Ascites in Ovarian Cancer Progression: Opportunities for Biomarker Discovery and New Avenues for Targeted Therapies

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http://dx.doi.org/10.5772/intechopen.70993

Abstract

Until recently, ovarian cancer research has mainly focused on the tumor cell themselves ignoring for the most part the surrounding tumor environment. However, one of the major conceptual advances in oncology over the last few years has been the appreciation that major aspects of cancer biology are influenced by the tumor environment. Malignant ascites accumulates in the peritoneal cavity during ovarian cancer progression and constitutes a unique pro-inflammatory tumor environment providing a framework that orchestrates cellular and molecular changes contributing to aggressiveness and disease progression. The composition of ascites, which includes cellular and acellular components, constantly adapts during the course of the disease in response to various cellular cues originating from both tumor and stromal cells. Increasing evidence now supports an active role of ascites in the progression of ovarian cancer. Although much work is still needed to fully understand the contribution of ascites to ovarian cancer aggressiveness, this tumor environment potentially provides a wealth of opportunities for translational research including biomarker discovery and novel therapeutic target identification. In this review, we discuss recent advances in our understanding of ascites pathophysiology, the characterization of its cellular and acellular contents, the intercellular crosstalks, and how these data can be used to improve the outcome of ovarian cancer.

Keywords: ascites, cytokines, ovarian cancer, progression, metastasis

1. Introduction

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer-related death among women in the Western world [1]. Early stage diseases are difficult to detect because of the location and size of ovaries and fallopian tubes, the lack of specific symptoms and the absence of reliable screening methods. Consequently, most women with EOC display advanced diseases (stage III/IV) with metastases throughout the pelvic and peritoneal cavities, as well as large amount of ascites, when they seek medical care [2, 3]. The presence of large volume of ascites correlates with poor prognosis and pelvic and peritoneal metastases [4, 5]. EOC encompasses five histopathological subtypes with unique characteristics: high-grade serous carcinoma (HGSC), low-grade serous carcinoma (LGSC), endometrioid carcinoma (EC), mucinous carcinoma (MC), and clear cell carcinoma [6, 7]. High-grade serous ovarian carcinoma (HGSOC) is by far the most common subtype and development of malignant ascites during the course of the disease is particularly common with this subtype [3]. Due to the accumulation of large volume, ascites can be debilitating for patients causing pain, early satiety and respiratory distress [8]. The standard of care for women with high-grade serous ovarian carcinoma (HGSOC) consists of debulking surgery together with platinum-based combination chemotherapy resulting in a median progression-free survival (PFS) of 16–22 months and a 5-year survival rate of 10–30% [1, 9]. This high mortality rate results from the biologic complexity of EOC, from the difficulty of resecting multiple peritoneal tumor implants and from the frequent occurrence of drug resistance, whether intrinsic (primary) or acquired (secondary), the latest being the most frequently observed. Treatment options for women with resistant diseases remain very limited and relapsing diseases are almost always incurable. In contrast, women with localized disease (tumor limited to the primary site) have a 95% 5-year survival [3]. Therefore, it is essential to gain a better understanding of the mechanisms involved in EOC dissemination and how the tumor environment participates to this process in order to develop novel therapeutic approaches that target crucial steps involved in cancer dissemination that could improve long-term survival.

In most human cancers, the tumor microenvironment is heavily altered compared to its normal counterpart [10, 11]. The importance of the tumor microenvironment in cancer progression is now well appreciated. Indeed, bidirectional communications between tumor cells and their surrounding environment influence disease initiation and progression and patient prognosis [12]. In response to evolving environmental conditions and signals from tumor and stromal cells, the surrounding tumor environment is continually changing over the course of cancer progression, underscoring the need to understand how the environment drives the metastatic process. As opposed to the surrounding microenvironment in solid tumors, malignant ascites constitutes a unique form of environment. Recent evidence suggest that ascites plays a major role in tumor progression, emphasizing the necessity to understand its pathophysiology and its impact on the biology of tumor cells, including its role in drug resistance, spheroid formation, tumor dissemination and progression. Here, we discuss the recent advances in our understanding of the role of ascites in ovarian cancer progression. In particular, we address its effects on spheroid formation, dissemination, chemoresistance and metastasis. Pinpointing key molecules in ascites that promote EOC dissemination and progression will provide new strategies to improve EOC survival.

