Chapter 3

Ovarian Cancer Metastasis: A Unique Mechanism of Dissemination

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Additional information is available at the end of the chapter

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Abstract

Ovarian cancer is the most lethal of all gynecologic malignancies and has witnessed minimal improvements in patient outcomes in the past three decades. About 70% of ovarian cancer patients present with disseminated disease at the time of diagnosis. The standard of care remains a combination of debulking surgery and platinum- and taxanes-based cytotoxic chemotherapy. Even though metastasis is the leading cause of ovarian cancer related fatalities, our understanding of the process remains limited. Ovarian cancer has a unique pattern of metastasis where the hematogenous spread is less common. Ovarian cancer cells mainly metastasize within the peritoneal cavity, which involves exfoliation from the primary tumor, survival, and transport in the peritoneal fluid followed by metastatic colonization of the organs within the peritoneal cavity. A key step for successful metastasis is their attachment and productive interactions with the mesothelial cells covering the metastatic organs for the establishment of metastatic tumors. This chapter provides an overview of ovarian cancer metastasis highlighting the unique dissemination and the underlying mechanisms of regulation of the steps involved. The role of the microenvironment in the process of metastasis will also be reviewed.

Keywords: ovarian cancer, metastasis, omentum, microenvironment, ascites

1. Introduction

Ovarian cancer is the most lethal of all gynecologic malignancies. It accounts for a fifth of all cancer-related deaths among women in the United States of America. It is estimated that 22,280 women will be diagnosed with ovarian cancer and 14,240 will die of the disease in the United States in 2016 [1]. This makes it a relatively less prevalent but a very deadly form of cancer. About

90% of all ovarian cancers are epithelial in origin, which are classified into high-grade serous, low-grade serous, endometrioid, clear cell, and mucinous subtypes [2]. Of these, the high-grade serous ovarian cancer (HGSOC) is the most common subtype and is characterized by mutations in p53 and genomic instability [3]. A detailed characterization of the HGSOC tumors was done by The Cancer Genome Atlas Network, which mapped the deregulated pathways involved. In the past, these tumors were thought to originate from the ovarian surface epithelium. However, more recently researchers have started to believe that they may actually originate from the fallopian tube fimbriae based on the analysis of prophylactic salpingo-oophorectomy samples [4].

One of the reasons for the poor prognosis of ovarian cancer is the fact that most patients are diagnosed late [5]. It is a highly metastatic cancer and more than 70% of ovarian cancer patients are diagnosed with metastasis [6]. As the tumor grows within the peritoneal cavity, the symptoms produced are abdominal pain or bloating and may be confused with other bowel diseases like irritable bowel syndrome [7, 8]. Ovarian cancer is often called 'the silent cancer' or 'the disease that whispers' because of these diffuse symptoms. The presence of high levels of cancer antigen 125 (CA-125) is used as a diagnostic marker of disease progression. Pelvic ultrasound, MRI, and CT scanning is also used to determine the extent of the disease. Patients undergo a 'debulking' surgery usually conducted by a gynecologic oncologist with a goal to remove as much of the tumor masses as possible from the abdomen [9]. In addition, the tumors are also staged histopathologically as per the International Federation of Gynecology and Obstetrics (FIGO) guidelines (**Table 1**) [10]. Minimal residual disease after the surgery is considered one of the strongest prognostic factors and is highly desirable [11]. The surgery is followed by adjuvant cytotoxic chemotherapy consisting of a combination of carboplatin and paclitaxel. The response to therapy is determined by measuring the serum CA-125 levels and by imaging techniques [2]. If the disease relapses within 6 months, it is considered chemoresistant and if relapse occurs after 12 months, it is considered chemosensitive. While a majority of the patients respond well initially to chemotherapy, most eventually end up developing chemoresistance [12]. Bowel obstruction by the metastatic tumors is the predominant cause of ovarian cancer-related mortality [13]. Since many parts of the bowel get affected, it becomes extremely difficult to surgically treat this condition. In addition, extensive ascites is a cause for major discomfort. Palliative measures such as control of nausea, abdominal pain, draining ascites, and modified diet are typically resorted to [2]. Since most of the ovarian cancer patients are diagnosed with advanced disease, in effect, it is metastasis that is being treated [6]. Therefore, a greater understanding of the process and regulation of ovarian cancer metastasis is essential.

