followed by chemotherapy (6). Surgery alone is often not curative due to the persistence of microscopic, metastatic, residual disease (6). With the addition of chemotherapy, the potential for a cure is increased through the attempted eradication of residual microscopic disease that cannot be seen during surgery (7). Despite these intensive therapeutic options, as many as 70% of patients experience recurrence of their cancer within 3 years because of metastasis and chemoresistance, ultimately succumbing to the cancer's relentless march (8). Given the grim prognosis of advanced ovarian cancer, patients need new treatments that effectively target metastasis and chemoresistance to provide new hope for a cure.

As new modern therapies are developed, epigenetic therapies are promising in the treatment of tumor metastasis. Epigenetic therapy can directly target cells with aberrant epigenetic modifications; so, cancer cells and its associated tumor microenvironment can be specifically targeted while sparing healthy tissue from toxic side effects (6). Epigenetics, the study of the alteration of gene expression without

associated changes to the DNA sequence, involves methylation or acetylation of DNA by specific enzymes that alter gene transcription (6). Ovarian cancer cells develop these aberrant epigenetic modifications that are preserved during cell division and help these cells to gain invasive, chemoresistant, and metastatic properties. Even more importantly, ovarian cancer cells are unable to achieve their full potential on their own; so, they trigger epigenetic changes in the neighboring cells within the tumor microenvironment, converting these once normal cells into engines that drive the metastasis of the malignant cells (9). With the advent of new epigenetic treatments, we can combine them with current chemotherapies to specifically reverse aberrant epigenetic changes in cancer cells and the tumor microenvironment while sparing healthy normal tissues and therefore avoiding side effects (9).

BACKGROUND INFORMATION ON OVARIAN CANCER

The term *ovarian cancer* refers to a morphologically, histologically, and genetically diverse spectrum of malignant neoplasms. Approximately 80% of ovarian cancer cases involve epithelial neoplasms, most of which are high-grade serous neoplasms (10). Other types of epithelial ovarian cancers include endometrioid carcinoma, and clear cell carcinoma. While other types of malignant neoplasms of the ovary exist, such as germ cell tumors that arise from cells that typically develop into gametes and sex-cord stromal tumors that arise from stromal tissue within the ovary, this chapter focuses on epithelial ovarian cancers since they are the most common malignancy of the ovary (10).

One common way of organizing these epithelial ovarian cancers is to divide them into groups based on their clinical behavior (Figure 2). Type I ovarian cancers include low-grade malignancies that are typically confined to one ovary and have a favorable prognosis (10). In contrast, type II epithelial ovarian cancers include high-grade serous carcinoma, clear cell carcinoma, and endometroid carcinoma—diseases which are more aggressive, presenting at advanced stages and having a worse prognosis (10). While this distinction fails to capture the complete breadth of unique behaviors and genetic differences among these ovarian cancer subtypes, it still helpfully provides a framework for discussing similar methods of tumor spread. Within this chapter, type II ovarian cancers, particularly high-grade serous carcinoma, will dominate the discussion since it is the most common and best understood histology of epithelial ovarian cancers.

Site of origin of ovarian cancer: does it actually start in the ovary?

Another important point to address is the site of origin for ovarian cancer. For many years the precursor lesion for ovarian cancer was unknown, with scientists assuming that most ovarian cancers arose from the epithelial surface of the ovary (11). With studies observing a decreased rate of ovarian cancer in patients with decreased lifetime ovulation from factors such as pregnancy and oral contraceptive pills, Fathalla proposed in 1971 the incessant ovulation theory to explain how a combination of inflammation and stress at the ovarian surface caused by monthly ovulation could trigger the development of ovarian cancer (12). One issue that

