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Chapter 4

A LOOK AT THE MANAGEMENT AND USAGE OF MALIGNANT ASCITES IN OVARIAN CANCER

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ABSTRACT

Ovarian cancer is known to be the most lethal gynaecological cancers because of the late-diagnosis. A common complication found in patient with advanced ovarian cancer is the abnormal buildup of ascitic fluid in the peritoneal cavity. Malignant ascites correlates with a dismal quality of life, high morbidity, and other troublesome symptoms. Consequently different therapies have been investigated and tested on patient in order to control ascites production and improve quality of patient life. In parallel, since malignant ascites is considered as a critical point, studies have been assessed on the role of malignant ascites in ovarian cancer. In this chapter, we reported information about the management of malignant ascites in ovarian cancer and discuss the

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involvement of malignant ascites in dissemination, angiogenesis, migration, invasion and apoptosis of ovarian cancer cells.

INTRODUCTION

Ovarian cancer remains the most lethal gynaecological cancers and the seventh highest cause of cancer death in women worldwide. Overall, 75% of patients with ovarian cancer are diagnosed with advanced-stage disease [1]. This is characterized by widespread metastasis throughout the peritoneal and abdominal cavities [2].

Ovarian cancer is often classified using the International Federation of Gynaecology and Obstetrics (FIGO). FIGO uses 4 main stages to identify the progression of the cancer's severity. Stage 1 is when the cancer is limited to ovaries. When cancer has spread to some other organs within pelvis, but not to the lymph nodes, it is in stage 2. Ovarian cancer at stage 3 has spread to the peritoneal cavity and to the lymph nodes. At Stage 4, a high number of organs have affected such as the liver, lungs, and spleen [3].

Current treatment for women at these stages combines surgical cytoreduction and chemotherapies. Even with aggressive primary therapy and good initial response rates, the majority of patients with advanced staged-disease develop recurrent, chemotherapy-resistant cancer. Because of this, the patients face extended exposure to multiple cytotoxic and debilitating chemotherapies [1, 4, 5]. A common complication associated with advanced ovarian cancer is the abnormal buildup of ascitic fluid in the peritoneal cavity, which correlates with a dismal quality of life, high morbidity, and other troublesome symptoms [6].

In other solid malignancies, ascites indicate a poor prognosis, with only 11% of patients surviving longer than 6 months. Unlike this, EOC patients with ascites can expect a median progression-free survival of 16-22 months. 5 year survival rates lie around 27% [7]. This difference can be partially explained by the biology of ovarian cancer and the etiology of abdominal fluid accumulation. Malignant ascites in EOC patients largely contribute to lymphatic obstruction, increased neo-angiogenesis, and its subsequent hyperpermeability of microvessels lining the peritoneal cavity [4].

In this review, we want to report on general information about malignant ascites and their management, as well as the different hypotheses about the role of ascites in ovarian carcinogenesis (cell dissemination, angiogenesis, migration/invasion and response to therapy).

1. ASCITES

1.1. Definition of Ascites

Ascites are defined by an abnormal accumulation of liquid in the abdomen and, more specifically, in the peritoneal cavity. The composition of malignant and non-malignant ascites is different: malignant ascites contain tumour cells, mesothelial cells, fibroblasts, macrophages, white blood cells and red blood cell [7]. Ascites can be classified in two ways: by how easily they can be detected or by their composition. For example, Grade 1 is only visible on ultrasound and CT; Grade 2 is detectable with flank bulging and shifting dullness; and Grade 3 is directly visible. With respect to composition, ascites were previously categorized as either transudates or exudates, depending on the fluid's protein concentration. They were classified as exudates if the protein concentration is higher than 2.5g/dL, and as transudates if lower. Exudates are associated with lymphatic obstruction and increased capillary permeability, whereas transudates are linked to portal hypertension caused by liver metastasis. Transudates ascites are also linked to the spread of cancer cells in peritoneal cavity. Recently, another classification system replaced that of transudates and exudates: the serum-ascites albumin gradient (SAAG). This is calculated by subtracting the ascitic fluid albumin value from the serum albumin value. A high SAAG (>1.1g/dL) in ascites corresponds to an increase in blood pressure and portal hypertension; a low SAAG (<1.1g/dL) in ascites suggests a peritoneal causes of ascites [8].

