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Ovarian Cancer Ascites as a Liquid Tumor Microenvironment

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Abstract: Ovarian cancer is a leading cause of death among women in most developed countries. This malignancy is characterized by rapid growth and spread of intraperitoneal tumors, leading to ascites, which is accumulation of fluid in the peritoneum. Despite proof that the accumulation of peritoneal fluid signifies the poorest outcome for cancer patients, the role of malignant ascites in promoting metastasis and therapy resistance remains poorly understood. Malignant ascites presents a unique tumor microenvironment to the tumor cells, non-tumor cells, and various biofactors such as growth factors, cytokines, and lipids. Interest in the characterization of the components of the microenvironment of malignant ascites and their role in the progression of ovarian cancer has increased over the years. In this chapter, we summarize the role of malignant ascites as a liquid tumor microenvironment in the development and progression of ovarian cancer.

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INTRODUCTION

Ovarian cancer is a leading cause of death among women in most developed countries (1). This malignancy is characterized by rapid growth and the spread of intraperitoneal metastasis (2). Ovarian cancer is distinct from other malignancies in some specific characteristics: (i) the origin of primary tumors can be from multiple sites, such as, the ovarian epithelium, the Fallopian tubes, the endometrium, or the peritoneum; (ii) tumor cells can disseminate by exfoliation from the ovaries (or the tubes) and migrate through the peritoneum; and (iii) secondary tumors do not have additional genetic mutations from that of the primary tumors (3). The World Health Organization classifies ovarian tumors as epithelial (~90% of the cases), germ cell (~3%) and sex cord-stromal (~2%) (4). Epithelial ovarian carcinoma (EOC) comprises five main types (Figure 1) based on its histopathology, immune, and molecular profile: (i) high-grade serous carcinoma (HGSC, 70%); (ii) low-grade serous carcinoma (LGSC, 5%); (iii) endometrioid carcinoma (10%); (iv) clear cell carcinoma (6%); and (vi) mucinous carcinoma (3–4%) (4). These subtypes are distinct but are clinically managed as a single entity, i.e., cytoreductive surgery followed by platinum-taxane combination chemotherapy. The response rate to first-line therapy is around 80–90%, but most patients relapse and develop chemotherapy resistance contributing to a poor 5-year survival rate of <35% (5, 6). Heterogeneity is a key feature of these tumors, explaining, in part, the lack of successful treatment. With the development of molecular tools such as deep sequencing, along with RNA sequencing, epigenomics, proteomics, and

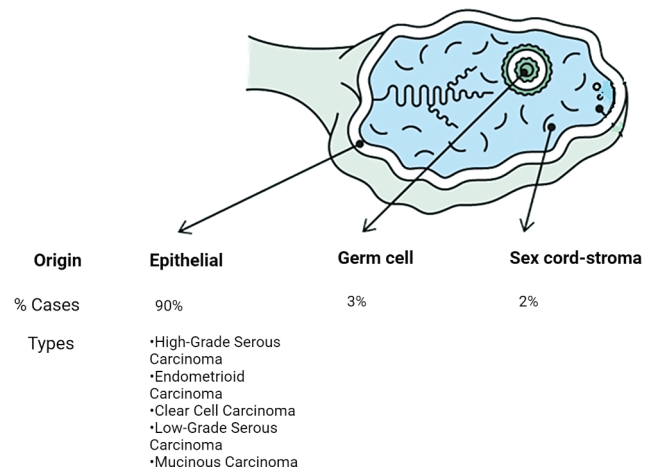

Figure 1 Origins of ovarian tumors. The EOC type comprises several subtypes. Created by Biorender.

immunologic studies, we are gaining further insight into the complexity of heterogeneity within these subtypes and within individual patient tumors (1).

