Emerging perspectives on growth factor metabolic relationships in the ovarian cancer ascites environment

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Abstract

The ascites ecosystem in ovarian cancer is inhabited by complex cell types and is bathed in an environment rich in cytokines, chemokines, and growth factors that directly and indirectly impact metabolism of cancer cells and tumor associated cells. This milieu of malignant ascites, provides a 'rich' environment for the disease to thrive, contributing to every aspect of advanced ovarian cancer, a devastating gynecological cancer with a significant gap in targeted therapeutics. In this perspective we focus our discussions on the 'acellular' constituents of this liquid malignant tumor

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microenvironment, and how they influence metabolic pathways. Growth factors, chemokines and cytokines are known modulators of metabolism and have been shown to impact nutrient uptake and metabolic flexibility of tumors, yet few studies have explored how their enrichment in malignant ascites of ovarian cancer patients contributes to the metabolic requirements of ascites-resident cells. We focus here on TGF-βs, VEGF and ILs, which are frequently elevated in ovarian cancer ascites and have all been described to have direct or indirect effects on metabolism, often through gene regulation of metabolic enzymes. We summarize what is known, describe gaps in knowledge, and provide examples from other tumor types to infer potential unexplored roles and mechanisms for ovarian cancer. The distribution and variation in acellular ascites components between patients poses both a challenge and opportunity to further understand how the ascites may contribute to disease heterogeneity. The review also highlights opportunities for studies on ascites-derived factors in regulating the ascites metabolic environment that could act as a unique signature in aiding clinical decisions in the future.

Keywords

Growth factors; metabolism; ascites; ovarian cancer; TGF-β; VEGF

Introduction

A major clinical feature of tumors with peritoneal carcinomatosis is fluid accumulation in the peritoneal cavity. Ovarian cancer (OC) is the most common cancer associated with development of malignant ascites[1]. The American Cancer Society estimated approximately 21,410 new OC cases and 13,770 OC-related deaths for 2021. Roughly 90% of these OCs are epithelial ovarian cancers (EOC), classified histologically as either Type 1 (endometroid, mucinous, clear cell, low grade serous) or Type 2 (poorly differentiated, carcinosarcoma, and high grade serous: HGSOC)[2 3]. Recent copy number analysis at single cell resolution reveals heterogeneity between high-grade and low-grade tumors as anticipated, but less heterogeneity in metastases as compared to primary tumors[4]. Additional molecular subtype signature-based classifications have also emerged for high grade serous cancers (HGSOC) from single-cell (sc) RNA-seq and drop-Seq studies indicating the presence of an epithelial to mesenchymal (EMT)- high subtype that can identify with poor prognosis[5]. Regardless of the classification, most ovarian cancers lead to ascites[6]. However much of our information comes from HGSOC, primarily due to the highest incidence of this subtype in the patient population. Recurrent HGSOC is incurable, and the presence of ascites is a common feature. Over 70% of patients will have recurrent disease with the likelihood being 90–95% in patients with stage 4 disease.

Ascites accumulation in patients frequently correlates with the extent of metastatic disease, and both ascitic volume and components in ascites can be used to grade, stage and predict survival and chemoresistance outcomes, which have been proposed as independent prognostic factors[7–9]. In some instances, ascites accumulation can lead to infections (bacterial peritonitis) and hernia, due to increased abdominal pressure, and can directly contribute to morbidity due to complications associated with gastrointestinal problems. Much like edema (capillary permeability and hydraulic and oncotic pressure gradients),

arterial vasodilation and venous obstruction are the main physiological causes of ascites accumulation[10]. In advanced EOC, increased vascular permeability of vessels lining the peritoneum and/or lymphatic obstruction by disease burden leads to altered lymph drainage and angiogenesis and thereby ascites accumulation[1,11,12]. While in most cases treating the underlying disease will reduce ascites, untreatable ascites can be a recurrent and frequent problem, requiring drainage and paracentesis.

The malignant ascites environment is comprised of diverse cell types (reviewed in detail recently in[13 14] and summarized in Fig 1). A subset of these include macrophages, fibroblasts, endothelial cells, lymphocytes and mesothelial cells and a small fraction of tumor cells[15,16]. In depth scRNA-seq has confirmed and identified 18 distinct cell clusters including epithelial, macrophage, cancer associated fibroblasts (CAF), dendritic cells, B cells, T cells and erythrocytes with immune cells being the most abundant (~65%). Layered on the complexity of these cell types, is the additional patient by patient variability in the percentage of these cells present, as described recently[16]. Notably, malignant cells exhibited an inflammatory transcriptional program[16].

In addition to the cellular make-up of ascites, significant variability exists in the acellular components found in the ascitic fluid, including growth factors, cytokines and metabolites that result from secretion by, and metabolic activity of, the cell types found in the ascites (Fig 1). Much has been discussed about the signaling pathways associated with ascites-derived growth factors and cytokines and their effects on cellular programs driving ovarian cancer progression[17 18 19 20]. However, limited integration of information exists on the link between growth factors and the metabolite environment of the ascites fluid, and regulation of the metabolism specifically of ascites resident cells. Undoubtedly, changes to cell signaling by growth factors in the ascites impacts nutrient uptake and energy production required for tumor cell survival and the interaction between the different cell types in the ascites. While an in-depth analysis of every cytokine and growth factor is beyond the scope of the review, we have discussed those that have established direct roles in ascites development and have been explored as treatment options for ovarian cancer in the clinic, particularly focusing on VEGF and TGF-β, and a subset of interleukins (IL). We focus on their relationship to metabolism and discuss the impact on nutrient balance in subsets of cell types found within the OC ascites, drawing inferences from other cancer types.