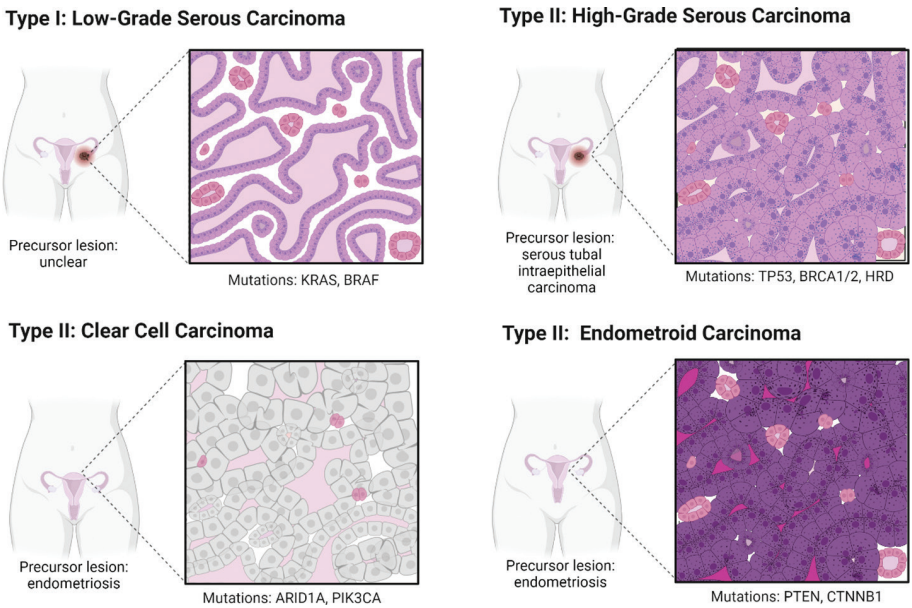

Figure 2. Common types of epithelial ovarian cancer. Epithelial ovarian cancers are classified into type I and type II. Type I cancers are described as low grade and chromosomally stable with a lower likelihood of metastasis and higher cure rates. Type II cancers are described as high grade and chromosomally unstable with a higher likelihood of metastasis and lower cure rates. Adapted from "Ovarian Carcinoma Histology," by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates. Created with BioRender.com. Used under BioRender's Academic License Terms.

always limited this theory is that ovarian cancers often resemble cells of Müllerian origin which is unusual because the epithelial surface of the ovary arises from coelomic origin (13). To explain this contradiction, researchers subsequently discovered that the precursor lesion to serous ovarian cancer develops in the fallopian tube and then is shed onto the ovary and directly into the peritoneal cavity (14). Given that the site of origin is likely shared between fallopian tube, ovarian, and peritoneal carcinoma and that these malignancies behave similarly and have similar responses to treatment, they are all considered the same entity for diagnostic and treatment purposes (10).

Clinical presentation of ovarian cancer

Traditionally, ovarian cancer has been recognized by the nickname *the silent killer* because patients are diagnosed at an advanced stage without much warning (15). When patients develop ovarian cancer, they present with a well-recognized constellation of symptoms that include the gastrointestinal and genitourinary tracts (15). Specifically, early satiety, abdominal bloating, increased abdominal girth, abdominal and pelvic pain, urinary frequency, and urinary urgency are hallmark symptoms of this disease process (15). While many of these symptoms are

frequently experienced by patients without ovarian cancer, when patients have a combination of 6 of these symptoms occurring for less than 1 year and for more than 12 times monthly, researchers have shown that patients within this subgroup have an elevated risk of ovarian cancer with a sensitivity of 79.5% for advanced-stage disease and a specificity of 90% for women that are older than 50 years (15). By increasing awareness about these symptoms, patients and clinicians can diagnose ovarian cancer at an earlier stage and potentially improve clinical outcomes.

OVARIAN CANCER METASTASIS

Compared to other malignancies, ovarian cancer is unique in the methods that it employs for metastasis. Classically, malignancies metastasize through hematogenous spread, invading into blood vessels and traveling to distant organs such as the brain, bones, and the liver (16). Epithelial ovarian cancer, however, is unique, with tumor cells detaching from the primary site and passively traveling to the omentum and peritoneum through the natural movements of peritoneal fluid and ascites (17). However, ovarian cancer does not act alone. One of its defining characteristics is its ability to manipulate nearby normal cells, known as the tumor microenvironment, and to transform these typically benign cells into agents that produce many of the tools that the cancer needs to grow, invade, and metastasize (9). With these patterns of behavior, ovarian cancer overwhelms a patient's normal physiologic processes, ultimately causing fatal obstruction of intraabdominal organs (10).