1.2. Malignant Ascites in Ovarian Cancer

About 10% of all ovarian cancers present ascites [4]. Malignant ascites are produced more frequently in ovarian cancer than in other tumour types because ovarian cancers commonly develop intraperitoneal metastasis [9]. Symptoms of ascites include anorexia, dyspnea, fatigue, vomiting, abdominal pain, and abdominal swelling [7]. Ascites production was mainly observed in patients with advanced stages: stages II and III. In stage I, few patients present a production of ascites (around 17%) [10]. At advanced stages, cancer cells from ovaries can migrate and disperse into the peritoneal cavity.

A combination of factors can contribute to the formation of ascites in ovarian carcinoma. Cancer cells can reduce lymphatic drainage from the peritoneal cavity by obstructing lymphatic vessels and preventing absorption.

Also, ascitic fluid can increase as a result of hyperpermeability of microvessels lining the peritoneal cavity and tumor neoangiogenesis. The subsequent increase in vascular permeability releases inflammatory cytokines. In the case of ovarian cancer, ascites is classified as exudative and malignant [1, 11].

The formation of ascites is dependent on abnormal tumor vascularity and permeability, which was demonstrated by a series of molecular biology and xenograft model studies [4]. Byproducts of newly formed and leaky capillaries, such as increased capillary permeability of the microvasculature and extravasation of plasma proteins and water in the tumor milieu, can also cause ascites formation [4, 12].

1.3. Management of Malignant Ascites in Ovarian Cancer

At this moment, different strategies have been proposed to manage ascites in ovarian cancer, but there is not yet one specific method that definitively manages them. These therapies include non-molecular therapy such as paracentesis, catheter drainage, diuretic therapy or Concentrated Ascites Reinfusion Therapy, and molecular therapy such as Catumaxomab or anti-angiogenesis treatment [4, 13].

1.3.1. Non-Molecular Therapy

1.3.1.1. Paracentesis

Paracentesis is a method used to drain and evacuate excess fluid from the peritoneal cavity. This technique is the most common one to treat ascites in ovarian cancer [14]. To remove excess fluid or to inject a therapeutic agent, a small incision is made in the peritoneal cavity and a trocar is inserted. The first drainage can remove up to 5 litres of ascetic fluid, but if the patient is too frail, the drainage can be stopped at 2 litres [15]. The drainage relieves the patient's discomfort, and also allows the attending to investigate infections. However, these treatments can have a negative impact on patients. Multiple paracentesis induce protein loss and hypovolemia, increasing the frequency of circulatory problems [16]. Despite this, it remains popular and is the preferred method of treatment for patients with especially bulky and chemo-resistant tumors.

1.3.1.2. Catheter Drainage

Another alternative method is catheter drainage. Studies report that this is a safe and effective palliative strategy associated with low complication [17,

18]. A recent study on 38 patients diagnosed with metastatic disease shown that a PleurX catheter can have a high rate of procedural success and a low rate of infections and complications [19]. Catheter drainage is recommended in resistant cases and in recurrent production of malignant ascites. A recent study done on 55 patients with malignant ascites, 43 of whom had ovarian cancer, confirms that catheter drainage is a safe, simple, and cost-effective method for patient with recurrent malignant ascites [13].

1.3.1.3. Diuretic Therapy

Diuretic therapy is another way to manage malignant ascites. However, few studies have been done on the concrete benefit of diuretic therapy [20]. Moreover, one study shows that this treatment can dehydrate patients [21]. It has been demonstrated that diuretic therapy is not overall efficient in the case of malignant ascites, but more information and studies are still necessary to definitively assess its efficiency.