Ovarian cancer predominantly metastasizes within the peritoneal cavity and through the pelvic lymph nodes (**Figure 1**) [14, 15]. However, recent evidence suggests the possibility of hematogenous metastasis of ovarian cancer (**Figure 1**) [16]. This chapter will discuss the steps involved in the unique metastatic dissemination of ovarian cancer and will highlight what is known about the regulation of the steps involved.

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Stage I: The disease limited to ovaries only

- Ia: Tumor in only one ovary or fallopian tube with intact capsule
- Ib: Tumor in both the ovaries
- Ic: Tumor on the surface of one or both ovaries/fallopian tubes with ruptured capsule and cancer cells present in peritoneal washings

Stage II: Tumors spread to the pelvis but limited to below the pelvic brim

- IIa: Tumors spread to the fallopian tubes or uterus or both
- IIb: Tumors spread to other pelvic tissues within the peritoneum

Stage III: Tumors have spread to the abdomen beyond the pelvis or has metastasized to the lymph nodes or both

- IIIa: Microscopic involvement of extra pelvic peritoneal regions
- IIIb: Tumors up to 2 cm diameter
- IIIc: Disease greater than 2 cm with or without lymph node involvement

Stage IV: Distant metastases: pleural effusions contain cancer cells and metastasis to the liver and spleen parenchyma

Table 1. International Federation of Gynecology and Obstetrics staging of ovarian cancer [10].

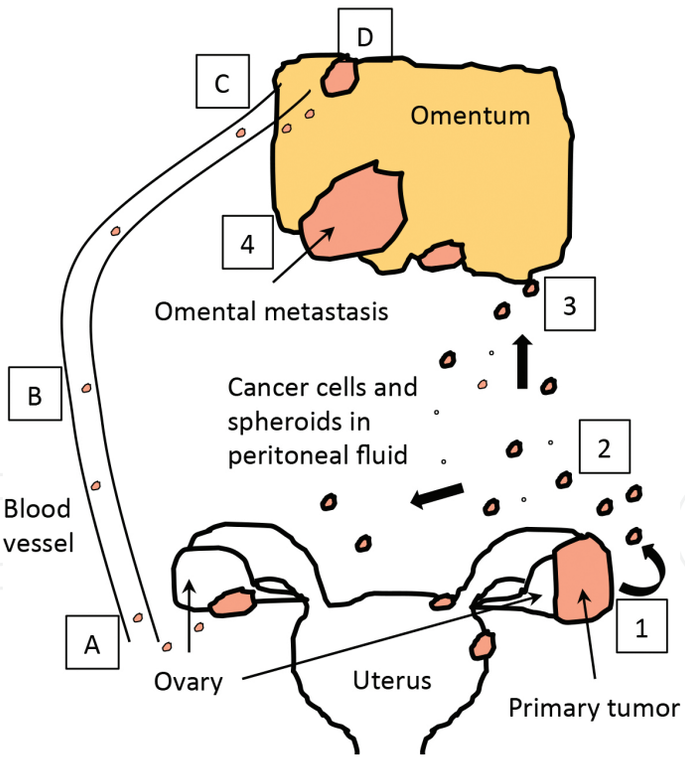

Figure 1. Mechanisms of ovarian cancer metastasis: Transcoelomic dissemination. (1) The cancer cells loose cell–cell contact and exfoliate into the peritoneal cavity. (2) They float in the peritoneal fluid and are carried all over the peritoneal cavity. (3) Attachment to the peritoneal organs like the omentum. (4) Formation of the metastatic tumor. Hematogenous metastasis. (A) Invasion and intravasation. (B) Transport of circulating cancer cells through the blood vessels. (C) Extravasation from the omental capillaries. (D) Formation of the metastatic tumor in the omentum.