2. What is the tumor environment of ascites

As previously mentioned, EOC progression is characterized by the progressive accumulation of peritoneal fluids, which presumably provides a supportive local environment. Because of its large volume (up to 10 L), its high cell density and lack of anchorage support for cells, the accumulation of peritoneal effusions occurring during EOC progression can be seen as a particular environment. The pathophysiology of ascites accumulation involves decreased clearance of peritoneal fluids, blockade of lymphatic channels drainage, increased permeability of capillaries due in large part to vascular endothelial growth factor (VEGF) [13, 14], decreased protein levels in blood, and decreased hepatic clearance. Ascites is characterized by cellular and acellular fractions. The cellular fraction is populated by a heterogeneous mixture of tumor and stromal cells, which includes mesothelial-derived cells, adipocytes, endothelial and immune cells. These stromal cells account for >99% of the cellular composition of ascites which contrast with the stromal content of tumor tissue which has a median relative proportion of 50% [15]. In solid tumors, stromal cells significantly contribute to malignant progression. In particular, cancer-associated fibroblasts (CAFs) promote cell survival, growth and progression by expressing a pro-inflammatory gene signature leading to secretion of a number of growth factors, including transforming growth factor-β1 (TGF β1), IL-6, CSCL1, and CXCL2 among others [16]. By analogy, stromal cells found in malignant ascites could play a similar role in ovarian cancer progression. Indeed, recent studies suggest that stromal cells in ascites facilitate tumor growth, survival and invasion [17–19].

The acellular fraction of ascites constitutes a dynamic reservoir of cytokines, growth factors, bioactive lipids and extracellular matrix (ECM) components that may have either pro- or anti-tumorigenic effects [20–25]. A number of factors in ascites, including CCL18, HGF, LPA and VEGF, have been shown to promote cell migration, invasion and tumorigenesis [20, 26–29].

3. Cellular contents: contribution to EOC metastasis

The origin and phenotype of the stromal cells in ascites is still not well understood. However, ascites is characteristically populated by mesothelial cells [30]. Mesothelial cells exfoliate from the peritoneal lining and accumulate in ascites [31]. Upon sustained inflammation, mesothelial cells lose their epithelial-like characteristics, including dissolution of cell-cell junctions and their apical-basolateral polarity, and acquired a mesenchymal phenotype (mesothelial-to-mesenchymal transition (MMT) giving rise to myofibroblastic-like cells, which are characterized by increased migration and invasion capacities [32]. Lineage-tracing experiments suggest that a sizeable subpopulation of cancer-associated fibroblasts (CAFs) found in ascites probably originates from mesothelial cells through MMT [33]. Mesothelial-derived CAFs share characteristics with myofibroblasts, such as the expression of alpha-smooth muscle actin (αSMA), fibroblast activation protein-α (FAPα) and fibroblast-specific protein 1 (FSP1) [33]. TGF-β has been implicated in mesothelial cell activation leading to MMT [34]. In EOC ascites, myofibroblastic-like cells are present in aberrantly high numbers and are different from normal mesothelial cells. Once these cells accumulate in ascites they can be "educated" by growth factors and cytokines in the surrounding environment to support tumor growth [19]. Upon stimulation by ascites, myofibroblastic-like cells have been shown to produce dipeptidyl peptidase IV [35], which is a multifunctional protein that have been associate with tumor growth in some context [36]. Exposure of myofibroblastic-like cells to ascites increased the secretion of VEGF and other pro-survival soluble factors [19, 37]. Furthermore, data from our laboratory suggest that ascites stimulates the expression and release of MUC16 from the mesothelial cell membranes [38]. MUC16 is an oncogenic high molecular weight mucin that promotes EOC progression [39–42] and regulates the formation of multicellular spheroids [43]. Therefore, through ascites exposure, myofibroblastic-like cells become a major source of secreted factors, which in turn, further contribute to the evolution of the tumor environment. This dynamic interaction between the surrounding environment and stromal cells provides favorable conditions for tumor progression.