1. Acellular Components in Malignant Ascites

1.1 Growth factors, cytokines and chemokines

Ascitic fluid is a reservoir of soluble factors that directly contributes to transcoelomic, hematogenous and lymphatic metastasis of OC[21,22]. Thus, characterization of these soluble factors is essential to understand how the ascites tumor environment affects OC progression[23–27], and as a resource for staging, diagnosis and prognostic biomarker development in the clinic. Several studies have implicated the presence of multiple cytokines, chemokines and growth factors within ascites with OC etiology (Table 1), with significant variation seen between studies in part due to sample handling[28] and sensitivity of the detection method used (Table 1). Several of these factors are elevated specifically in malignant ascites in comparison with benign ascites or in normal or diseased sera indicating

OC-dependent increases[29–31]. Chemokines including CCL2, −3, −4, −5, −8 and −22 as well as chemokine receptors CCR1, −2a, −3, −4, −5, and −8 are also found in OC ascites with studies indicating CCL2 as being the most abundant[32]. Among the pro-tumor cytokines, IL-4, −5, −6, −8, −10, −13, and TGF-β exhibit strong immune modulatory and angiogenetic functions[33 34 35 36]. Increased levels of IL6, IL8, IL10, CCL2 (MCP-1), IFNγ, and TNF-α have been linked to poor prognosis and IL6 has been proposed as an independent factor for predicting overall survival (OS) in advanced ovarian cancer[20]. Specifically, IL6 has been linked to paclitaxel and cisplatin resistance in multiple in vitro OC studies[37]. Further, neo adjuvant chemotherapy can lead to lowered IL6 and IL8 levels and improve survival outcomes[9,38–43]. Comparisons of IL6 levels in matching specimens indicates an almost 100 times increase within peritoneal fluid as compared to plasma levels in the circulation[44], suggesting that OC ascites is a highly pro-metastatic and inflammatory environment. Indeed, incorporation of IL6 levels as a biomarker improves the Risk of Malignancy Index (RMI)[45], an algorithm used widely for assessing ovarian cancer risk along with CA125 and HE4. Notably, IL6 was most effective at distinguishing advanced malignant disease[46]. IL10, is also present at higher concentrations in ascites and is linked to shorter progression free survival (PFS) in OC patients[40,43]. IL-6 and −10 have also been shown to stimulate CA-125 expression[47], and enhance ascites accumulation in OC patients[48]. However, a recent cell-free and concentrated ascites reinfusion therapy (CART) study indicated that higher levels of IL10 after CART, correlated with longer survival in response to CART[49], suggesting some variability in outcomes associated with IL10 levels.

The significance of VEGF in the ascites is exemplified by the use of bevacizumab as standard of care for homologous recombination (HR) proficient patients, and the ability of such anti-VEGF therapies to reduce ascites burden and prolong the time between paracentesis[50]. Findings from the 2014 AURELIA trial have also led to bevacizumab use in platinum resistant patients[51,52], where progression free survival (PFS) was improved, and ascites control observed. It remains unclear whether the improved PFS was due primarily to ascites control, or if a reduction in ascites was the result of delayed recurrence. Adverse side effects such as gastrointestinal events, hypertension, bleeding, thromboembolism and delayed wound healing remain with anti-VEGF therapies[53], and identification of additional non-toxic angiogenic targets remains and an active area of investigation to combat OC progression and ascites formation.

In addition to VEGF, which is arguably the most dominant driver of angiogenesis in cancer, TGF-β, IL6 and IL8 are considered angiostimulatory and are negatively associated with progression free survival[54 17 55]. Consistently, TGF-β2 and particularly TGFβ1 are elevated in the ascites of EOC patients[56 57] (Table 1). Not all TGF-β superfamily members are elevated however, as a subset of BMP's can be present below the levels found in normal circulation[57] and caution must be used in treating all TGF-β superfamily members equally. The significance of the presence of high TGF-β and engagement of its signaling machinery in ascites-resident cells[17] is underscored by TGF-β pathway blockade strategies that not only abolish ascites formation in preclinical models but also suppress VEGF production and improve ascites drainage by lymphatic vessels[58 36,58]. In addition to potential direct contributions to ascites development via the vasculature, TGF-βs are also key drivers of epithelial mesenchymal plasticity in OC cells and in multicellular spheroid

aggregates[59,60 61]. In addition, TGF-βs regulate mesenchymal cells and the immune cell population[62], which together can increase tumor burden and the formation of malignant ascites.

The source of cytokines and growth factors is as varied as the growth factors themselves and includes secretions by tumor cells, immune cells, stromal cells, as well as contribution from the diseased host tissue, including the omentum and peritoneal organs. For instance, besides tumor cells, CD163 positive macrophages enhance tumor angiogenesis and metastasis by inducing secretion of VEGF, IL6 and IL10, in the ovarian tumor microenvironment[63]. What is evident from cytokine screens from different studies is that significant heterogeneity and variability exists in the levels of these soluble factors between patients (Table 1). The types of growth factors, cytokines and chemokines detected often differ between patients and between studies. The relative levels detected can also be highly variable. This underscores the need for a standardized screening method if these soluble factors are to be used as bio- and prognostic markers in future.

1.2 Metabolites

Although less well studied than the growth factor and cytokine components of ascites, metabolites are an important subset of the acellular components in this liquid tumor environment. Metabolomic, lipidomic and proteomic studies of EOC patient ascites and *in vivo* tumor models have highlighted the presence and importance of some key signature metabolites in the ascites fluid, which are integral to regulating the metabolism of tumor and tumor associated cells in the ascites[64–67] (Fig 1). Malignant ascites display a distinct metabolite pool relative to benign ascites. For example, principal component analysis can distinguish OC ascites from cirrhosis derived ascites by the presence of amides of linoleic acid and oleic acid, and high glucose-1-phosphate, glycero-3-phosphate, cholesterol and ceramide levels. Conversely, 2-hydroxyisovalerate, glycolate, glucose, furanose, and fructose were less abundant in malignant ascites relative to cirrhosis-derived ascites[65] (Fig 1). Several glucose metabolism intermediates are altered in a way that suggest increased consumption of glucose by ascites resident cells. For example, levels of glucose, furanose, glycolate and fructose are significantly reduced in the malignant ascites, whereas glycero-3-phosphate (G3P), and glucose-1-phosphate (G1P) are significantly elevated[65,68] (Fig 1).

Lipid accumulation is also a common feature of malignant ascites[65,66], and has been linked to both increased lipogenesis and fatty acid oxidation by ascites resident cells. Lipid species detected include long chain fatty acids that are derived from lipid biosynthesis in tumor and adipocytes in the peritoneal tissues, as well as signaling lipids. The types of long chain fatty acids also appear to be specific to ascites. For example, lipidomic profiling found a higher accumulation of unsaturated fatty acids (UFA) compared to saturated fatty acids (SFA) in patient ascites[66]. In addition, increased ratios of UFAs to SFAs were found in OC cell lines cultured in conditioned ascites or omentum-derived media[66]. The higher UFA levels in ascites likely reflect increased biosynthesis of long chain fatty acids, which are both synthesized, stored and used as fuels for beta-oxidation by tumor cells. Examples of PUFAs enriched in ascites are prostanoids, hydroxy eicosatetraenoic acids, leukotrienes, linoleic acid, docosahexaenoic acid and prostaglandin E2.