CLASSIFICATION OF OVARIAN CANCER

LGSC and HGSC represent two separate tumor types with different morphology, pathogenesis, molecular events, and prognosis (4). HGSC usually occurs in older patients, is detected at an advanced stage, and is responsible for most ovarian cancer deaths. Morphologically, HGSCs are composed of ciliated, columnar cells that form papillae, solid masses, or slit-like spaces with high-grade nuclear atypia. Immunohistochemistry is positive for cytokeratin 7 (CK7), paired box gene 8 (PAX8), Wilms tumor gene product (WT1), but negative for cytokeratin 20 (CK20). The cell cycle checkpoint p53 is generally mutated, resulting in overexpression, or null mutation which translates to a negative immunohistochemistry result. The genomic analysis of HGSC demonstrated a few recurrently mutated genes, such as TP53 (96% of the cases) and BRCA1/BRCA2 (22% of the cases) (7). Most of these carcinomas arise from the distal fimbrial end of the Fallopian tube from a precursor lesion known as serous tubal intraepithelial carcinoma (STIC) (4). Primary peritoneal HGSCs are extremely rare (4).

LGSC are uncommon, comprising 2% of all ovarian carcinomas, and are more frequently found in younger women (median 43 years) (4). These tumors are slow-growing, arising from benign and borderline serous tumors (4), and have a 10-year survival rate of 50% (8). Morphologically, these carcinomas seem like HGSC but with less atypia and an immunohistochemical profile also similar to HGSC (positive for CK7, WT1) however, the expression of p53 is normal-like. LGSCs are genomically stable and display somatic mutations in KRAS and BRAF in approximately half of the cases; these mutations are mutually exclusive (8, 9).

Endometrioid carcinoma usually presents as unilateral solid masses, low grade, and associated with a good prognosis (10). Most of these tumors arise from transformed ovarian endometriosis or benign and borderline tumors (4). Histologically, they are composed of glands resembling endometrial epithelium and typically exhibit a glandular architecture with squamous differentiation, but solid areas can be seen. The immunohistochemistry profile shows positivity for CK7, PAX8, and hormone receptors, and negativity for WT1 and CK20 (10). Endometrioid carcinoma displays somatic mutations of CTNNB1, PI3KCA, PPP2R1A, PTEN, and ARID1A genes (11, 12). Based on its analogous molecular features, seromucinous carcinoma is considered a subtype of endometrioid carcinoma (4).

Clear cell carcinoma is quite uncommon, and some studies show that it has the worst prognosis of all EOCs subtypes (13). These carcinomas occur at a younger age and have a clear association with endometriosis (14–16). The pattern of growth is in the form of a large pelvic mass; it is rarely bilateral, and associated with thromboembolic complications, hypercalcemia, and lymph node metastases (17–19). Histologically, they are composed of glycogen-laden, large, cuboidal, hob-nailed, or flattened clear cells and display an admixture of growth patterns including solid, tubulocystic, or papillary (20). The immunohistochemistry profile of clear cell carcinoma is characterized by the expression of napsin A and the absence of WT1, p53, and ER expression (21, 22). Some studies show that the

tumor suppressor ARID1A is mutated in most clear cell carcinoma cases (12, 21). PI3KCA exhibit activating mutations (22). Recent studies showed that clear cell carcinoma is resistant to platinum-based chemotherapy, but, despite this, its management is similar to the rest of EOC (18).

Mucinous carcinomas are rare, and patients are usually diagnosed at an early stage with an excellent prognosis after surgery. However, when patients have relapses (or metastatic mucinous carcinoma) they have a worse prognosis (23, 24). Usually, these type of EOC are unilateral, large, multicystic tumors filled with mucus and frequently containing solid areas. Morphologically, mucinous carcinoma is composed of cysts and glands of variable size with a confluent pattern and back-to-back glands. The cells are tall, columnar, and stratified, with a large cytoplasm containing mucin (25, 26). Immunohistochemistry of mucinous carcinoma shows CK7 and CK20 positivity but are usually negative for hormone receptors and WT1. These carcinomas seem to arise from borderline mucinous neoplasms and show a heterogeneous pattern with coexisting mucinous, benign, borderline, and adenocarcinoma areas (27). The most common molecular alterations are KRAS and TP53 mutations (both 64%) (4), which have been identified in benign and borderline areas as well as in adjacent carcinomas (28–30). HER2 amplification is also found in around 20% of mucinous carcinoma, as well copy-number loss of CDKN2A (76% of cases) (4). These genomic abnormalities are mutually exclusive (31).