2. Overview of ovarian cancer metastasis

The lack of an anatomic barrier allows the ovarian cancer cells to very conveniently spread into the peritoneal cavity. The cancer cells on the surface of the primary tumors start loosing cell–cell contact and become loosely attached to each other. As a result of this, they become prone to exfoliation into the peritoneal cavity (**Figure 1**). Exfoliation is promoted by the mechanical forces like rubbing of neighboring peritoneal organs during respiratory movements and flow of the peritoneal fluids. The cancer cells may come off as single cells or as clumps. This is a passive mode of dissemination unlike the typical invasion followed by intravasation observed in tumors undergoing hematogenous metastasis [13, 17, 18]. The peritoneal fluid naturally flows within the peritoneal cavity upward, toward the head, and then back downward, toward the feet, as a result of the diaphragm movement during respiration and gravitational pull, respectively [19]. The exfoliated ovarian cancer cells from the primary tumor are disseminated throughout the peritoneal cavity by this natural flow of the peritoneal fluid (**Figure 1**). Since normally there is only a small volume of the peritoneal fluid present, dissemination is predominantly limited to the organs in the vicinity of the primary tumor [17]. As the disease progresses, more and more ascites is produced and this enables the spread of the cancer cells to more distant sites in the abdomen. One of the predominant sites of ovarian cancer metastasis is the omentum which is a fatty double fold of the peritoneal membrane, about 8 by 8 inches in size, covering the bowels [13]. It is important to note that this mode of spreading typical of ovarian cancer is very different in terms of the hydrodynamic forces experienced by the cancer cells when they are carried rapidly in the blood vessels during hematogenous metastasis [20, 21].

Epithelial cells tend to undergo anoikis in the absence of attachment to a substratum. Therefore, the main challenge faced by the cancer cells floating in the peritoneal fluid is overcoming anoikis and surviving floatation. In addition, they have to avoid immune surveillance. The cancer cells either form aggregates or spheroids or exist as single cells (**Figure 1**) [22]. The spheroids may also contain embedded cancer-associated fibroblasts as well as activated mesothelial cells, which contribute to the development of the ascetic microenvironment [22]. The subsequent challenge for these floating cancer cells is to successfully attach to the surface of the organs in the peritoneal cavity (**Figure 1**). Debulking surgery often reveals such spheroids loosely attached to the peritoneum. The mesothelial cells covering the peritoneum and the bowels secrete mucus like substances, which help in reducing friction between surfaces as they brush against each other during the course of the organs' natural movements. The same also helps in preventing attachment of the cancer cells to some extent. However, the integrins expressed by the metastasizing cells help them to attach to the extra cellular matrix proteins (ECMs) secreted by the mesothelial cells. Thereafter, the cancer cells are able to push apart the mesothelial cells forming the protective barrier and invade into the organ [23, 24].

Having invaded through the mesothelium of the site of metastasis, the cancer cells have to now revert back to their normal self of growing attached to a substratum. However, since they are now encountering a new microenvironment with a potentially different ECM and secreted factors, they have to now adapt to these new conditions. The adaptive process involves

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extensive and productive reciprocal interactions between the cancer cells and the normal microenvironment of the metastatic site [6]. Those cells, which are able to successfully adapt to this new microenvironment, go on to eventually establish metastatic colonies. On the other hand, the cells that cannot productively interact and adjust to the new microenvironment eventually perish or remain dormant. The cells that are successful eventually reprogram the microenvironment to form and 'activated tumor stroma,' which include cancer-associated fibroblasts, endothelial cells, immune cells, and modified ECMs that promote tumor growth at the metastatic site. In addition to the peritoneal dissemination described above, ovarian cancer cells have been found in blood circulation and recent reports have indicated the existence of an alternative hematogenous mode of metastasis [16, 25].

The process of attaching to and developing metastatic tumors in the new organ is known as metastatic colonization (**Figure 1**). It is considered the least efficient step in the whole process of metastasis [21, 26]. This is also evidenced in mouse xenograft experiments to study ovarian cancer metastasis where many millions of cancer cells are injected intraperitoneally and result in about a hundred tumors or even less [6, 27, 28]. At the same time, the mechanism of regulation of this step and the initial cross talk between the cancer cells and the microenvironment remains a mystery for the obvious difficulty in getting access to this window. Greater understanding of the biology of this process will enable the identification of key regulators that can be targeted therapeutically to hit the metastatic disease at its most vulnerable phase.