In addition to the complex nature of stromal cells present in ascites, this environment also appears to contain distinct populations of tumor cells displaying different phenotypic characteristics. A population of non-adherent tumor cells in 2D cultures expressing E-cadherin, EpCAM, CA125, Oct4 and STAT3 were particularly associated with diseases recurrence [44]. Tumor cells are shed from the primary tumor and aggregate in ascites. Exfoliated tumor cells will form free-floating multicellular spheroids in ascites, which range from 50 to 750 μM in size [45]. These multicellular spheroids probably represent the invasive and metastasis-forming intermediate [46]. In addition, aggregation of tumor cells is essential for anchorage-independent growth and survival. Indeed, once suspended in the peritoneal fluid, cancer cells must resist anoikis, a specialized form of apoptosis triggered by a lack of attachment to other cells or to the extracellular matrix (EMC). Recently, we have characterized multicellular spheroids from HGSOC ascites. Interestingly, we found that these spheroids contained one

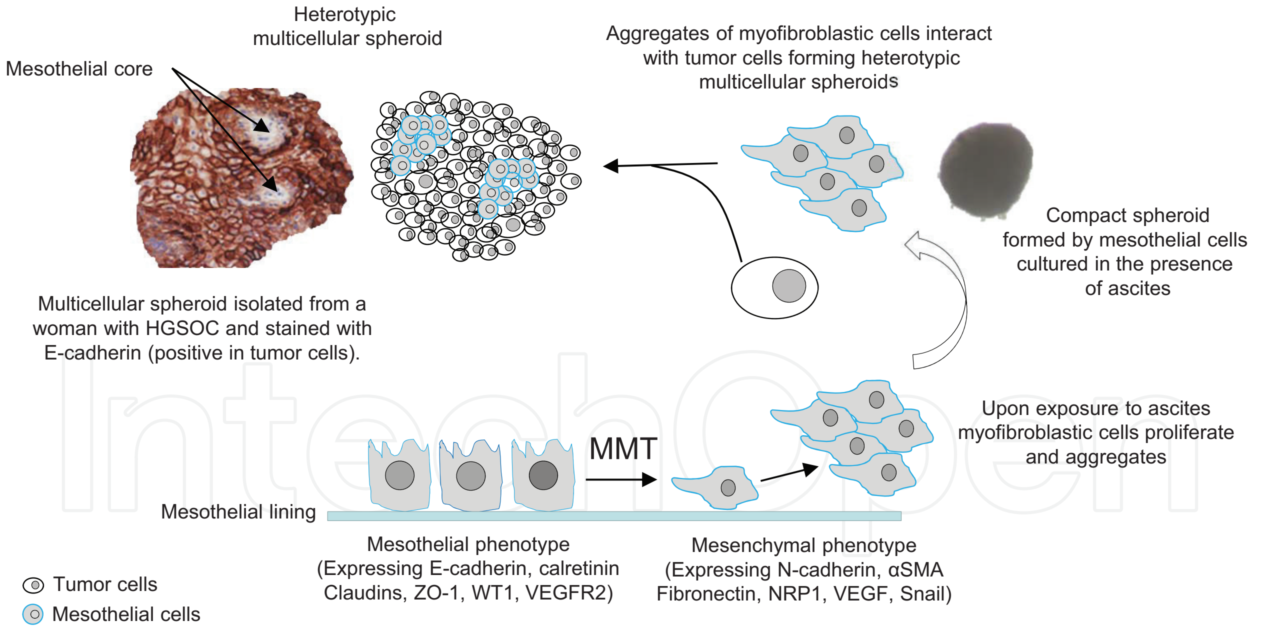

Figure 1. Model for myofibroblast cell interactions with tumor cells and spheroid formation. In response to extracellular cues in the local environment, particularly TGF-β1, mesothelial cells lining the peritoneum undergo a mesothelial-to-mesenchymal transition (MMT) characterized by dissolution of cell-cell junctions, actin reorganization and stress fiber formation. This mesenchymal phenotype is characterized by increased migration and invasion. MMT enables cells to exfoliate from the peritoneum into the existing peritoneal fluid. Unpublished results from our laboratory suggest that, upon exposure to malignant ascites, myofibroblastic-like cells aggregate to form very compact spheroids. These myofibroblastic-like cell aggregates interact with exfoliated tumor cells to form heterotypic multicellular spheroids. Mesothelial cells located in the center of spheroids may provide initial matrix support for EOC cells to avoid anoikis. Extracellular cues from the surrounding environment can induce the secretion of prosurvival factors in mesothelial cells.