The role of epigenetics in ovarian cancer

Ovarian cancer develops key genetic mutations that allow cells to grow uncontrollably, invade into nearby tissues, and to evade immune system defenses (6). However, recent discoveries show that epigenetic modifications are also important for ovarian cancer cell to propagate and metastasize. Instead of waiting for new errors in the genetic sequence to occur during cell division, ovarian cancer cells manipulate the expression of critical genes through two major processes: DNA methylation, and histone deacetylation (9). DNA methylation is the silencing of gene expression by adding a methyl group to clusters of CpG nucleotides (6). While this process occurs normally in healthy cells, cancer abuses this system, leading to hypermethylation and subsequent suppression of gene expression. One important example of hypermethylation is seen with the silencing of BRCA genes (6). BRCA genes serve as tumor suppressors by repairing double-stranded DNA breaks. However, when silenced, patients have increased rates of numerous malignancies including breast and ovarian cancer (6). Histone deacetylation is another common epigenetic process in which acetyl groups are removed from histones resulting in silencing of gene transcription (6). Typically, DNA is stored as chromatin that is bound around histone proteins when it is not actively being transcribed (6). By removing acetyl groups, the DNA becomes tightly wound; and expression of the related genes is decreased (6). While this process is not as well understood, it has been associated with an increased risk of high-grade serous and

clear cell ovarian carcinoma and conveys a poor prognosis (18). Pharmaceutical agents are in development that target these and other epigenetic methods of modifications. By using these drugs alone or in combination with other treatments, we can reverse the aberrant changes present in cancer cells and inhibit the cancer's ability to grow, invade, metastasize, and resist chemotherapy.

Epigenetic aberrations in the tumor microenvironment

While epigenetic therapies directly affect ovarian cancer cells, the tumor microenvironment is another crucial target that can be treated with these novel agents. Ovarian cancer cells take advantage of intercellular communication systems to influence the gene expression of cells within the omental tumor microenvironment, causing aberrant epigenetic modifications that transform normal cells into accomplices that aid cancer progression (19). Omental stem cells become powerful allies for the malignancy, responding to the epigenetic changes by differentiating into different cell types such as fibroblasts that fulfill a complimentary role, producing factors and resources that cancer cells themselves cannot produce that are important for the metastatic process. Additionally, as demonstrated in Figure 3, cancer cells interfere with immune cells such as macrophages, T-cells, and dendritic cells, instructing these cells to release different growth factors and cytokines that shield the cancer cells from the patient's own immune defenses (19). One of the issues with epigenetic therapies is that they cause significant side effects at higher dosages that were initially intended to kill cancer cells (20). Alternatively, lower dosages may reverse the aberrant modifications found in the

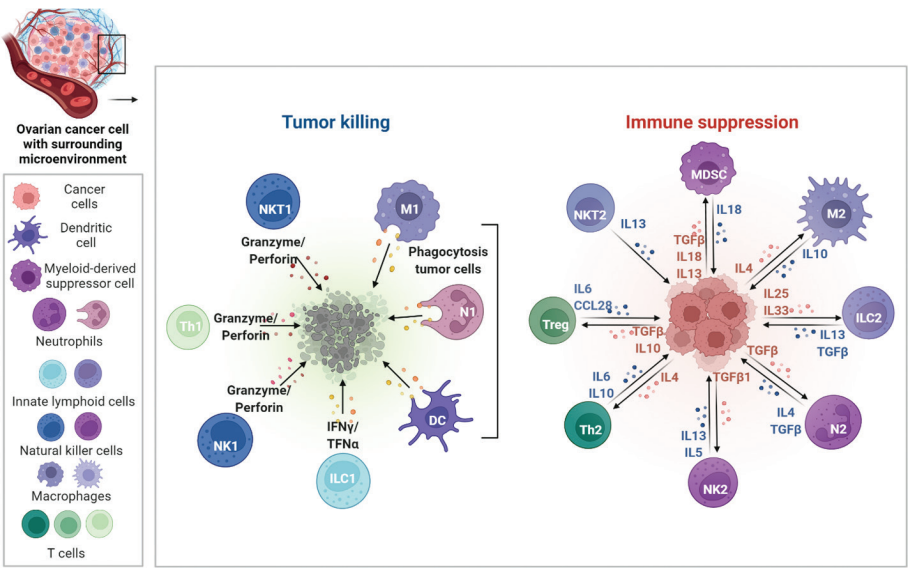

Figure 3. Components of omental tumor microenvironment. Adapted from "Tumor Microenvironment with Callout (Layout)," by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates. Used under BioRender's Academic License Terms.

tumor microenvironment by reversing the pathologic changes in the omentum and peritoneum and allowing the immune system, together with standard chemotherapies, to eradicate the cancer specifically, avoiding negative effects to normal tissues. Epigenetic treatments are exciting and, when combined with standard chemotherapy, may provide new hope for patients who seek disease control and a cure.