MALIGNANT ASCITES AS A LIQUID TUMOR MICROENVIRONMENT

Several studies associate different ovarian cancer characteristics with the intrinsic properties of tumors and their microenvironment (32–34). In ovarian cancer, most patients are diagnosed at advanced stages (stage III/IV), presenting metastasis throughout the pelvic and peritoneal cavities, and by the accumulation of a large volume of peritoneal fluid (malignant ascites, MA) (35). The role of MA is to facilitate the spread of tumor cells to other pelvic and peritoneal organs, serving as a vehicle for tumor cells (36). This form of transcoelomic dissemination is crucial to the adhesion of tumor cells to the omentum and serous membranes lining the peritoneal organs, leading to metastatic lesions in the peritoneal cavity, instead of invading the lamina propria like the majority of other solid tumors (37). Ovarian cancer cells disseminate into peritoneal sites such as the hepatic, omentum, spleen, uterus, etc, using the MA flux. MA comprises not only tumor cells, but also many other non-tumor cell types (Figure 2), which produce a unique microenvironment that can modify the neoplastic properties of tumor cells (38).

The peritoneum is lined by mesothelial cells that cover and protect the viscera and the stroma that contains a collagen-based matrix, activated fibroblasts, blood vessels, and lymphatics vessels. This conjugation creates a unique milieu full of factors secreted by all tumor cellular components that support metastatic seeding and tumor proliferation (39). MA is an exudative fluid composed by a cellular fraction with highly tumorigenic cancer cells (40), immune cells, including different types of T cells (41), tumor-associated macrophages (42), and other host cells.

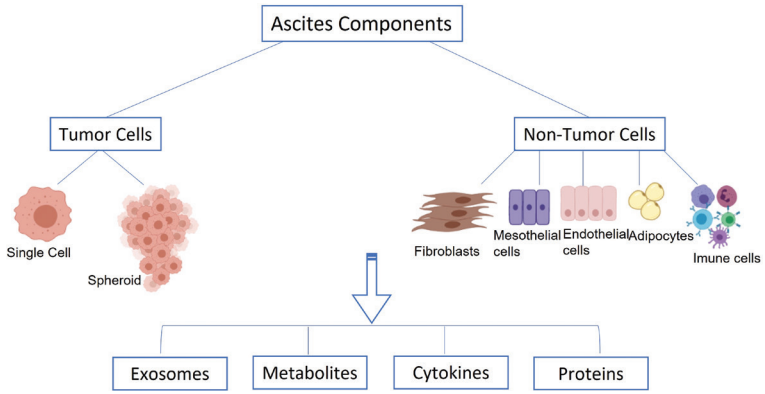

Figure 2 Scheme of cellular and acellular components of ascites. Ascites is composed by tumor cells (single cells and spheroids), and non-tumoral cells, including fibroblasts, mesothelial cells, endothelial cells, adipocytes, and immunologic cells. These types of cells communicate with each other through acellular factors, including cytokines, proteins, metabolites, and exosomes. Created by Biorender.

The acellular fraction contains tumor-promoting soluble factors, bioactive lipids, cytokines, and extracellular vesicles (43).