1.3.1.4. Concentrated Ascites Reinfuision Therapy (CART)

In 1977, concentrated ascites reinfusion therapy, or CART, was developed by Japanese research groups as a method that improves the quality of life of patients with malignant ascites without adding therapeutic molecules to their bodies [22, 23]. With this method, the cellular fraction of ascites is filtered out to remove bacteria and cancer cells, concentrated, and then this protein-rich acellular fraction is re-injected to improve the quality of life for patients who go through paracentesis, or abdominal tapping. As discussed before, paracentesis, though effective, is not without complications. Patients experience discomfort from the sudden fluid and protein loss, and repeated taps are usually necessary. However, by filtering the ascites and injecting them back into the body, some of these unfavorable side effects can be avoided. Several studies about the application of CART for patients with gastrointestinal cancer have demonstrated a real benefit to the quality of patient life by decreasing in malignant ascite symptoms [24-26]. Regarding ovarian cancer, studies are more recent but show encouraging results. In fact, in studies evaluating symptoms before and after the first CART, there was a significant decrease in the severity of symptoms such as abdominal tension and fatigue after CART treatment [27-30].

1.3.2. Molecular Therapy

Most patients relapse and continue production of ascites after non-molecular therapy. Thus, investigation groups are trying to find new molecules

to control production of ascites. For example, studies have shown that after injection of octreotide (inhibitor of growth hormone), the volume of malignant ascites was reduced and the period before it was necessary to have another paracentesis was shortened, but the difference was not significant [31, 32]. Then, other studies using mixantrone (using to block cell division) and tumor necrosis factor (TNF) have demonstrated a reduction of ascites reduction and a reduction of the frequency of paracentesis [33-35]. However, no other studies have investigated the effect of TNF or mixantrone or octreotide on recurrent malignant ascites in ovarian cancer. Nevertheless, other molecules have been introduced and are the subject of promising research for the management of malignant ascites.

1.3.2.1. Catumaxomab

A number of investigations have been done on catumaxomab, a trifunctional bispecific monoclonal antibody. It binds epithelial cell adhesion molecule (EpCAM) to tumor cells and binds the CD3 antigen to T-cells and to type I, IIa and II Fcɣ receptors on accessory cells [36]. In 2007, a pilot study on 23 patients with recurrent malignant ascites showed a decrease in accumulation of ascites after treatment with catumaxomab [37]. It was approved in Europe in April 2009 for the intraperitoneal treatment of patients with malignant ascites [38], making it the first drug approved for the treatment of malignant ascites [39]. In 2011, Ott et al. did a pilot study on 258 patients with malignant ascites and found a correlation between humoral response (human antimouse antibodies (HAMA)) and clinical outcome after catumaxomab treatment, indicating that HAMAs could be good biomarkers for the response to catumaxomab [40]. In 2013, Goéré et al. investigated the immunomodulatory effect of catumaxomab and concluded that it enhanced T-cell activation and stimulated inflammatory molecules by activating TRAIL. They also demonstrated that catumaxomab promotes cell death [41]. However, the side effects of catumaxomab treatment include pyrexia, nausea, vomiting and abdominal pain. Recently, Shouli et al., did a study with a combination of catumaxomab and prednisone to try to reduce side effects. They did not observe improvement from the addition of prednisone, but their results did further confirm the safety and efficacy of catumaxomab for the treatment for malignant ascites [42]. In addition, catumaxomab treatment increased the puncture-free interval [43].

1.3.2.2. Anti-Angiogenesis Treatment

Angiogenesis and vessel hyperpermeability occur during ascites formation. Consequently, many studies have been published about inhibiting angiogenesis. Molecules such as Albendazole [44], TGF-β Receptor II [45], batimastat [46], and endostatin [32] have been tested to inhibit angiogenesis and, thus, to control malignant ascite formation. But, at the moment, the best angiogenesis inhibitor in use is avastin (bevazimuab), which is an antibody against the vascular endothelial growth factor (VEGF).

Numnum et al. investigated the effect of bevacizumab to palliate symptomatic ascites in patient with ovarian cancer. 4 patients were treated with bevacizumab and, for each case, symptomatic relief of ascites was observed [47]. A 2007 study demonstrated that after an intraperitoneal dose of bevacizumab, there was no reaccumulation of malignant ascites and thus no needed repeat of paracentesis [48]. In 2008 and 2010, three more case reports showed that treatment with bevacizumab reduced ascites and prevented paracentesis repetition. These suggest that bevacizumab could be used for the management of recurrent malignant ascites [49-51]. A recent study of 58 patients in stage III cancer also supports claims of the drug's efficiency. In this one, patients received a combination of bevacizumab and cisplatin or cisplatin-only. In patients treated with cisplatin/ bevacizumab, VEGF levels in ascites and the volume of ascites were lower compared to the cisplatin-only group. In addition, no serious adverse effects have been observed [52].