or more cores of myofibroblastic-like cells encased in a shell of tumor cells suggesting that free-floating tumor and stromal cells in peritoneal effusions can interact with each other to form heterotypic spheroids (**Figure 1**). The analysis of multicellular spheroid cell components isolated from EOC ascites revealed that myofibroblastic-like cells were present in all spheroids studied [47]. Based on data derived from a 3D *in vitro* model, the interaction between myofibroblastic-like cells and tumor cells is mediated, at least in part, by β1-integrin [45, 47]. In addition, β-catenin-regulated ALDH1A1, a known cancer stem cell marker, has also been implicated in the formation of multicellular spheroids [48]. Recent studies suggest that tumor cells possess varying capacity for spheroid formation [45, 47, 49]. A positive correlation has been reported between compact spheroid formation and a mesenchymal phenotype of tumor cells [47, 49]. Therefore, aggressive cancer cell populations (mesenchymal phenotype) could gain a survival advantage through their propensity to form more compact spheroids. Recent data suggest that the presence of myofibroblasts in multicellular spheroids promotes the invasion of tumor cells [50]. These data suggest that spheroid-associated myofibroblasts may play an important role in EOC progression. In addition, these stromal cells may play a role in the early steps of spheroid formation before peritoneal implantation. Myofibroblasts located within the center of spheroids may provide initial matrix support for tumor cells to avoid anoikis. Spheroid-associated myofibroblasts may also secrete factors within the microenvironment of the spheroids that induce signaling events in tumor cells to further inhibit anoikis. Recent data suggest that tumor-associated macrophages (TAMs) may promote spheroid formation and tumor growth in a mouse model [51]. This group found that nearly 80% of macrophages infiltrated in the peritoneal cavity were detected in spheroids. Spheroid-associated TAMs were shown to secrete large amounts of epidermal growth factor (EGF), which leads to upregulation of integrin and ICAM-1 expression in tumor cells to form a positive autocrine feedback loop [51].

4. Cell-free ascites: biomarkers and EOC progression

As mentioned above, the presence of ascites is correlated with poor prognosis. In a study limited to patients with stage III/IV EOC, women without ascites had a 5-year survival rate of 45% compared to 5% for those with ascites [52]. The composition of cell-free ascites is also a major predictor of clinical prognosis. For example, EOC patients with ascites containing high IL-6 levels (>2662 pg/ml) at diagnostic had a worse outcome [53]. In that study, IL-6 was found to be an independent factor for progression-free survival. Patients with EOC and higher IFN-γ expression levels in ascites have shorter disease-free progression and overall survival [54]. Measuring cytokines in ascites may also provide a novel approach to discriminate patients with intrinsic resistance to first-line therapy [55]. The authors found that the combination of serum CA125 and ascites leptin levels was a strong predictor of clinical resistance to first-line therapy. The biochemical composition of ascites, particularly the levels of chemokines, chemokines receptors and growth factors, including CCL2, CXCL1, CXCL5, CXCL8, CXCL12, HGF, TGF-β1 and VEGF, in undifferentiated tumors could explain, to some extent, the aggressive behavior of this histotype [56]. Ascites is therefore an attractive biofluid for biomarker discovery as it is easy and minimally invasive to obtain. There is indeed growing evidence showing that proximal fluids such as ascites are valuable sources for biomarker discovery as they reflect events in ovarian tumorigenesis earlier than in peripheral blood circulation [57, 58]. The concentration of soluble factors is usually much higher in ascites compared to serum, which increases the likelihood of detecting low abundance proteins [24, 57]. In that context, proteomic/peptidomic profiling of ascites has been employed for biomarker discovery [59–61]. Different experimental approaches were used leading to the identification of various sets of biomarkers all of which requiring further validation to determine their true potential. Nonetheless, ascites profiling represents a potentially new approach for much needed new biomarkers in the context of EOC.