Phospholipids are also detected in the malignant EOC ascites[69]. Levels of the bioactive lipid molecule LPA (lysophosphatidic acid) and sphingosine1-phosphate (S1P) are elevated in malignant EOC ascites fluid compared to benign ascites[27], and high levels of LPA are associated with adverse outcomes[70]. Furthermore, concurrent with elevated levels of LPA, human EOC ascites also had high levels of cytosolic and calcium-independent Phospholipase 2 (PLA2) activity[64]. PLA2 is critical for breakdown of phosphatidylcholine (PtdCho), the major membrane lipid, to form lyso- phosphatidylcholine (lyso-PtdCho) which is present at higher levels in EOC ascites compared to benign ascites[71].

Other metabolic intermediates have also been noted to be significantly altered in ascites. For example, presence of BHB (β-hydroxybutyrate), a ketone body formed from acetyl-CoA and two other metabolites of the TCA cycle, citrate and maleic acid, is elevated in ascites in *in-vivo* tumor studies[72]. In contrast, 2-hydroxyisovalerate is one of the most depleted metabolites in malignant ascites compared to benign liver cirrhosis ascites. Even though, the cause of this decrease in 2-hydroxyisovalerate is not clearly understood, it might be the result of increased amino acid catabolism as 2-hydroxyisovalerate is produced upon breakdown of branched amino acids[68]. Many of the causes and consequences of changes in metabolites such as amino acids still require further investigation and analysis.

2. Linking growth factors and metabolism in malignant ascites

Metabolic flexibility is an important hallmark of OC to allow cells to survive under fluctuations in nutrient availability in response to changing tumor microenvironments. Several growth factors and cytokines that are elevated in ascites have also been implicated in regulating proliferation and metastatic progression by impacting nutrient uptake and metabolic flexibility in tumors. TGF-βs, VEGF and ILs have all been described to have direct or indirect effects either by gene expression of enzymes involved in metabolic reactions in autocrine or paracrine fashions. Additionally, targeting these growth factors, particularly VEGF, can lead to metabolic alterations and impact therapeutic response and resistance mechanisms.

2.1 Glycolysis & Pentose phosphate pathway

Malignant ascites of EOC patients have lower levels of glucose and a higher level of G1P and G3P as compared to benign ascites (Fig 1). This decrease in glucose levels is thought to be reflective of enhanced consumption of glucose by ascites resident cells[65] (Fig 2). Increased consumption of glucose in the tumor microenvironment (TME) is frequently associated with enhanced glycolytic activity by tumor cells, shifting mitochondrial ATP production to macromolecular biosynthesis pathways to support proliferation (Fig 2). Of the 2000 proteins and peptides identified in metastatic EOC ascites[73,74] two glycolytic enzymes pyruvate kinase isozymes M1/M2 (PKM1/2) and glyceraldehyde phosphate dehydrogenase (GAPDH) showed significant difference between patient and control ascites and serum samples. In addition to promoting glucose metabolism, PKM2 has been implicated with platinum-resistance in ovarian cancer cell lines[75]. While an increase in aerobic glycolysis, otherwise known as the Warburg effect, is a common phenotype of cancer cells, the fate of glucose in tumor cells is cell state dependent, as highlighted in subsequent sections, and

the depletion of ascites glucose could have consequences on rewiring towards utilization of alternate fuel sources. Moreover, increased glucose utilization by tumor cells decreases glucose availability to other tumor associated cells, such as effector T-cells[76] (Fig. 2). Thus, tumor cells and T-cells (and potentially other immune cells) have distinct metabolic profiles as a consequence of glucose competition within ascites, which has also been observed in other solid tumor tissues[77].

PKM2 is also a downstream target of TGF-β during epithelial to mesenchymal transition (EMT) and this is accompanied by increases in glucose transporters[78] (Fig 2). Notably, several lines of investigation in non-ovarian models link TGF-β during the process of EMT to metabolic changes associated with a shift to aerobic glycolysis[79 80 81]. This glycolytic reprogramming is also partially required for TGF-β-induced EMT[82]. A reciprocal relationship between glucose metabolism and the TGF-β's exists (Fig 3), wherein glucose can also stimulate TGF-β signaling by increasing TGF-β cell surface receptor levels and activating latent TGF-β in the extracellular space[83]. Energy production is an accepted requirement for EMT as metabolic changes accompany EMT. However, it is currently difficult to distinguish if metabolic changes are by products or drivers of the process. Moreover, with the significant plasticity of the process of EMT itself, it is likely that the metabolic flexibility mirrors the EMT process[84].

Epithelial to Mesenchymal Transition (EMT) is an important reversible transcriptional program process during metastasis that shares gene expression signatures with cancer stem cells[85]. EOC subpopulations isolated from malignant ascites that are enriched in stem like cells[86,87] may be prone towards a rewiring of metabolism towards glycolysis. Specifically, an ALDH+/CD44+ cancer stem cell subpopulation has been shown to be reliant on aerobic glycolysis, which is dependent on their upregulation of pyruvate dehydrogenase kinase 4 (PDK4)[88]. Interestingly, the increased activity of PDK4 in ALDH+/CD44+ ascites-resident cancer stem cells also increases IL-8 expression[88], suggesting that metabolic changes lead to alterations in ascites cytokine levels. Whether or not cytokines like IL-8 and IL-6 also conversely contribute to metabolic reprograming of cells within malignant ascites remains to be further explored. However, evidence from other tumor types suggests that this interplay is possible. In pancreatic cancer, stromal IL6 increases glycolytic flux in tumor cells, leading to lactate efflux and selection of cancer stem cells[89]. Whether IL6 in the ascites could favor EOC stem cell enrichment via metabolic changes is speculative, yet intriguing (Fig 3).