3. Mechanism of peritoneal metastasis

The first step in the peritoneal metastasis is exfoliation of the ovarian cancer cells from the primary tumor into the peritoneal cavity. The prerequisite for this step is the loss of cell–cell contact between the cancer cells. As mentioned earlier, ovarian cancer can potentially arise from the fallopian tube epithelial cells or ovarian surface epithelium. Both express the classic epithelial marker epithelial cadherin (E-cadherin) [29]. E-cadherin plays a key role in epithelial cell behavior, tumor suppression, and tissue architecture through its function as a cell–cell adhesion molecule [30]. It is associated with the actin cytoskeleton through α, β, and γ catenins. While E-cadherin is directly involved in the formation of adherens junctions between adjacent epithelial cells, it can also regulate the formation of tight junctions and desmosomes [30, 31].

As the cancer progresses from a benign to a malignant form, the cells undergo an epithelial to mesenchymal transition (EMT). This involves molecular and morphological changes wherein they loose their epithelial characteristics and gain mesenchymal traits. This includes a loss of the compact cell-to-cell attachment, polarity, and cuboidal shape. The cells become more spindle shaped and motile. EMT also involves a change in the expression of epithelial and mesenchymal markers [32]. A very important aspect of this transition is the loss of expression of E-cadherin and a concomitant increase in the expression of neural cadherin (N-cadherin). This results in a reduction in the cell–cell interaction between cancer cells through their adherens junctions and an increase in the ability of the cancer cells to interact with the normal stromal cells present in the microenvironment. In ovarian cancer, E-cadherin expression can

be regulated transcriptionally and post-transcriptionally [33]. ZEB-1, ZEB-2, Snail, and Slug are known to repress E-cadherin and can be regulated by several external cues. The signaling pathways that regulate EMT and E-cadherin expression include transforming growth factor β (TGF-β), epidermal growth factor (EGF), hepatocyte growth factor (HGF), endothelin-1 (ET-1), and bone morphogenetic protein 4 (BMP-4) [32]. Moreover, the miR-200 family of micro-RNAs can also indirectly regulate EMT by targeting ZEB-1 and ZEB-2, which results in the derepression of E-cadherin [34]. Decreased expression of miR-200 family resulted in an increase in the expression of ZEB-1 and ZEB-2, which repressed E-cadherin transcription and induced EMT in ovarian cancer.

The loss of E-cadherin expression and the resulting decrease in the cell–cell attachment promotes the dissemination of the cells into the peritoneal cavity. Interestingly, the loss in E-cadherin expression was found to lead to an induction of expression of α_5-integrin [35]. α_5-integrin forms a heterodimer with β_1-integrin that binds to fibronectin and hence is called the fibronectin receptor. The induction of α_5-integrin was not through the canonical β-catenin pathway. Instead, it was through the epithelial growth factor receptor (EGFR)/focal adhesion kinase (FAK)/mitogen-activated protein kinase (MAPK) pathway. The increase in fibronectin receptor expression was found to help the disseminated ovarian cancer cells attach to the fibronectin secreted by the mesothelial cells lining the omentum and peritoneum [35]. This is an evidence of how the loss of E-cadherin—which facilitates shedding—is coupled to preparing the cells to reattach at the distant metastatic site.

Once the cancer cells have been shed into the peritoneal fluid, it significantly affects the prognosis of the patient as evidenced by the 29% relapse rate of stage 1A ovarian cancer compared to 59% relapse rate of stage 1C [36]. However, once detached from the tumor mass, the cancer cells face several challenges in surviving in the peritoneal fluid. The peritoneal fluid is a result of continuous secretion of fluids by the peritoneal capillaries. This helps in lubricating the adjacent organs in the peritoneal cavity and allows uptake of soluble factors through the peritoneum. A majority of the peritoneal fluid is returned to the circulation through lymphatic drainage. However, in ovarian cancer patients, the increased leakiness of vasculature induced by high vascular endothelial growth factor (VEGF) levels accompanied by blocking of the lymphatic vessels by cancer cells results in ascites formation [22]. This ascites is called malignant ascites because of the presence of floating cancer cells. The malignant ascites facilitates the spread of the cancer cells throughout the peritoneal cavity.

The disseminated ovarian cancer cells floating in the ascites either as spheroids or as single cells develop resistance to anoikis and acquire cancer stem cell-like properties [37, 38]. Interestingly, the single-cell population was found to have a greater percentage of cancer stem cells [39]. The cancer stem cells enriched from ascites have highly elevated ability to form mouse xenograft tumors [40]. Just like cancer stem cells, the floating spheroids and single cells are resistant to chemotherapy. The compact nature of the spheroids serves as an additional physical barrier for the chemotherapeutic agents, preventing the inner cells from exposure to the drug [22]. Taken together, this indicates that the cancer cells floating in the ascites are stem-like and chemoresistant and have the potential to seed new metastatic tumors within the peritoneal cavity.