Another way to inhibit VEGF is through VEGF trap. VEGF trap, or aflibercept, prevents VEGF receptor binding. In ovarian cancer mouse models, it was observed that VEGF-trap treatment strongly reduced accumulation of ascites [53]. Combined with paclitaxel, VEGF-trap facilitated a complete resolution of symptoms associated with ascites [54]. Two different studies, on patients with epithelial ovarian cancer and malignant ascite production, published in 2012 have concluded that aflibercept can reduce and control malignant ascites and decrease the repetition of paracentesis. However, these studies also warned against serious side effects such as hypertension, headache, anorexia, dysphonia, and fatal bowel perforation [55, 56]. At the beginning of 2015, a case report was published on a patient whose ovarian cancer had relapsed and was subsequently treated with aflibercept. This treatment had a prolonged response with good tolerance and also inhibited formation of ascites [57]. Still, though, few studies have been studied aflibercept, so further clinical studies are essential before accepting this drug as a true option for management of malignant ascites in ovarian cancer.

Table 1. Methods for the management of malignant ascites in ovarian cancer

Non-molecular therapy	Molecular therapy
• **Paracentesis:** most popular method despite an increase in the frequency of circulatory problem (14-16) • **Catheter drainage:** safe, simple, cost effective method, low complication (13,17-19) • **Diuretic therapy:** not very efficient, dehydration of patients, few studies (20,21) • **CART:** improves to the quality of patient life with decreasing of symptoms severity (22-30)	• **Catumaxomab:** trifunctional bispecific monoclonal antibody activating TRAIL leading cell death, approved in Europe in 2009, several side effects and increase the puncture free-interval (36-43). • **Anti-angiogenesis treatment:** = Antibodies against VEGF : bevacizumab (Avastin) (47-52): • Reduces ascites formation • No serious side effect = VEGF trap : Aflibercept (53-57): • Prevents VEGF receptor binding • Reduces ascites formation • Presence of serious side effects

At this moment, no guidelines for the management of ascites in ovarian cancer have been solidified. However, different ways to manage ascites have been proposed and practiced resumed in Table 1. Out of these, antiangiogenesis treatments are more used than other treatments. However, Catumaxomab is also a very good option for the treatment. Finally, CART seems to be a promising therapy to improve quality of patient life. More knowledge about malignant ascites could help to develop targeted or palliative therapies for patients with ovarian cancer.

2. ROLE OF MALIGNANT ASCITES IN OVARIAN CANCER

2.1. Dissemination

2.1.1. Routes of Dissemination

Epithelial ovarian carcinomas (EOC) disseminate contiguously [58]. After direct extension into adjacent organs, dissemination can occur via the transcoelomic, hematogenous, or lymphatic routes. Among these routes, transcoelomic metastasis is the most common type of dissemination, which is often associated with the formation of malignant ascites [11].

Lymphatic dissemination occurs in about 14-70% of patients and is mainly found in the pelvic and para-aortic lymph nodes [59]. In a clinical trial of Ayhan et al. the number of metastatic lymph nodes and the presence of lymphatic metastasis were shown to be significantly higher in advanced staged disease and patient groups with ascites. Furthermore, intestinal or omental metastasis was also correlated with the presence of ascites, which explains the natural parallel spreading behavior of intraperitoneal ovarian tumors to the retroperitoneum [60].

Interestingly, unlike most other cancers, ovarian carcinoma barely disseminates through the hematogenous route [1]. This implies that malignant cells are capable of acquiring nutrients to survive and proliferate while lacking normal solid-phase scaffolding [2].