High glucose consumption by tumor cells in malignant ascites is likely also driven by a need for macromolecular biosynthesis pathways. Glucose is frequently wired into the Pentose phosphate pathway (PPP), which provides anabolic substrates for ribonucleotide synthesis to support cancer growth, and the production of NADPH (Fig 4), which is necessary for maintenance of a reduced glutathione (GSH) pool[20] The PPP thus represents an important mechanism for tumor cells to manage oxidative stress. For example, cancer cells are able to cope with the oxidative stress associated with loss of matrix attachment by reroute glucose metabolites into the PPP[90 91] (Fig 4). This metabolic adaptation is important for anoikis resistance of breast cancer cells[90], and was demonstrated in OCs cultured in anchorage independent spheroid conditions, which were shown to exhibit increased glycolytic flux and NADPH generation[91]. Moreover, enhanced activity of 6-phosphogluconate dehydrogenase

(6PGD), an important enzyme for NADPH synthesis in the PPP has been associated with cisplatin resistance in OC[92]. Whether ascites fluid or specific growth factor cooperate to regulate PPP enzymes in OCs or tumor associated cells remains to be explored. However, a link to VEGF signaling has been made in endothelial cells, wherein G6PD positively regulates VEGFR2 signaling and VEGF-induced EC proliferation, migration, and tube formation[93].

Like other cancers, OCs require increased supplies of nutrients and thereby depend on angiogenesis, which concomitantly contributes to ascites formation. VEGF is the predominant driver of angiogenic changes in cancers including OC, and much of VEGF's role in metabolism has been delineated in endothelial cells. Similar to tumor cells, the tumor vasculature prefers glycolysis, as 2-Deoxy-glucose was shown to be highly toxic to endothelial cells[94], likely due to the need to preserve oxygen for blood supply to the tumor/tissues. Endothelial cells are also highly plastic and are poised to proliferate in response to tumor secreted VEGF (angiogenic switch), and during such bursts, can significantly increase their VEGF-dependent glycolytic flux[95] (Fig 3). However, VEGF can influence additional cells in the ascites, including tumor cells, stromal/fibroblasts and immune cells. Approximately 85% of human ovarian tumors express VEGFR2 receptor, with most ovarian cancer cells expressing functional VEGFR2[96]. Notably short-term pharmacological inhibition of VEGFR2 signaling in EOC cells is a strong suppressor of survival in suspension cultures[97]. It is possible that VEGF may also contribute to metabolic reprograming of ascites resident EOCs, yet this remains to be clearly defined (Fig 3).

The mildly hypoxic environment of the ascites and hypoxic micro-environments found within the core of multicellular tumor spheroids aggregates suspended within ascites can stimulate VEGF synthesis[98–100], likely further contributing to a re-wiring of metabolism, in addition to increases in factors, such as hypoxia inducible factors (HIF). HIF activation can also be driven by LPA, (Fig 3) which is highly abundant in malignant ascites and has been shown to be secreted by both tumor cells and Tumor associated macrophages (TAMs). LPA induces pseudohypoxia in OC cells with the resultant expression of HIF1α via Gαi2, Rac1, NOX2 and the generation of reactive oxygens species (ROS)[101] (Fig 3). This may lead to increased expression of glucose transporter-1 (GLUT1/SLC2A1) and the glycolytic enzyme hexokinase-2 (HK2), leading to an LPA-mediated glycolytic shift in EOC cells[70]. Like the effect of LPA, both GLUT1 and HK2 are also established targets of TGF-β in non- ovarian cancer models, and corelate strongly with EMT and glucose uptake during TGF-β-induced EMT[78] (Fig 3).

Aside from tumor cells, TGF-β are well-known regulators of the stromal environment driving fibroblast reprogramming[102], which mimics the EMT process of tumor cells. TGF-β pathways increase oxidative stress, autophagy/mitophagy and glycolysis in fibroblasts in part via caveolin regulation[103]. TGF-β can enhance autophagy in cancer associated fibroblasts, which further fuels metabolism of tumor cells by providing metabolites such as pyruvate, lactate and ketone bodies[104] [105] (Fig 2).

Another scenario that presents in the clinic, is with the use of anti-angiogenics (anti-VEGF) to manage ovarian cancer, that induce vessel pruning and leads to starvation of

tumor cells. It is widely appreciated that such starvation can intensify the hypoxic/acidic environment[106] [107] and may explain the dramatic depletion of glucose and ATP in tumors seen. These metabolic changes are accompanied by partial tumor regression in highly glycolytic ovarian tumors[108], with poorly glycolytic ovarian tumors being more growth arrested by anti-VEGF, and highly glycolytic ones becoming resistant more rapidly[108]. Thus, the metabolic profile may impact response to anti-VEGF and potentially other targeted therapies

2.2   Mitochondrial Oxidative Phosphorylation (OxPhos)

While the Warburg effect is a major pathway for the consumption of glucose by tumor cells, OC cells do not exclusively rely on glycolysis as a metabolic pathway (Fig 3,4). This is supported by OC cells exhibiting both oxidative phosphorylation and glycolysis and an increased ability to survive under anchorage-independent conditions[109] (Fig 3). Similarly, CD44+/CD117+ ovarian cancer stem cell spheroids isolated from patients' ascitic fluid exhibited not just enhanced glucose uptake as described in the previous section, but also had heightened OxPhos[110]. OC tumor initiating cells have been shown to have increased flexibility in mitochondrial function in response to mitochondrial uncouplers[72]. Chemo resistant OC cell lines are also less sensitive to glucose deprivation and display increased ability to switch from glycolysis to OxPhos[111]. Given the ability to upregulate both glycolysis and OxPhos, it appears that OC cells are highly metabolically flexible, which may provide survival adaptations under conditions of nutrient depletion. Interestingly, TGF-β present in ascites fluid can promote anchorage independent survival via increasing the stemness gene *SOX2*[57] [112]. Whether this also contributes to a shift in metabolism towards OxPhos remains untested but anticipated. As pointed out above, EMT is frequently associated with a shift toward aerobic glycolysis. However, in non-small cell lung cancer, TGF-β treatment leads to increased OxPhos and a shift away from glycolysis, via the repression of repression of pyruvate dehydrogenase kinase 4[82]. Conversely, TGF-β may have negative effects on mitochondrial function of tumor associated cells, which contributes to its immunomodulatory function. TGF-β blocks mitochondrial OxPhos in CD4+ T cells, leading to repression of IFN-gamma production[113]. Thus, it is possible that the role of ascites-derived TGF-β on mitochondrial function is cell type dependent.