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The spheroids have elevated levels of E-cadherin and EpCAM and concomitant diminished expression of vimentin, matrix metalloproteinases (MMPs), and CD44 [37, 39]. Therefore, the metastasizing cancer cells demonstrate plasticity in terms of their ability to switch back and forth from epithelial and mesenchymal phenotypes as per the demands of the different steps of metastasis. In addition to the cancer cells, the ascites has several normal cell types that together form the malignant ascites microenvironment and supports the floating cancer cells. The main non-cancer-cell types include cancer-associated fibroblasts (CAFs), mesothelial cells, immune cells, mesenchymal stem cells, and platelets [22]. These cells can be associated with the cancer spheroids or the single cells. They can also exist by themselves, floating in the peritoneal fluid. These supporting cells produce a milieu of factors that assist the cancer cell survival and subsequent colonization of the metastatic site. Cells like platelets also offer protection from immune surveillance by coating the cancer cells.

Having successfully survived flotation in the peritoneal fluids, the next goal of the metastasizing ovarian cancer cells is to attach to the various organs present in the peritoneal cavity. Electron micrographs of sections of normal peritoneum and omentum have revealed the architecture of the mesothelium covering them. The mesothelium consists of a monolayer of mesothelial cells that are very tightly joined end to end to form a protective barrier [24]. These mesothelial cells serve to provide a slippery surface—through the secretion of glycosaminoglycans and lubricants—facilitating normal coelomic movement as well as preventing infection and attachment of cancer cells [41]. The mesothelial cells can perform diverse functions such as secretion of ECMs, growth factors, and inflammatory cytokines for tissue repair and regeneration, proteases for fibrinolysis, and prevention of adhesions [41]. They are also actively involved in the movement of fluids and solutes across serosal cavities [42].

Early *in vitro* experiments revealed that the ovarian cancer cells force the retraction of the mesothelial cells upon attachment to the mesothelium [43]. More recently, Iwanicki et al. have demonstrated the role of the fibronectin receptor ($\alpha_5\beta_1$-integrin) expressed on the surface of the ovarian cancer cells help them attach to the fibronectin secreted on the surface of the mesothelial cells and promote the displacement of mesothelial cells through myosin-mediated traction forces [24]. Subsequent studies revealed the ovarian cancer cells with a mesenchymal phenotype had a greater propensity for mesothelial clearance [44]. The fibronectin secretion by the mesothelial cells was found to be induced by their interaction with the metastasizing ovarian cancer cells. The TGF-β secreted by the cancer cells activated a RAC1/SMAD-mediated signaling pathway in the mesothelial cells, which resulted in the transcriptional upregulation of the fibronectin gene and also induced an EMT-like phenotype in the mesothelial cells [45]. This would probably help in subsequent mesothelial clearance and also may potentially serve as a source of cancer-associated fibroblasts in the microenvironment of the metastatic tumor [46].

The increased expression of the fibronectin receptor in the ovarian cancer cells is also beneficial in coupling attachment to growth factor signaling to promote metastasis. Inhibition of the interaction of $\alpha_5\beta_1$-integrin on the cancer cells with the fibronectin on the surface of the omentum and peritoneum in mouse xenograft models of ovarian cancer metastasis resulted in a decreased metastatic burden in both prevention and intervention settings [28]. Since

inhibition of $\alpha_5\beta_1$-integrin can also inhibit angiogenesis, it was further investigated whether the effects on metastasis were actually due to disruption of the human cancer cell $\alpha_5\beta_1$-integrin interaction with fibronectin or that of the mouse endothelial cell $\alpha_5\beta_1$-integrin. The effect of an anti-murine $\alpha_5\beta_1$-integrin-blocking antibody was compared to that of the anti-human $\alpha_5\beta_1$-integrin-blocking antibody. Interestingly, the murine-blocking antibody did not show any significant effect and, therefore, confirmed the key role of the interactions of the cancer cell $\alpha_5\beta_1$-integrin with the fibronectin of the microenvironment in promoting ovarian cancer metastasis [28]. Further investigation revealed that the activation of $\alpha_5\beta_1$-integrin resulted in the activation and phosphorylation of the receptor tyrosine kinase c-Met independent of its ligand—hepatocyte growth factor (HGF) [28]. This attachment induced activation of the growth factor receptor lead to increased invasiveness and growth through the subsequent activation of the FAK/Src signaling pathways in the cancer cells. The expression of a constitutively active FAK could abrogate the inhibitory effects of the $\alpha_5\beta_1$-integrin-blocking antibody on the ovarian cancer cells [28].