Several studies have demonstrated an "activated" phenotype of the peritoneal environment associated with ovarian cancer, as opposed to its quiescent state in benign conditions (44). The pro-inflammatory signature, associated with cancer, promotes angiogenesis, and exerts chemotactic and protective effects on cancer cells. Chemokines, cytokines, and growth factors commonly secreted in the tumor microenvironment include the stromal cell-derived factor, interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemoattractant protein 1, Chemokine (C-C motif) ligand 5 and 7 (CCL5 and CCL7), transforming growth factor- β1, tumor necrosis factor α (TNFα), fibroblast growth factor, and others (44–46). While tumor cells play a role in the secretion of factors that modulate angiogenesis, non-transformed tumor-infiltrating cells such as fibroblasts, myeloid cells, immune cells, and endothelial precursors also play a crucial role in modulating neo-vascularization (47). All these factors present in the MA microenvironment induce tumor cell proliferation, progression, chemoresistance, and immune evasion (3) unveiling a key role of this serous liquid in the development and progression of ovarian cancer (48).

The cellular components of malignant ascites

Cancer cells in MA can be found as single cells with adherent properties or multicellular spheroids with no-adherent properties (49), being the major contributors to the peritoneal dissemination (50). The multicellular spheroids are key mediators of peritoneal dissemination since they have low expression levels of E-cadherin (49) and allow ovarian cancer cells to resist anoikis and apoptosis, including that induced by chemotherapeutic agents, since drugs do not penetrate in such multicellular structure (35, 51, 52).

The stromal cells, such as fibroblasts, endothelial or mesothelial cells, adipocytes, adipose tissue-derived stromal cells, bone marrow-derived stem cells and

immune cells (53, 54), can regulate the extracellular matrix composition and produce molecules that attract ovarian cancer cells to specific sites (55, 56). These tumors are typically highly vascularized, because some of these cells show abnormal activities, like the stimulation of growth and angiogenesis (57, 58), which correlates with a poor prognosis and contributes to tumor development (38, 59) (Figure 3).

The malignant role of cancer-associated fibroblasts is to promote proliferation, migration, and invasion of cancer cells. Cancer-associated fibroblasts secrete factors that can transduce signals to cancer cells as well as to themselves, establishing reciprocal reinforcement of growth and migration signals and contributing to chemoresistance (60). Mesothelial cells lining the peritoneum are also important for tumor progression (57), as they secrete factors that promote tumor growth. Lysophosphatidic acid is produced by immortalized peritoneal mesothelial cells and it was shown to improve adhesion, migration, and invasion of ovarian cancer cells (61). In addition, mesothelial cells produce dipeptidyl peptidase IV and vascular endothelial growth factor (VEGF) in response to MA environmental exposure (62, 63).

The complex immune suppression system that efficiently neutralizes antitumor immunity is one of the reasons for disease progression and treatment failure (64) as cancer cells are able to subvert the natural purpose of immune cells for their own benefit. The equilibrium between these immune reactive and immune suppressive cells defines the immunosuppressive and pro-tumoral properties of MA microenvironment (38, 39) (Figure 4).

The immune reactive cells include cytotoxic T lymphocytes and activated $CD4^+$ T cells. The immune-suppressive cells are myeloid-derived suppressor cells,