It should be noted that an increase in OxPhos presents challenges to tumor cells due to increased generation of mitochondria-produced reactive oxygen species, derived from electron leakage at the electron transport chain (Fig 4). OC cells can overcome this in anchorage-independent conditions by upregulating mitochondrial antioxidant defense mechanisms[114]. Coping with lethal oxidative stress is a necessary adaptation for tumor cell survival, as similarly illustrated above through increased pentose phosphate pathway - dependent NADPH generation. However, sublethal levels of ROS can conversely also be drivers of tumor progression. An example being NOX-derived ROS in stimulating pseudohypoxia via the regulation of HIF, as mentioned above. Growth factors can also manipulate ROS generation via the mitochondria. For instance, VEGF is widely reported to stimulate ROS production in endothelial cells[115] (Fig 4), and can induce the production of mitochondrial ROS by enhancing mitochondrial function[116]. Mitochondria-derived ROS can have a number of consequences on endothelial cell function (reviewed in[117]), including

enhanced migration by ROS-mediated Rac1 activation in response to VEGF[116]. VEGF-dependent regulation of OxPhos and subsequent mitochondrial ROS generation might thus be contributors to tumor angiogenesis. ROS can also increase VEGFR2 phosphorylation demonstrating a reciprocal link between redox and VEGF signaling[117]. Whether the same mechanisms utilized by endothelial cells are also seen in OC or other cells in the ascites that also express VEGF receptors is currently unclear (Fig 3). It is worth mentioning that cancers with HR defects, as is the case for a proportion of HGSOCs', are sensitive to OxPhos inhibition, as a consequence of their high need for NAD^+ and ATP, substrates required for PARP-dependent DNA repair[118]. Thus, the current therapeutic regimen of anti- angiogenic strategies (VEGF) along with PARP inhibitors being evaluated as a combination strategy in the clinic is highly timely[119] [120], and metabolic links to their mechanistic synergy should be further explored.

2.3 Glutamine

Glutamine is an important carbon source for the TCA cycle and is frequently used by tumor cells, stromal cells and immune cells in the tumor environment as an alternate fuel source (Fig 4). OC cells require glutamine for optimal proliferation, survival and metastasis as evidenced by highly invasive cells exhibiting higher glutamine dependency[121,122]. Such glutamine dependency may be regulated by growth factors like TGF-β that can increase glutamine uptake in cancer cells, including driving expression of the glutamine transporter *SLC7A5*[123]. During TGF-β induced EMT genes involved in glutamine metabolism, particularly Glutaminase (*GLS*), the first enzyme of glutaminolysis necessary for shuttling of glutamine to the TCA cycle are also increased[123] [124]. TGF-β's effects on glutamine uptake by tumor cells could be indirect as well, where TGF-β dependent fibroblast reprogramming leads to production of glutamine that can then be utilized by neighboring cancer cells[103] (Fig 2). Whether TGF-β in the ascites and in OC models plays similar roles needs to be determined and may depend on the immune environment as well, since both TGF-β and glutamine are immunosuppressive. Specifically, in an OC model, immunosuppressive $CD11b^+Gr1^+$ myeloid cells are primed by exposure to ID8 mouse ovarian tumor cells to increase their glutamine metabolism to fuel mitochondrial respiration[125]. Together with myeloid-derived suppressor cells (MDSCs), Tregs contribute to the immunosuppressive environment of ovarian and other cancers[62]. In contrast to MDSCs glutamine deprivation has been shown to promote TGF-β-mediated conversion of CD4+T cells into Foxp3 T regs[126], implicating this axis in immune tolerance. Decreased glucose uptake by ascites-resident T-cells, potentially as a consequence of tumor cell depletion of ascites glucose, may lead to the reduced glutamine uptake seen by T-cells, that inhibits mitochondrial OxPhos and decreases anti-tumor immunity[76] (Fig 2). This is consistent with findings that glutamine depletion blocks T effector cell proliferation[127], much like the effects of TGF-β on effector Tcells[62]. However, the effect of glutamine in Treg homeostasis could be cancer model dependent and remains to be clarified in the OC ascites environment. Given the direct, established role of TGF-β in promoting expansion of Tregs, it is possible that glutamine uptake as impacted by the competition for ascites metabolites also plays a direct role in immunosuppression in OC.

In addition to effects on the adaptive immune system, exposure to tumor cell metabolites and alterations in macrophage metabolism within the ascites environment regulate macrophage polarization[128–130]. For example, addiction to extracellular glutamine by OC cells results in increased glutaminolysis and secretion of N-acetylaspartate (NAA), which is abundantly found in ovarian cysts and ascitic fluids[131]. NAA treatment can increase glutamine synthetase (GLS) expression in macrophages, a marker upregulated in M2 macrophages, leading to and enhancing M2 reprogramming. NAA may also work synergistically with cytokines in the ascites including IL10[132] (Fig 2).

Glutamine metabolism is also crucial for VEGF dependent angiogenesis as endothelial cells were found to consume glutamine more than any other amino acid[133]. However, endothelial cells may differ from tumor cells in their response to glutamine deprivation in their ability to use asparagine instead. Indeed, asparagine suppresses glutamine deprivation-induced death, but not the proliferation defect of cancer cells[134].

The diverse dependencies on glutamine are being explored for therapeutic intervention[124] by ways of inhibiting the enzyme GLS1 which is required for incorporation of glutamine into the central carbon metabolism. Most current trials are exploring the use of GLS1 inhibitors in a broad spectrum of OCs including clear cell carcinomas with ARID1A mutations, which demonstrate increased GLS1 expression[135]. Glutamine metabolism has also been associated with OC chemoresistance[136 137] and as such GLS1 inhibitors are currently in Phase I trials alongside PARP inhibitors (Niraparib) for platinum resistant homologous recombination (HR) proficient OC patients as well.

2.4   Fatty acid metabolism

The interaction between ascites and the adipocyte rich omentum and peritoneum allows exchange and supply of free fatty acids to the tumor cells in the ascites fluid. Importantly, migration and proliferation of human OC cells are significantly increased when cultured with omentum adipocytes, or their conditioned media[138 139]. In these studies, adipocytes increase the lipolysis of triglycerides to produce free fatty acids. Lipolysis and lipogenesis are important in maintaining high ATP production in OC cells, necessary for proliferation, increased lipid metabolism and increased lipid synthesis by OC cells in response to ascites[66]. The lipolysis also likely contributes to the high free fatty acid content in ascitic fluid (Fig 1) that may contribute to the metabolic reprograming of OC cells from aerobic glycolysis to fatty acid β-oxidation (Fig 4). It has been shown that OC cells utilize free fatty acids from ascites or omentum-conditioned media and in response alter expression of genes involved in fatty acid oxidation and lipogenesis[66]. Anchorage-independent cancer cells, which are commonly enriched in cancer stem cells can also switch metabolism to utilization of fatty acids for beta-oxidation as an alternate fuel source including the production of ATP and NADPH[71,140]. Multicellular aggregates found in ascites are also frequently hypoxic, and hypoxic cells have elevated FA uptake[141].