The epithelial-to-mesenchymal transition: detaching from the primary tumor

The early stages of metastasis are highlighted by the transformation of ovarian cancer cells from an epithelial type to a mesenchymal phenotype. Known as the epithelial-to-mesenchymal transition, cancer cells lose the properties that anchor them in place, allowing them to dislodge from the primary tumor into the adjacent peritoneal environment. Typically, epithelial cells line the surface of organs and are held in place by integral membrane proteins such as cadherins (21). Cadherin proteins belong to a superfamily of glycoproteins that facilitate intercellular adhesions through calcium-mediated mechanisms (21). With epithelial cells relying on cadherin-mediated adhesion, the cadherins interact with intracellular cell adhesion molecules known as catenins to anchor the cells (22). As benign epithelial cells transform to malignant cells, they lose either function or expression of catenins and cadherins that then compromises a cell's adhesive capability and stimulates the cancer's invasive potential (21). In ovarian cancer cells, β-catenin levels function as anchoring points that allow the malignant cells to attach to omental and peritoneal surfaces (23). For epithelial cells, E-cadherin is the specific cadherin that connects to microfilaments within the cytoplasm through alpha- and beta-catenin (23). Studies have shown that metastatic cancers have exceedingly low or even undetectable levels of E-cadherin (21). Through a phenomenon known as a "cadherin switch," ovarian cancer cells exchange E-cadherin for other cadherins such as P- and N-cadherin that are associated with a mesenchymal phenotype, allowing the malignant cells to exfoliate from the tumor and attach to mesothelial surfaces such as the omentum and peritoneum (17). In considering possible treatment options, we can target cadherins that are present on malignant cells potentially by using monoclonal antibody as a way to direct the immune system specifically against malignant cells while sparing healthy tissues.

The importance of spheroid formation in ovarian cancer metastasis

Once ovarian cancer cells break free from the primary tumor, they will then aggregate together to form a spheroid structure (9). By achieving this three-dimensional formation, cancer cells gain numerous survival advantages. As demonstrated in laboratory models, ovarian cancer spheroids secrete more growth factors than single cells in single two-dimensional layers (24). Additionally, cells in spheroids develop chemoresistance that limits the effectiveness of current treatments (24). Studies have shown that ovarian cancer spheroids shield malignant cells from apoptosis that is triggered by cytotoxic treatments such as paclitaxel and radiation (25, 26). Finally, the three-dimensional structure creates areas of necrosis and

hypoxia that stimulate survival mechanisms and differentiation, driving cells in the interior to develop stem cell-like properties that facilitate growth and invasion (27). The typical first-line treatments of surgery and intravenous chemotherapy do not address these free-floating spheroids in the abdomen which contributes to the high recurrence rate for this disease. Novel treatments are urgently needed to counteract three-dimensional spheroids to improve patient outcomes.

In developing new therapies, one promising idea that researchers have explored is the utilization of intraperitoneal chemotherapy. In the 2000s the implementation of intraperitoneal chemotherapy was heralded as promising treatment that could effectively increase the overall survival of patients with ovarian cancer (28). With this treatment, chemotherapy was administered to patients under hyperthermic conditions directly into the peritoneal cavity to increase the penetration of the chemotherapy into the surface of the peritoneal surfaces and to directly attack floating cancer cells (29). Additionally, hyperthermia stimulates the immune response by inducing apoptosis, thus activating heat-shock proteins that function as receptors that attract natural killer cells (29). However, further studies questioned the efficacy of the intraperitoneal chemotherapy; in the setting of increased morbidity from the treatment as well as decreased compliance with the regimen related to toxicities, this intervention was largely discontinued (29). One promising avenue that needs to be explored is the combination of epigenetic drugs with intraperitoneal chemotherapy. By reverting the aberrant epigenetic modifications on ovarian cancer cells in spheroids, the chemoresistance could be reversed, decreasing recurrence rates caused by metastasis and improving survival rates for patients.

The role of ascites in metastasis

Regarding the development of ascites in ovarian cancer patients, it is currently unclear whether ascites is already present at initial time of metastasis, facilitating spread, or if it is a consequence of a large quantity of intraperitoneal disease (30). The etiology of ascites formation is thought to be multifactorial, with cancer cells potentially disrupting lymphatic drainage by invading lymphatic channels. Additionally, ovarian cancer cells secrete vascular endothelial growth factor which increases vascular permeability and facilitates ascites formation. In recent years, bevacizumab, a monoclonal antibody that inhibits vascular endothelial growth factor, has proven beneficial for targeted treatment of patient with ovarian cancer especially for the control of ascites (31).