Although studies have tried to find evidence of metastatic behavior of serous ovarian carcinoma outside of the peritoneal cavity, it has been shown that it is confined to within the peritoneal cavity [1]. This was confirmed by a study in which patients were treated with peritoneovenous shunting for malignant ascites. Shunting, or surgical insertion of a shunting tube to drain ascites from peritoneal cavity to the venous system, is used for palliative clinical intervention, instead of repeated paracentesis. Although the patients received direct infusion of billions of malignant tumor cells into the venous system for up to 2 years, most patients of this study did not become overwhelmed by metastases, and some were completely free of it [61].

2.1.2. Ascites as a Carrier

Dissemination occurs when cancer cells detach from the primary tumor and are transported away within the abdominal cavity. This can occur through a series of proteolytic pathways and epithelial-to-mesenchymal transitions (EMT), which are up-regulated in ascitic environments. Thus, extensive seeding of the exfoliated tumor cells throughout the peritoneal cavity is often correlated with malignant ascites [1].

EMT occurs under normal physiological conditions in response to regenerative stimuli such as ovulatory rupture. The process of repairing the surface after ovulation and modifying its extracellular matrix are physiologically balanced. However, within the tumor microenvironment, models of carcinogenesis in vitro showed that ovarian surface epithelium cells are capable of converting themselves to have a mesenchymal and fibroblast-like phenotype. Cells seem to use this as a rapid tool for acquiring metastatic potential [1, 11]. Before exfoliated cells start their metastatic journey, they

undergo EMT to acquire an invasive and migratory phenotype, which confers the hallmark survival advantage of cancer cells. The transformed cells, looking more like fibroblasts, survive in the hypoxic condition. EMT allows epithelial cells to attach to the basement membrane and provides signaling pathways with stromal cells, which results in increased proliferation and motility, respectively [1, 11].

Once malignant cells detach from the primary ovarian tumor as single cells or as multicellular spheroids, they implant in static sites along the peritoneal fluid circulation through a passive mechanism. They are carried to the peritoneum and omentum by a peritoneal fluid vehicle, whose movement is influenced by hydrodynamics and gravity factors. In the absence of ascites, this process does not occur and the movement of cancer cells is largely restricted to the primary site [5].

In a study regarding dissemination, Carmignani and collaborators suggested that intraperitoneal fluid and peritoneal surface motions were prominent mechanisms controlling patterns of spread in carcinomatosis. They concluded that the direction that the detached cancer cells take within the peritoneal cavity depends on the histological type of tumor involved, as well as other factors such as gravity, negative sub-diaphragmatic pressure, organ mobility, peristalsis, reabsorption of peritoneal fluid, and the viscosity and volume of fluid within the abdomen [62].

Ongoing research is directed toward having a better understanding of the peritoneal environment and its participation in EOC carcinomatosis. Serous lining of mesothelial cells covers the peritoneum cavity and forms a potential space for ascites development around the major abdominal and pelvic organs. Under normal homeostatic conditions, the peritoneal cavity permits important passive and active exchange of substances between peritoneal fluid and lymphatic and blood vessels [11, 63].

Ovarian carcinomas most commonly metastasize in the omentum and peritoneum. Within the peritoneum, tumors preferentially colonize in the right diaphragm and small bowel mesentery. This indicates that the distribution of ovarian carcinoma cell metastasis might be not completely random. However, at this time, we do not know if these preferential sites are primed to be metastatic niches or if there is a mutual regulatory relationship between specific peritoneal cells and EOC cells that may create a microenvironment favorable to carcinomatosis [1, 63].

2.2. Angiogenesis

2.2.1. General Information

The formation of new blood vessels, or angiogenesis, is mediated via multiple proangiogenic and antiangiogenic molecules under physiological homeostasis. The ovary is a unique organ characterized by physiological-balanced angiogenesis, in response to stimulation of follicle cells and to increased permeability of follicle vessels [64].

In the tumor microenvironment, this process plays a key role in growth beyond 1-2mm^3 in size. The new tumor vasculature is highly disorganized, tortuous, and leaky when compared with normal vessels, which implies that angiogenesis may be the pivotal regulator for both ovarian tumor growth and ascites formation [4, 65].