FA uptake is mediated primarily by the FA transport family protein CD36, which is corelated with poor prognosis in OC and drives metastasis[142]. Interestingly, both free fatty acids and CD36 exacerbate TGF-β driven EMT[143]. Besides CD36, FABP4, frequently overexpressed in OC cells is important in the uptake of FAs[138]. FABP4 is also regulated by

VEGF to facilitate free fatty acid transport across the endothelium[144] (Fig 3). However, the contribution of fatty acid oxidation (FAO) to energy production in endothelial cells can be variable. Given the use of anti- VEGF therapies as front line treatment for ovarian cancers, it is worth noting that OC cells can switch their lipid metabolism and storage in response to anti-VEGF leading to alterations in the lipidomic profile that has been shown to confer antiangiogenic drug resistance[145]. Notably, anti- angiogenic treatment mediated oxygen deprivation can lead to tumor cells switching from glycolysis to FAO metabolism upon treatment in non-ovarian cancers, particularly in tissues proximal to adipose deposits[146].

Fatty acid metabolism is also important for tumor associated cells. For example, tumor cell line derived high molecular weight hyaluronic acid can alter macrophage membrane composition by significantly decreasing membrane cholesterol content. Consequently, this was found to alter macrophage activation by promoting IL-4-mediated pro-tumor reprogramming[147]. Peritoneal resident macrophages also exhibited tumor cell induced increases in fatty-acid oxidation and production of itaconic acid, which increased OxPhos mediated ROS generation in macrophages and tumor cells[148]. Poly unsaturated fatty acids (PUFA), such as prostaglandin E2, can also exert immune suppressor functions by inhibiting expression of T_H1 cytokines TNFα, IFNγ and IL-2, and increasing expression of T_H2 cytokines IL-4, IL-10 and IL-6 (reviewed in[149]). PUFAs have also been implicated in mediating T-cell suppression by OC ascites[150]

LPA, which is abundant in ascites can increase de novo lipid synthesis in OC to promote proliferation[71,101]. Both LPA and S1P stimulate expression of IL8 in OC cells[151], thus contributing to the high cytokine levels in ascites. It is widely accepted that obesity and adipocytes recapitulate persistent inflammation that is marked by increased cytokines including IL6, IL8 and VEGF[152] which are all elevated in ascites and contribute to the reciprocal crosstalk. Such interactions provide a rationale for targeting the availability of free fatty acids for ovarian cancer management.

3. Summary and Future Perspectives

VEGF and TGF-β represent two highly abundant growth factors in the OC ascites and in combination with other abundant factors including LPA, IL6, IL10 and additional chemokines, are likely contributors to metabolic alterations in ascites resident cells, and thereby the metabolite environment of the ascites. We highlight here the existence of metabolic plasticity in OC cells and the tumor associated cells in the ascites environment. This flexibility confers a higher level of 'cellular fitness' especially to tumor cells that can switch between glycolysis, fatty acid oxidation, glutaminolysis, pentose phosphate pathways and OXPHOS. The flexibility is likely a feature of OC cells that evade anoikis and survive under anchorage independent stress, leading to shifts in the metabolite pools in the ascites. As a consequence of the metabolic flexibility, a possible outcome is also immune evasion by eliciting metabolic changes in the immune cells and the creation of a tolerogenic environment.

The described examples also highlight potential links to reprograming of metabolism, particularly glycolysis in the ascites tumor environment that maybe influenced by the

growth factors and cytokines enriched in the ascites. No discussion on metabolism and signaling is complete without appreciating the significance of nutrient sensing mechanisms likely to be active in all the cell types found in the ascites. Rewiring and changing metabolism in cells is controlled by the key transcription factors HIF, SREBP and ATF4 and the signaling pathways including mTORC1 and AMPK. It is also widely appreciated that mTORC1 activation is mediated by both growth factors and amino acids[153 154]. The processes regulated by these mechanisms include scavenging, autophagy, mitophagy and pinocytosis. All these mechanisms can in turn be regulated by growth factors as well, to balance proliferation and survival[155 154]. Since nutrient availability is challenging to analyze, growth factor measurements may be more feasible in a clinical setting. Hence defining the relationship between the two is central to understanding the metabolic outcomes of ascites accumulation and in defining treatment modalities that impact it.

At present it also is unclear how heterogeneity and individual variations in patient ascites metabolite pools and cytokine and growth factor levels as described here influence, tumor progression, response to therapy and patient survival. The lack of studies on the interplay between metabolites and GFs, exposes a knowledge gap and presents an important opportunity for future investigations. Together, the advances in standardization in measuring growth factors and metabolites, which are greatly influenced by sample handling and detection methods, and understanding of this link, could in the future lead to the utilization of the composition of ascites as a significant individualistic parameter to consider for personalized medicine approaches.