Beyond the contribution of specific cell types in ascites, extracellular cues from cell-free ascites have the potential capacity to drive disease progression. Cytokine profiling of EOC ascites has demonstrated elevated levels of various pro-tumorigenic cytokines including adiponectin, CXCL1, CXCL10, CCL2, CCL4, ICAM-1, IL-6, IL-8, IL-10, IL-15, PDGF-BB, RANTES and VEGF [24, 25]. Theses cytokines contribute to create an inflammatory environment that sustains chronic inflammation. Chronic inflammation, in turns, promotes tumor growth and peritoneal spread [62]. IL-6 is probably the best studied cytokine in that context. IL-6 signaling is known to be associated with specific immune and metabolic alterations that lead to cancer cachexia, which is often seen with advanced diseases. IL-6 plays an important role in the development of ascites as well as the spread of EOC through, at least in part, its induction of tumor angiogenesis [63]. In support for the role of IL-6, we found that IL-6 and sIL-6R are significantly higher in ascites obtained from women with advanced diseases compared to women with stage I/II EOC (**Table 1**). VEGF is a well-established factor that increases vascular permeability. VEGF binding to its receptor activates focal adhesion kinase (FAK) which localizes to the cytoplasmic tail of VE-cadherin at endothelial cell-cell junctions. FAK phosphorylates β-catenin, which destabilizes the cell-cell junctions, resulting in increased vascular permeability [64]. Metabolome profiling of ascites has revealed significant differences in fatty acids, cholesterol, ceramide, glycerol-3-phosphate, glucose and glucose-3-phosphate compared to non-cancerous peritoneal effusions [65]. Whether these changes directly contribute to oncogenic signaling or they merely reflect upregulation of pathways of the fatty acid synthesis associated with increased metabolic activity in tumor cells remains to be determined.

There is extensive cellular crosstalks and signaling events between the surrounding environment and tumor cells during EOC dissemination and progression. As a result, ascites is constantly adapting in response to the different cues. In order to characterize the changes in ascites during EOC progression, we have performed cytokine profiling of stage I/II and III/IV serous ascites. As shown in **Table 1**, 29 cytokines/chemokines/growth factors out of 120 tested were present at significantly higher levels in stage III/IV ascites supporting the idea that ascites evolve during EOC progression. Consistent with the critical role of IL-6 in EOC progression, we found several components of the IL-6 trans-signaling system, including IL-6, IL-6 receptor (IL-6R), and soluble glycoprotein 130 (sgp130), elevated in ascites of women with advanced diseases. Factors such as CCL2 have been implicated in CAFs activation [12]. As mentioned above, once stimulated myofibroblastic-like cells in ascites provide a source of secreted factors that support tumorigenesis.

Cytokines	Serous Stage I–II RFU[a] (SEM) (n = 2)	Serous Stage III–IV RFU (SEM) (n = 5)	Fold change	*p*
IL-6	1352 (302)	14183 (10619)	10.5	<0.0001
Angiopoietin-2	6293 (4081)	14683 (11235)	9.8	0.0076
IL-10	457 (45)	4220 (3752)	9.2	0.0003
Leptin	561 (102)	4991 (5849)	8.9	0.0031
sTNF RI	828 (260)	5238 (2768)	6.3	<0.0001
uPAR	1092 (488)	6417 (3387)	5.9	<0.0001
CXCL1	2569 (1386)	14926 (12569)	5.8	0.0003
HGF	1017 (212)	5504 (4708)	5.4	0.0004
OPG	978 (394)	3493 (1606)	3.6	<0.0001
CCL2	1918 (480)	8032 (5439)	4.2	0.0001
Fit-3 ligand	739 (129)	3092 (2119)	4.2	0.0001
CCL16	593 (146)	2313 (1713)	3.9	0.0003
CCL7	630 (119)	2366 (2184)	3.8	0.0021
IL-1 R4/ST2	709 (188)	2194 (2078)	3.1	0.0049
CCL22	776 (131)	2301 (1246)	3.0	<0.0001
ICAM-1	4107 (861)	11832 (4961)	2.9	<0.0001
EGFR	871 (239)	2514 (1937)	2.9	0.0013
IGFBP-6	1274 (594)	3668 (1537)	2.9	<0.0001
IL-16	654 (106)	1814 (1738)	2.8	0.0077
CXCL13	679 (98)	1851 (1477)	2.7	0.0022
Axl	1039 (412)	2578 (856)	2.5	<0.0001
CXCL9	773 (103)	1903 (1308)	2.1	0.0017
sTNF RII	2393 (759)	5301 (1694)	2.5	0.0011
Fas	1487 (557)	3779 (3301)	2.5	0.0067
IL-3	734 (191)	1720 (1058)	2.4	0.0006
CCL4	1312 (369)	2739 (1704)	2.2	<0.0001
CCL19	797 (184)	1712 (1521)	2.2	0.0155
IGFBP-1	2827 (1092)	6007 (4692)	2.1	0.0091
IL-6 R	3602 (1009)	7160 (4835)	2.0	0.0048
MIF	2920 (916)	5460 (3396)	1.9	0.0051
sgp130	1510 (359)	2510 (852)	1.7	0.0002
TIMP-1	1189 (233)	1669 (833)	1.5	0.0268

SEM: standard error of the mean.