Among the numerous pro-angiogenic factors that have been described, VEGF family and its receptors represent an essential pathway. VEGF is a glycoprotein that stimulates the full cascade of events required for angiogenesis *in vitro* and *in vivo* [12]. VEGF-A was first identified and cloned in 1989, and it is known that the VEGF family is composed of VEGF A-E and the placental growth factors, PIGF-1 and PIGF-2 [4, 11, 12, 66].

This signaling pathway includes the cell surface tyrosine kinase receptors VEGFR-1, VEGFR-2, and VEGFR-3. The ligand-receptor interaction occurs to provoke the downstream signaling pathway [12]. Consequently, this ligand-receptor axis interaction via has emerged as a very promising therapeutic target and clinical trials [5].

2.2.2. Angiogenesis and Ascites

Ascitic fluid is complex and heterogeneous. It contains variable proportions of suspended cells and debris. Many of these soluble factors provide a protective environment for tumor cells, and enhance tumor cell proliferation through autocrine and paracrine networks [67]. For example, a study on VEGF expression in ovarian cancer showed that lysophosphatidic acid (LPA) in ascitic fluid binds to a receptor for the endothelial differentiation gene (Edg4). Once transcription of Edg4 is activated, expression of VEGF promoter increases. Thus, the level of angiogenesis is significantly increased [68, 69].

High concentrations of VEGF have been found in the ascitic fluid of patients with ovarian cancer. In their experiment regarding ascitic fluid of

human OVCAR-5 ovarian cancer in mice, Yu-Long Hu et al. found that the VEGF concentration is 50-200 times higher in malignant ascites fluid than in non-malignant fluid [68]. Furthermore, reduction of malignant ascites in vitro is associated with inhibition of VEGF activity [11, 68].

There are a number of factors that may foster the expression of the VEGF family by OC cells, including hypoxia, acidosis, and mechanical stress, as well as changes in tumor suppressor gene expression [5]. Other factors that may augment production of VEGF are LPA, tumor necrosis factor, endothelin1, cyclooxgenase1, IL-1β, matrix metalloproteinase, insulin-like growth factor1, epidermal growth factor, platelet-derived growth factor, and transforming growth factor [7]. Among histological subtypes, higher VEGF levels are found in serous adenocarcinoma and clear cell tumors [12].

A significant correlation between VEGF and IL-8 in EOC patients' ascites and angiogenic potential was found in a study by Krzysztof et al. Peritoneal fluid was collected from advanced EOC patients and the angiogenesis index was assessed in mice by measuring VEGF and IL-8 levels in the supernatant and cellular suspension. IL-8 is a chemoattractant for neutrophils, lymphocytes T and basophiles, and also possesses proangiogenic activity. Both VEGF and IL-8 were at a higher concentration in the supernatant and cellular fraction rich in EOC cells. This study concludes that the ascites play an important role in stimulating angiogenesis and the mechanism is dependent mostly on cancer cells' activity and it is enhanced by infiltrating leukocytes [64].

While malignant ascites in EOC patients are generally known for their angiogenic properties, Jandu N. et al. observed antiangiogenesis in a chick chorioallantoic membrane assay (CAM). Among the immunopurified fibrin degradation products (FDPs), soluble FDPs were shown to contribute to the net antiangiogenic property of ascites fluid [70].

2.3. Migration and Invasion

Malignant ascites create a tumor microenvironment that encourages increased migration and invasion of cancer cells in the peritoneal cavity. This unique environment contains various growth factors, lipid signaling molecules, and enzymes. Discussed below are numerous studies that identify which particular aspects of ascites affect migration rates and the mechanism by which they act.

According to one study, different types of ascites with the same protein concentration exhibited either stimulatory or inhibitory effects on migration

when tested with OV-90 cells. They also noticed that there was a particular threshold at which, if surpassed, more stimulatory ascites would become inhibitory. This general look at ascites underlines that their interactions with cancer cells are complex [71].

2.3.1. Pro-Migratory Effects of Lysophosphatidic Acid

Lysophosphatidic acid (LPA) has been demonstrated to consistently increase migration and invasion of cancer cells. Particularly, the variants LPA_2 and LPA_3 directly stimulate invasion and enhance the adhesion of cancer cells once they are at their secondary sites. This effect was confirmed by a study that transfected siRNA against the three different types of LPA (the third being LPA_1) in SKOV3 cells [72].