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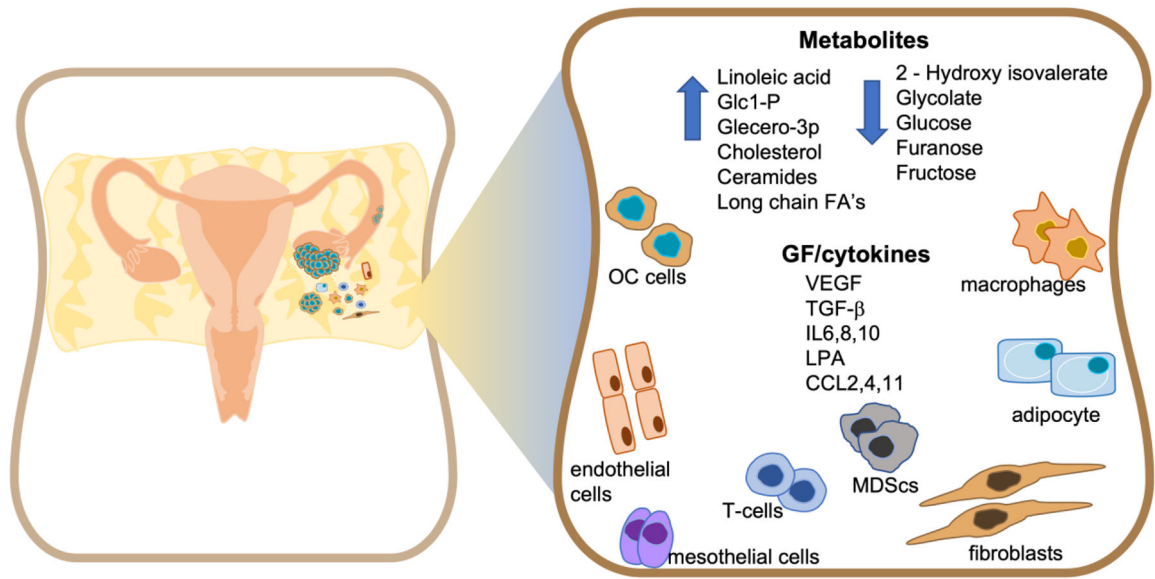
Figure 1.
Overview of the major cellular components, metabolites, cytokines and growth factors (GF) found in the ovarian cancer ascites (OC) environment.
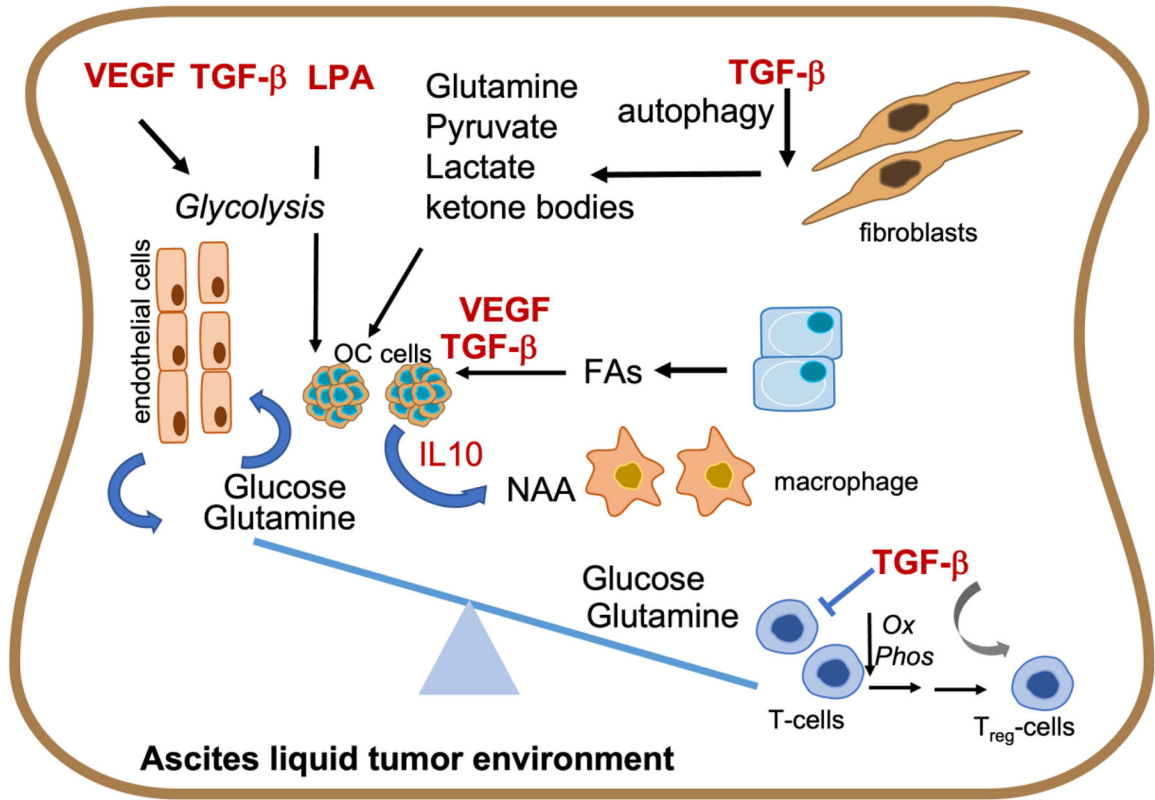
Figure 2. Potential GF-metabolic pathway interactions in the OC ascites.
TGF-β, VEGF and LPA are known regulators of tumor cell metabolism, including
increasing glycolysis and fatty acid (FA) uptake, and glutamine production from fibroblasts.
Increased use of metabolites, such as glucose and glutamine by OC tumor cells alters the
ascites metabolite environment. This can lead to depletion of the same metabolites for T-cell
metabolism resulting in reduced Oxphos and increase in differentiation and expansion of
the regulatory T cell population (T_{reg}) that can be enhanced by TGF-β. Addiction of OCs
to glutamine can also increase NAA to fuel M2 macrophages thus skewing the ascites
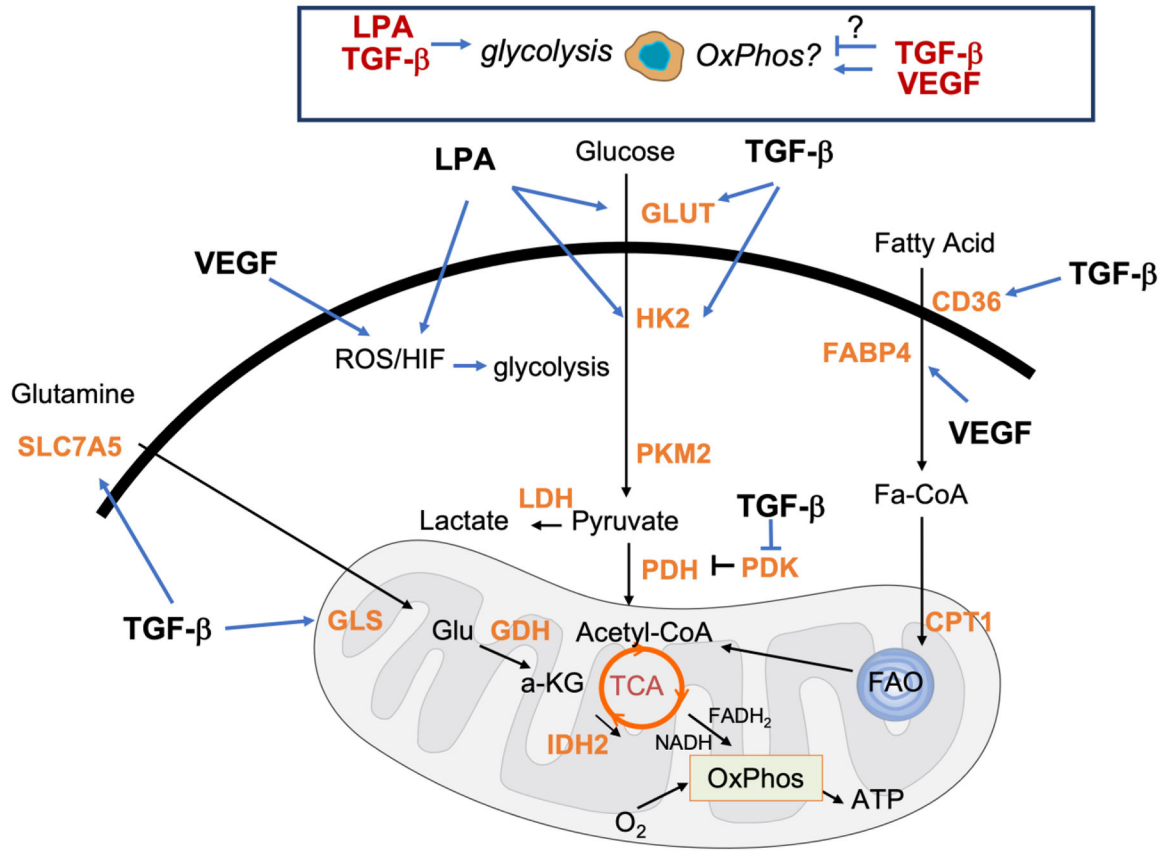metabolite pool to create a tumor permissive immune environment.