Another effect of adhesion of the ovarian cancer cells to the surface of the omentum is the increased secretion of the extracellular protease MMP-2. It cleaves fibronectin and vitronectin present on the surface of the mesothelium into smaller fragments, which enhances binding of the cancer cells to these ECMs through their specific integrin receptors $\alpha_5\beta_1$-integrin and $\alpha_v\beta_3$-integrin, respectively [47]. Inhibition of MMP-2 in the ovarian cancer cells as a prevention measure inhibited their adhesion to the omentum in nude mice. However, the host MMP-2 did not play a role in this process as evidenced in MMP-2 knockout mouse xenograft experiments [47].

Once the cancer cells attach to the mesothelial cells on the surface of the omentum, they embark on a process of adapting to the new microenvironment of the site of metastasis. As evidenced by the ECM-cancer cell interactions and their consequences above, more productive reciprocal interactions between the cancer cells and their new microenvironment are essential for successful establishment of the metastatic tumors. The cancer cells have to revert from surviving anoikis while floating in the peritoneal fluid to an attached growth in the presence of new ECMs and growth factors available in the microenvironment of the omentum and peritoneum. This involves significant changes in the gene expression profiles of the colonizing cancer cells and, therefore, would involve the activation/repression of transcriptional/translational regulators dependent on microenvironmental cues. One such important microenvironment regulated translational regulator was reported to be the micro-RNA miR-193b [6]. miR-193b is a tumor suppressor micro-RNA that was found to be downregulated in the metastasizing ovarian cancer upon their interaction with the mesothelial cells covering the surface of the omentum. This downregulation promoted growth and invasiveness of the cancer cells *in vitro,* colonization of human omentum *ex vivo,* and decreased metastasis in mouse xenografts [6]. Interestingly, the miR-193b downregulation was induced by the hypermethylation of its promoter as a result of the cross talk between the cancer cells and the mesothelial cells. The promoter hypermethylation was catalyzed by the increased expression of DNMT1 in the cancer cells stimulated by their interaction with the mesothelium [6]. miR-193b was found to directly target urokinase. A decrease in miR-193b expression resulted in increased

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expression of urokinase, which mediated the functional effects of miR-193b in driving metastatic colonization of the omentum [6].

As the cancer cells adapt to the new microenvironment of the metastatic site and start to proliferate, they also recruit resident and non-resident normal cells and convert them into the tumor-associated stroma or 'activated stroma' [48]. It is well known that the tumors consist of 10–50% of non-cancer cells or the tumor stroma [49]. The key components of this tumor stroma are the cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and other immune cells, endothelial cells, pericytes, adipocytes, extracellular matrix proteins, etc. [50]. All these stromal components are essential for successful growth and progression of the tumors as they are a critical source of growth and tropic factors, help in evasion of immune surveillance, angiogenesis, ECM remodeling, invasiveness, etc. Therefore, the eventual success of the cancer cells in colonizing the omentum will depend upon their ability to develop an active tumor stroma.

The metastasizing ovarian cancer cells were found to recruit the resident normal fibroblasts in the basement membrane of the omentum and reprogram them into CAFs. This reprogramming was driven by the decreased expression of miR-214, miR-31, and an increase in the expression of miR-155 in the normal fibroblasts induced by the cancer cells [49]. The resulting CAFs promoted ovarian cancer cell migration, invasion, and colony formation *in vitro* and tumor growth and metastasis *in vivo*. Interestingly, CAFs could be converted back into normal fibroblasts by the combined overexpression of miR-214 and miR-31 and inhibition of miR-155. The micro-RNAs mediated their effects through an array of targets, most of which were identified as chemokines and cytokines. The main mediator was found to be CCL5, which was a direct target of miR-214. Inhibition of CCL5 in nude mice injected with a mixture of ovarian cancer cells and CAFs significantly decreased the ability of the CAFs to promote tumor growth and metastasis [49].