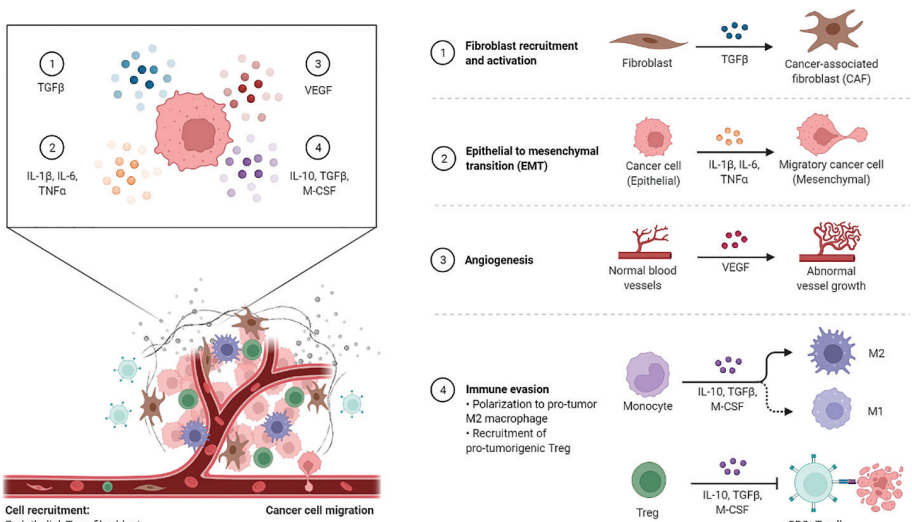

Figure 3 Cancer-associated changes in stromal cells. IL-10, interleukin 10; IL-1β, interleukin 1β; IL-6, interleukin 6; M-CSF, macrophage colony-stimulating factor; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α. VEGF, vascular endothelial growth factor. Created by Biorender.

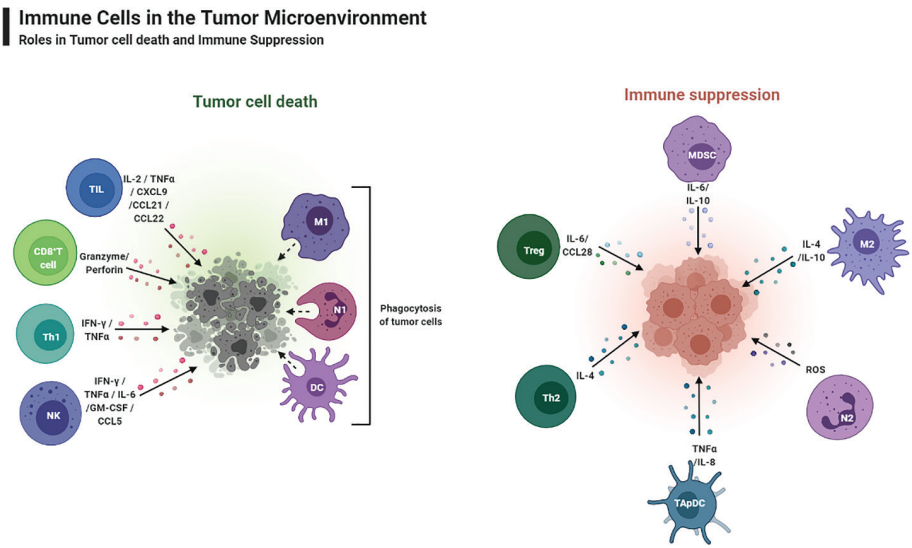

Figure 4 **Immune cells in the tumor microenvironment.** The left panel represents the immune cells that act as tumor killers by the production of cytokines that destroy tumor cells. The right panel represents the cells that contribute to immune suppression. $CD8^+$T, $CD8^+$ T cells; DC, dendritic cell; M1, macrophage type 1; M2, macrophage type 2; MDSc, myeloid-derived suppressor cells; N1, neutrophil type 1; N2, neutrophil type 2; NK, natural killer; TApDC, tumor-associated plasmacytoid dendritic cell; Th1, T "helper" 1 cell; Th2, T "helper" 2 cell; TIL, tumor-infiltrating lymphocytes; Treg, egulator T cell. Created by Biorender.

tumor-associated macrophages (especially M2 subtype), dendritic cells, lymphocyte T helper cells (Th2 subtype), and T regulatory cells (Tregs). The presence of $CD3^+$ tumor-infiltrating lymphocytes (TILs) in ovarian cancer is associated with increased survival (65). It was shown that, in patients whose tumors contained T cells, the 5-year overall survival was 38% compared to 4.5% in patients with tumors lacking T cells. In addition, a strong correlation between the presence of $CD8^+$ TILs and favorable clinical outcomes of HGSC (66–68) has been demonstrated. The ratio of $CD8^+$ T cells/Tregs cells is also related to increased survival of ovarian cancer patients (67). A positive correlation between the presence of oligoclonal expanding T cells and the regression or stabilization of metastases also demonstrates the value of the tumor immune microenvironment in the outcome of ovarian cancer patients (69). There is increasing evidence that non-tumoral cells in the tumor microenvironment have a key regulatory role in ovarian cancer and should be evaluated for diagnostic and treatment purposes (39).