The pro-migratory ability of malignant ascites decreased significantly after human peritoneal mesothelial cells (HPMCs) were incubated with heat-treated ascites. Since LPA is not heat-inactivated, these results indicate that other molecules found in ascites that are affected by heat must interact independently to stimulate migration [67]. Furthermore, the same group found that LPA levels were not consistently higher in malignant ascites OVC346 and OVC508 [73]. However, this particular type of ascites is noted for its growth-enhancing activity, rather than its migratory effect. Different ascites present different overall effects; multiple studies have acknowledged the balancing act that tumor-positive and -negative regulators present in malignant ascites, which allows some to be stimulatory and others to be non-stimulatory [71, 73].

It is also unclear at this point whether LPA is produced extracellularly and is then secreted, or if it is produced extracellularly, as in from shed microvesicles (which are discussed later). If this mechanism can be identified, it may provide opportunities for directed therapy against tumor cell migration and adhesion [72].

2.3.2. Hepatocyte Growth Factor's Effect on Migration

Hepatocyte growth factor (HGF) also plays a significant role in stimulating the migration of HPMCs. It acts as a ligand of the cMet receptor, whose expression is associated with a poor prognosis for ovarian cancer patients; the correlation between the HGF/cMet signaling pathway and migration was confirmed by adding a HGF-neutralizing antibody and observing the sharp decrease in HPMC migration. Like in the first study described, there is a concentration threshold of HGF above which stimulation does not directly increase. Furthermore, the HGF/cMet signaling relationship can be eradicated by heat treatment. Once activated, cMet induces the

downstream signaling pathways such as Akt and ERK1/2 in HPMCs. However, this ascites-induced phosphorylation is only partially affected by heat inactivation, which indicates that there are even more factors in play for Akt/ERK1/2 [67]. In addition, inhibiting Akt and ERK's activation individually revealed that ERK is much more involved in cell invasion. Its inhibition both delayed invasion and reduced the degree of migration, whereas there was only a delay in the invasion with Akt's inhibition [72].

2.3.3. Proteolytic Enzymes and Migration

The proteolytic enzymes MMP-2, MMP-9, and uPA have been identified as pro-migratory in ovarian cancers with ascites. These enzymes are found in association with membrane-bound vesicles within ascites, which indicates that these vesicles must also play a key role. Purified vesicles were added to DOV 13 and OVCA 429 ovarian cancer cells, and a consistent increase was observed in both cell lines. This increase was directly related to the dose of vesicles added. By adding inhibitors of MMP and uPA, it was found that this type of increased invasion depends on both of these enzymes' activities. These proteinases work to degrade the extracellular matrix, which is a key step for tumor cells to migrate effectively. Interestingly, ascites in early-stage patients present a lower concentration of vesicles than do late-stage, but the vesicles themselves are just as efficient in promoting invasion [74].

2.3.4. Relationship between Gene Expression Profiles and Invasion Capacities of Ascites

Ascites contain cellular and acellular characteristics. The cellular part contains epithelial ovarian cancer cells, lymphocytes, and mesothelial cells, and the acellular has cytokines and angiogenic factors. The gene expression profile of the cellular fraction of ascites can also have a net-positive migratory effect on tumors. For example, 10 genes (IRS2, CTSD, NRAS, MLXIP, HMGCR, LAMP1, ETS2, NID1, SMARCD1, and CD44) were confirmed to be up-regulated in ascites and this up-regulation increased invasion levels. In the same study, three significant, down-regulated genes (MDC1, SMARCA4, and GPR125) were found to have the opposite effect as expected and actually inhibited migration [75]. This opposite effect could be the result of a flawed study, or it could simply be another indicator of the ambiguous nature of ascites. For example, another study showed that there was no difference in proliferative activity of SKOV3 cancer cells when ascites were added to the medium [76]. This underscores the ambiguous role of ascites.