[a] Relative fluorescent unit.

Table 1. Levels of cytokines in stage I/II versus stage III/IV ovarian cancer ascites.

Therefore, disrupting specific factors in cell-free ascites may provide an additional level of therapeutic intervention.

5. How does the tumor environment affect EOC dissemination?

One of the reasons for unsuccessful EOC treatment is its insidious nature, resulting from an unusual mechanism of dissemination. In contrast to other tumors that spread predominantly through lymph and bloodstream, EOC has a distinct tendency for metastasizing via shedding of cancer cells from the primary tumor site into the peritoneal cavity and implanting onto the mesothelial lining of the peritoneal cavity. The current admitted model for pelvic and peritoneal metastasis involves the shedding of tumor cells from the primary tumor into the abdominal cavity, wherein they survive and travel as free-floating multicellular spheroids to disseminate at distant sites where they adhere onto the mesothelial lining of the peritoneum and disaggregate to form metastatic outgrowth (**Figure 2**). Although not clearly define, each of these steps must require adaptive changes in tumor and/or stromal cells to progress to the next step.

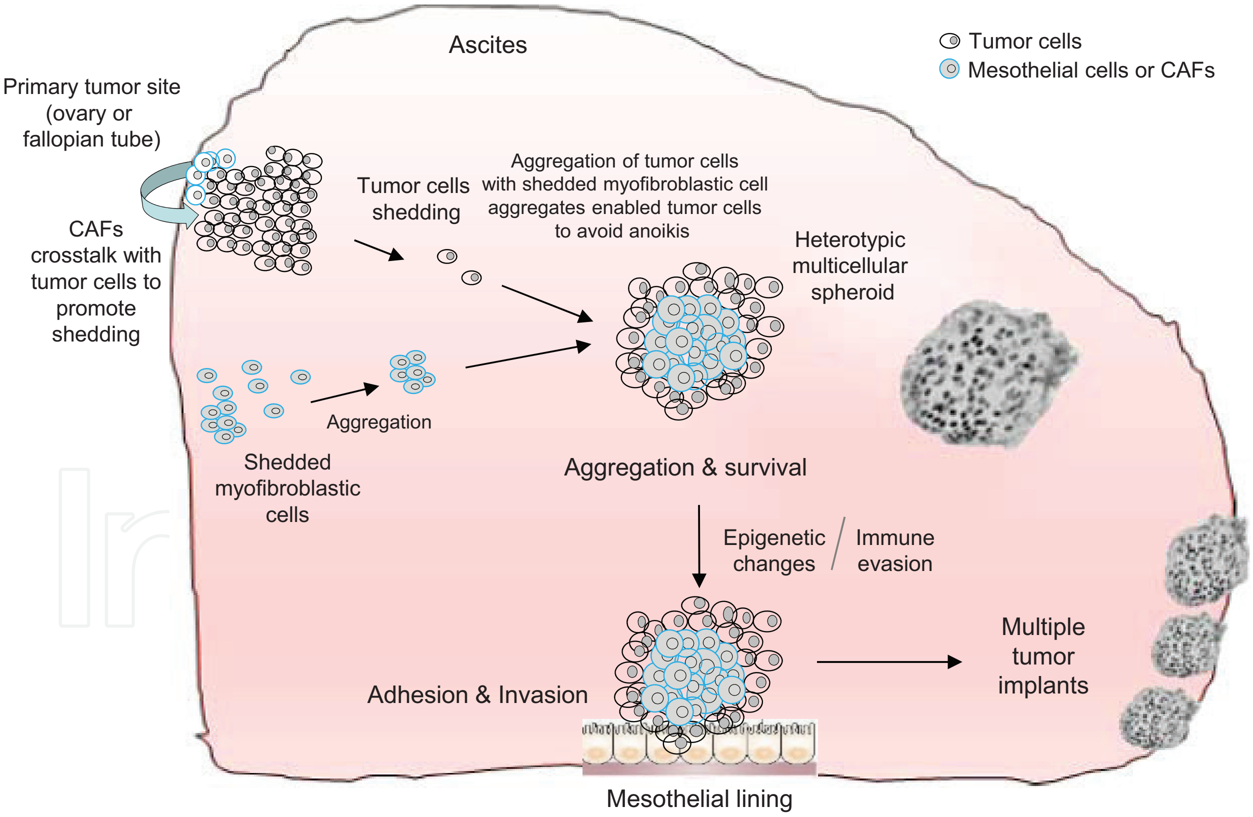

Figure 2. Model for EOC dissemination. CAFs in the primary tumor become educated by the tumor cells to acquire pro-tumorigenic functions. CAFs then in turns secrete a plethora of factors that enable tumor cells to exfoliate from the primary tumor. Once in the peritoneal fluid, tumor cells aggregate with free-floating mesothelial cells to form multicellular heterotypic spheroids, which enables tumor cells to avoid anoikis and gain a more invasive phenotype. Multicellular spheroids then attach to the mesothelial lining using various cell adhesion molecules. Mesothelial cells lining the peritoneum dissociate which enables tumor cells to invade to mesothelium lining.

A single mesothelial cell layer lines the pelvic and peritoneal organs including the diaphragm, bowel serosa, omentum and entire peritoneum. This mesothelial layer is highly receptive to ovarian cancer seeding [66]. Implantation of spheroids on the peritoneum involves interactions between cancer cells and the mesothelium. Adhesion of ovarian cancer cells to the mesothelial layer is facilitated by the expression of matrix metalloproteinase such as MMP-2 and MMP-9, and by fibronectin and vitronectin as well as their integrin receptors [67–69]. Once tumor cells have attached to the peritoneal surface, they gain access to the submesothelial environment by exerting force on the mesothelial lining, driving migration and clearance of the mesothelial cells [70]. Tumor cells undergo epithelial-to-mesenchymal transition (EMT) during the process [71].