The acellular components of malignant ascites

The cellular components of MA communicate with each other through soluble factors, including cytokines, proteins, metabolites, and the secretion and exchange of exosomes (2).

The cytokine profiles of ascites can be pro-tumorigenic or anti-tumorigenic (70–73). The pro-tumorigenic cytokines are regulated by Th2, such as, IL-4, IL-6,

IL-8, IL-10, IL-13, IL-15, CCL2 and VEGF and the anti-tumorigenic are regulated by Th1, such as, IL-2, IL-3, IL-5, IL-7, IL-17, CXCL-10, CCL4, INFγ, and TNFα (74, 75). These cytokines contribute to the creation of a pro-inflammatory and immunosuppressive tumor microenvironment (73).

The metabolome profiling of ascites has demonstrated important differences in fatty acids, cholesterol, ceramide, glycerol-3-phosphate, glucose, and glucose-3-phosphate. The MA present low levels of 2-hydroxyisovalerate, although glucose-1-phosphate is present in high levels in this liquid microenvironment. 2-hydroxyisovalerate is the result of breakdown of branched-chain amino acids (76) and is found in the urine of patients with lactic and ketoacidosis, which indicates an increase in amino acid catabolism (77). The glucose-1-phosphate is a product of glycogenolysis which is correlated with the increased use of glucose by the tumor cells in the MA microenvironment (78). The glucose transporter 1 or 3 and glycolytic enzymes, such as hexokinase II, are overexpressed in ovarian cancer, and are indicators of poor prognosis (36), as they are associated with chemoresistance and poorer progression-free survival (37). In addition, glycolate, glucose, furanose and fructose are found in low levels, while glycerol-3-phosphate, cholesterol, ceramide and monoacylglycerol are elevated in ovarian cancer patient-derived MA (38).

Proteomics of ascites has revealed the presence of over 2000 different proteins (79, 80). Examples of proteins found abundantly in MA are pyruvate kinase isozymes M1/M2, glyceraldehyde phosphate dehydrogenase and mesothelin (81). Moreover, the most abundant proteins are related to the components associated with RNA splicing (79). Exosomes were also detected in ovarian cancer MA. These nano-sized microvesicles (30–100nm of diameter) are membrane-bound extracellular vesicles that are produced in the endosomal compartment of most eukaryotic cells and carry various lipids, proteins and nucleic acids, within the membrane-covered vesicles (82). These structures have the molecular signature of donor cells and circulate in the organism, with the objective of transporting information between cells to change the gene expression of receptor cells (83). Exomes have disease-specific biomarkers in ovarian cancer, such as miR-200c, miR-214, CA125, Mucina-1 and CD24 (82, 84).

CONCLUSION

Metastatic ovarian cancer is a deadly disease. The mechanism of tumor dissemination in the peritoneal cavity leads to the formation of MA. MA constitute an easily accessible source of cancer cells and cancer-associated factors. The presence of this liquid tumor microenvironment is correlated with a poor prognosis in ovarian cancer patients but its association with chemoresistance is poorly understood. Further studies supported by technological advances are needed to better explore the multidimensional potential of this unique tumor microenvironment that supports ovarian cancer cell growth, progression, and metastatic outgrowth. The actual challenge is to understand the complexity of the multiple interactions between ovarian cancer ascites components and to develop new drugs to abrogate these tumor microenvironment communications routes.

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