2.4. Apoptosis

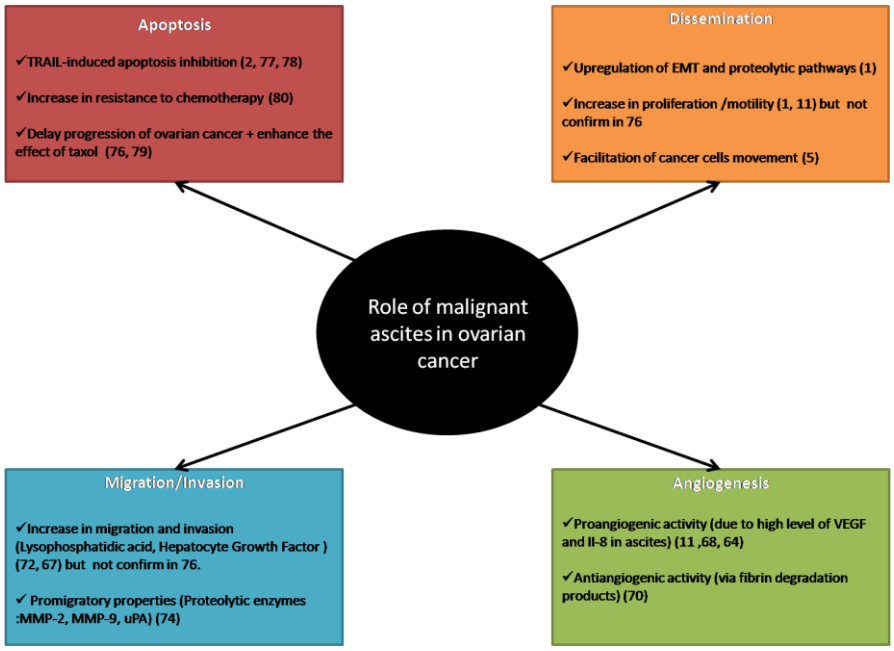
Malignant ascites' effect on apoptosis, or programmed cell death, is a current topic of controversy. Traditionally, data supported the idea that ascites inhibit TRAIL-induced apoptosis. Ascites act as an antagonist against apoptosis by activating kinase PI13 and the cytosolic protein kinase Akt, which is a downstream effector of PI13. It is suspected that Akt modulates TRAIL responsiveness by up-regulating the concentration of c-FLIP, which is another key protein involved in apoptosis [2, 77, 78]. However, Lane, et al. admits that the attenuating effect was variable among different malignant ascites. They explained this by suggesting that the concentration of pro-survival factors could vary in each one. These discrepancies in clinical presentation make the exact role of ascites in ovarian cancer difficult to understand.

A new look at ascites' role in apoptosis, though, found opposite results: that malignant ascites can actually delay the progression of ovarian cancer. Ascites can induce apoptosis by enhancing expression of Death-Domain Associated protein (Daxx) and phosphorylating the JNK kinase cascade to increase Fas-mediated apoptosis. The study claims that the discrepancies in Lane have come from the use of inconsistent lines of ascites [76]. More recently, another study has also observed that ascites treatment increase ovarian cancer cells apoptosis [79].

In study of Cohen et al. it was found that ascites could enhance the effects of taxol against tumor growth in human models [76]. However, a recent study published by Mo et al. contradicts this and suggests that ascites could increase resistance to chemotherapy by promoting efflux function and thus reduction of the intracellular drug concentration [80]. This study has been assessed on mouse so it would be very interesting to investigate it on patients.

The question about the role of ascites in apoptosis is very interesting for a future possible tandem between the palliative technique of concentrated ascites reinfusion therapy (CART), discussed before and molecular therapies to destroy cancer cells. In fact, one study dealing with gastric cancer has demonstrated that a combination of CART with paclitaxel can be used for the management of massive malignant ascites. However, this study is not sufficient to prove the benefit of CART for the survival of patient [26]. Another recent study suggests also a potential improvement in the prognostic for patient, with a continuous chemotherapy treatment, could be give rise to CART [81]. Therefore, other studies should be performed to determine the real efficacy of a combination between CART and drugs on malignant ascites [82].

Table 2. Role of malignant ascites in ovarian cancer



There is still plenty of ambiguity surrounding ascites exact role in ovarian cancer but some of them have been started to be described (Table 2). Soon, though, we may be able to target them effectively and safely or use their components as treatments on a large scale.

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