Implantation at the metastatic site

Besides the fallopian tubes and ovary, the omentum and peritoneum are the most common areas of metastasis (17). The peritoneum is a membrane consisting of mesothelial cells that envelopes the intraabdominal organs and lines the abdominal cavity, and the omentum is an extension of the peritoneum that drapes down from the stomach. When investigating why ovarian cancer exhibits such a high affinity for the omentum and peritoneum, it appears that the mesothelial cells present in these two areas are the main microenvironment that support the cancer's growth and invasion (32). As the malignant cells float throughout the

peritoneum, they are attracted to the mesothelial cells that line the basement membranes of omental and peritoneal surfaces (Figure 4) (32). The attraction between these two cell types is mediated by proteins known as integrins, which are receptors that span the cell membrane and are integral in facilitating cell-to-cell adhesion and adhesion between a cell and the extracellular matrix (Figure 4) (33). Specifically, β_1-integrin is essential for the connection between mesothelial cells on omental surfaces and ovarian cancer cells. (33). As research progresses, integrins are a potentially tantalizing target for new therapies that would leave the ovarian cancer cells and spheroids stranded in the peritoneal cavity and unable to metastasize, presenting a new potential for a cure.

When discussing metastasis to a secondary site, matrix metalloproteinases (MMPs) play an important role in this process. MMPs, a group of proteolytic enzymes, create splits within the extracellular matrix that allows ovarian cancer cells to invade into omental tissue after the initial attachment (17). The extracellular matrix is a network of proteins that supplies a structural scaffold to surrounding cells (34, 35). After attaching to mesothelial cells, ovarian cancer cells release MMPs such as MMP-2 and MMP-9 that fracture extracellular proteins and allow the cancer cells to firmly anchor in place (34). Since MMPs are important for early invasion into the omentum, they are exciting therapeutic targets that can be neutralized to prevent the formation of new metastatic implants.

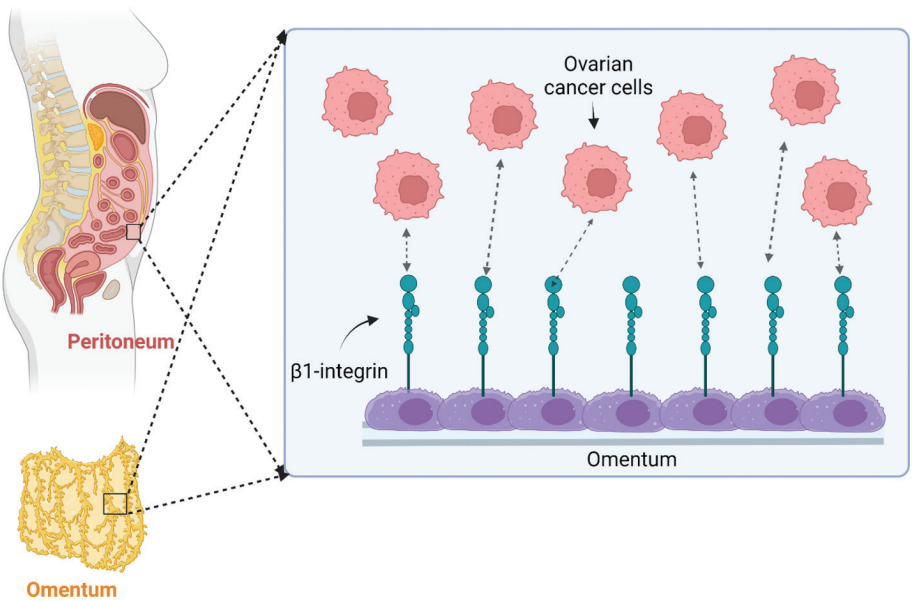

Figure 4. The role of β1-integrins in the metastasis of ovarian cancer to the omentum. β1-integrins on omental mesothelial cells attract ovarian cancer cells and facilitate attachment and invasion. Created with BioRender.com. Used under BioRender's Academic License Terms.

CONCLUSION

Despite numerous advancements in recent years, ovarian cancer is still a disease with a poor prognosis. One of the main reasons why treatments have a limited efficacy is because of the way that ovarian cancer metastasizes within the abdominal cavity, with cells shedding from the primary tumor and then thriving in the omental microenvironment. As knowledge about the mechanisms that underly tumor metastasis are better understood, researchers can continue developing novel tests and therapies that allow clinicians to detect ovarian cancer sooner and to improve survival for patients.

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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