An important and abundant cellular component of the omentum is adipocytes. Until recently, not much was known about the direct role of the omental adipocytes in promoting ovarian cancer metastasis to the omentum even though it is well established that omentum is one of the main sites of ovarian cancer metastasis and that it is a predominantly fatty tissue. Dr. Lengyel's group went on to demonstrate that the omental adipocytes secrete adipokines that promote the homing of the metastasizing ovarian cancer cells to the omentum [51]. The cancer cells, thereafter, could induce metabolic reprogramming of the adipocytes and induce lipolysis in them. The adipocytes in turn induced the expression of FABP4—a fatty acid transporter—in the cancer cells. As a result of this, the cancer cells efficiently take up the free fatty acids released by the adipocytes and utilize them as a source of energy and building blocks to drive tumor growth [51]. This explains why the omentum tumor is usually the largest one in the peritoneal cavity with sometimes the whole omentum getting converted into a solid, hard omental cake. By that time, all the adipocytes have been depleted and used for the growth of the metastatic tumor.

4. Other mechanism of dissemination

While the transcoelomic route of peritoneal dissemination is thought to be the predominant mode of ovarian cancer spread, other mechanisms do exist. The ascites produced in the peritoneal cavity is typically drained through the lymph vessels present in the diaphragm [36]. This provides the cancer cells present in the ascites, the opportunity to metastasize to the lymph nodes. Moreover, the lymphatic vessels drain into the left subclavian vein via the thoracic duct. This enables some cancer cells to enter into the blood circulation.

Although circulating ovarian cancer cells have long been reported to be present in the blood [52], it has typically been considered a mode of dissemination only in the very late stages of the disease. The prevalent reasoning being that although the ovarian cancer cells enter into the circulation, they are not yet adept at surviving in the circulation and establish metastatic tumors in a very different 'soil.' However, recent reports have suggested that hematogenous metastasis may be more commonly occurring in ovarian cancer that we had thought [16, 53]. Using a parabiosis model, Pradeep et al. have very elegantly demonstrated the haematogenous metastasis of the ovarian cancer cells from the primary tumor in one mouse to the omentum of the paired mouse [16]. The expression of $ErBB_3$ in the ovarian cancer cells entering into circulation and the omental expression of NRG1 was found to be the key players responsible for the hematogenous metastasis [16]. Interestingly, the use of mouse models of hematogenous ovarian cancer metastasis revealed a preferential homing of the cancer cells to the ovary followed by the development of ascites and subsequent peritoneal metastasis [53]. When the ovaries were removed before injecting the cancer cells, peritoneal metastasis and ascites formation were completely abolished [53]. Taken together, recent evidences point toward a more significant role of hematogenous dissemination in ovarian cancer that previously thought.

5. Conclusion

Ovarian cancer is a malignancy where most patients are treated for metastatic disease because they are usually diagnosed at an advanced stage. A better understanding of the process of metastasis and the underlying mechanisms of regulation is crucial for development of effective therapies. However, our knowledge in this field remains limited. It has become increasingly clear that there are multiple different ways in which the cancer cells disseminate, and the transcoelomic rout remains the most predominant mode. While it appears to be a relatively simpler way to metastasize, the steps involved pose their own unique challenges to the cancer cells. Moreover, the absence of the need for invasion, intravasation, and extravasation can potentially enable the cancer cells to metastasize earlier and in greater numbers than in case of hematogenous metastasis. Studying the underlying mechanisms have remained challenging but the evolution of *in vitro* organotypic 3D culture models have opened up opportunities to conduct more meaningful experiments and have lead to significant leaps in knowledge [6, 24, 45]. Use of ovarian cancer cell lines that closely resemble the mutational profile of clinical

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HGSOC samples will also contribute toward meaningful progress in research in this field [54, 55]. The use of such models will hopefully provide greater insights into the regulation of metastatic colonization and enable therapeutic targeting of the disease at the stage in which they are most vulnerable. Moreover, considering the key roles played by the microenvironment of the site of metastasis as well as the tumor stroma, these 'normal' components can be targeted as well as the cross talk between them and the cancer cells. Since these cells are genetically stable and provide multiple different modes of support to the cancer cells, there will hopefully be reduced chances of development of drug resistance.

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