Cells shed from the primary tumor aggregate to form free-floating multicellular spheroids in ascites, which initially spread to adjacent organs such as uterus, contralateral adnexa, bladder and rectum (stage II). After extension to the pelvic cavity, EOC will disseminate throughout a transcoelonic route to the peritoneal cavity forming multiple tumor implants (stage III), which are often difficult to remove completely at the time of the cytoreductive surgery and, substantially contribute to the high morbidity associated with this cancer. Metastasis can also occur beyond the abdominal cavity (stage IV). Whether the metastatic characteristics are already inherent in the primary tumor or are present only in subclone of metastatic cells within the primary tumor mass or occur in response to environmental cues remains unclear. This process of transcolonic seeding could be a continuing metastatic adaptive behavior or a passive process, in which exfoliated tumor cells that have already acquired all the necessary metastatic characteristics are merely transported via ascites into the peritoneal cavity to new sites. Comparative genomic studies showed similar genetic alterations in primary ovarian tumors and their respective metastasis supporting a passive transcolonic dissemination. However, transcriptomic analysis of matched primary tumors and peritoneal metastasis demonstrated the upregulation of certain pathways in metastatic lesions which suggest that the heterogeneity of tumor cells found in EOC is imposed, at least in part, by the nature of their surrounding environment [72]. The same group identified versican as a key upregulated gene in CAFs associated with the primary tumor, which promoted the motility and invasion of EOC cells by activating the nuclear factor-κB (NF-κB) signaling pathway and upregulating CD44, MMP-9, and hyaluronan-mediated motility receptor expression in cancer cells [73]. Versican expression was modulated by the activation of TGF-β signaling in CAFs induced by TGF-β ligands secreted by cancer cells. Therefore, these data further support the idea that ascites play an active, rather than a passive, role in EOC dissemination.

6. What are the effects of ascites on tumor cells?

The observation that ascites is often associated with the most invasive malignant tumors indirectly supports the notion that ascites is involved in the progression of EOC. Although different soluble factors in ascites have been implicated in EOC cell migration and invasion, the combined effect of the various factors found in cell-free ascites is also important to assess. Puiffe and colleagues have assessed the effect of 54 distinct ascites on growth, invasion and spheroid formation in comparison to serum in a single cell line [23]. They showed that ascites fell into one of two categories: stimulatory or inhibitory. The mechanisms or factors responsible for these opposite effects were not further investigated. Consistent with the results of Puiffe et al., Lane et al. showed that not all EOC ascites tested (2/6) promoted cancer cell migration [29]. In this study, the authors found that CCL18 was one of the factors in ascites implicated in ascites-induced cell migration. As such, CCL18 might represent a potentially new target in EOC treatment.

HGSOC ascites possess pro-survival properties. Ascites inhibits drug and TRAIL-induced apoptosis in EOC cells. Unsurprisingly, given the heterogeneity of ascites, the magnitude of the effects varies depending on the cell line and ascites tested [74, 75]. Multiple signaling pathways are activated by ascites in cancer cells, including up-regulation of anti-apoptotic protein Mcl-1 through ERK1/2-Elk-1 [76], up-regulation of anti-apoptotic protein c-FLIP [74], and activation of Akt through $\alpha v\beta 5$/FAK signaling [75, 77], all of which contributing to the pro-survival effect of ascites. Collectively, these data support the notion that ascites is a tumor environment enriched with pro-tumorigenic molecules. A considerable effort is required however to gain a comprehensive understanding of how the different factors in ascites may alter the properties of tumor and stromal cells. The complexity of these processes requires the development of models that reflect the *in vivo* conditions as close as possible.

7. How can we exploit ascites for developing new therapeutic strategies?

More effective therapies to combat metastatic disease are urgently required for EOC, particularly in the context where early detection of this disease remain a difficult goal to achieve. Since the prognosis of patients with peritoneal metastases is directly correlated with optimal surgical cytoreduction [78], and widespread metastases are not often entirely amenable to surgery, the development of novel strategies to limit or stop metastatic progression is imperative. In that context, novel strategies that target interactions between cancer cells and their environment and inflammation-driven modifications are likely to be broadly applicable to cancers that metastasize within the abdominal cavity. In addition, as stromal cells are genetically more stable compared to tumor cells, targeting stromal cells rather than tumor cells would be less prone to the development of resistance. Thus, targeting the tumor environment may be a more compelling option.

Based on our increasing knowledge of the role of ascites and its components, a number of targeted specific therapies have been developed to improve EOC outcome. Bevacizumab, an anti-VEGF targeted therapy, is probably the most studied VEGF-targeting agent in EOC patients in the setting of front-line, maintenance or salvage therapy [79]. Although VEGF-targeting agents have yielded promising results in EOC in the settings of front-line and salvage treatment, the efficacy of these agents has yet to be clarified. Therapies taking advantage of the immune system could represent another potential avenue. For example, intra-peritoneal infusion of Catumaxomab, an anti-epithelial cell adhesion molecule (EpCAM), provided a significant improvement of ascites-related signs and symptoms [80]. Catumaxomab mediates a T-cell-induced lysis of tumor cells. Abagovomab is a murine monoclonal anti-idiotypic antibody that mimics parts of CA125. It is designed to act as an active immunogen aimed at breaking immune tolerance to the antigen. Unfortunately, abagovomab showed no improvement in progression-free or overall survival in a phase III clinical trial [81]. Another anti-CA125 antibody, Oregovomab, also failed to show improved outcome in EOC patients [82]. Anti-IL6 chimeric antibody Siltuximab has been assessed in phase II clinical trial but has shown very limited clinical benefits [83]. Other emerging strategies include the concept of neutralizing tumor-associated chronic inflammation as ascites in a highly pro-inflammatory environment [84].

8. Conclusions and future directions

There is an increasing interest for understanding the role of the tumor environment in the context of ovarian cancer. Recent studies have revealed new biological concepts and identified new therapeutic strategies to target the ascites. As illustrated by the limited clinical success obtained thus far, many challenges remain, including how to identify and target susceptible molecules given the complexity and heterogeneity of the tumor environment. Although the heterogeneity of ascites is a potential limitation, it also provides a unique opportunity for the development of personalized medicine based on the patient's characteristics. In that context, the profiling of the cell-free ascites components could guide clinical decision making for patient management. An important aspect to overcome limitations to unsuccessful clinical trials is the development and implementation of suitable *in vitro* and *in vivo* pre-clinical models that accurately mirror the clinical situation. For example, mounting evidence suggests that cell behavior in 3D cultures differs from monolayer cultures and better reflects the *in vivo* situation.

The accessibility of ascites translates into a readily available source of proximal fluids. In that context, ascites is a milieu from which we could potentially derive diagnostic and prognostic biomarkers. With the advances in our understanding of the crosstalk between the different cellular components of ascites and the various cues that cells receive from the surrounding environment, it is anticipated that reliable biomarkers will become available in the near future.

Acknowledgements

This work was supported by internal funding from Université de Sherbrooke.

Conflict of interest statement

Author declares no conflict of interests for this article.

Author contribution

Piché A. drafted the paper and wrote the final version. Matte I. and Bessette P. reviewed the draft and approved the final version.

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