Figure 3. GF regulation of the metabolic pathways active in ascites resident OC cells

Ascites resident OC cells exhibit both glycolysis and oxidative phosphorylation with GF's been shown to regulate both processes in other cell types. TGF-β can increase expression of genes involved in glucose, glutamine and FA uptake and metabolism (GLUT, CD36 and SLCA5) and (HK2, GLS1, and FABP4) respectively. A mutual relationship exists between glucose and TGF-β wherein glucose can stimulate TGF-β signaling. LPA induces pseudohypoxia by increasing HIF1α and likely increased expression of GLUT1 and HK2, leading to an LPA-mediated glycolytic shift in OC cells. VEGF can regulate FABP4 and increase ROS production.
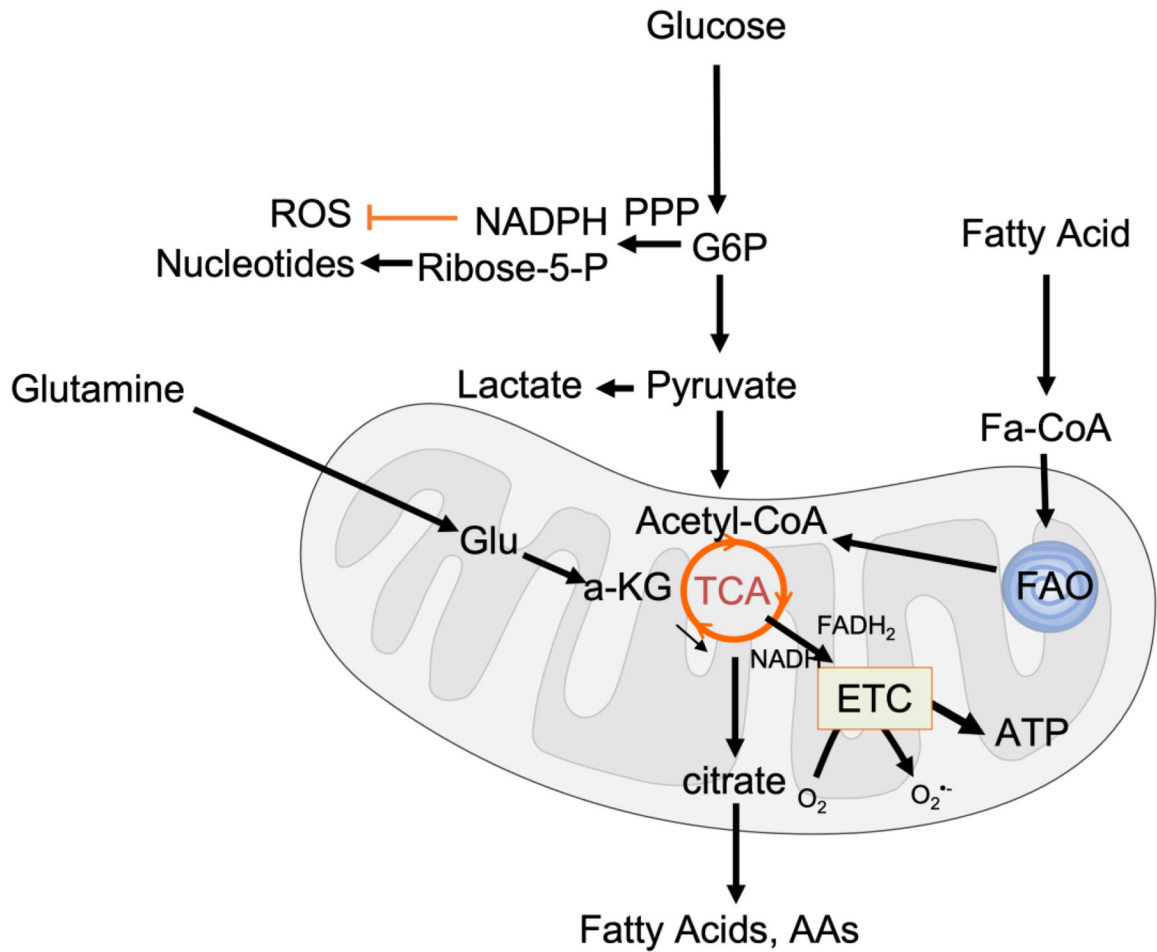
Figure 4. Metabolic pathways active in ascites resident OC cells
OC tumor cells demonstrate metabolic flexibility by their ability to utilize glucose, glutamine and fatty acids as fuel sources. Glucose-6-phosphate (G6P) can be routed into the Pentose phosphate pathway (PPP) for generation of NADPH and ribose 5-phosphate, that aids in ROS scavenging and nucleotide synthesis, respectively. Fatty acid oxidation (FAO) and glutaminolysis provide alternate carbon sources for the tricarboxylic acid cycle (TCA). The TCA provides metabolic substrates for macromolecular synthesis necessary for tumor cell proliferation and reducing equivalents FADH2 and NADH for Oxidative Phosphorylation and ATP production at the electron transport chain (ETC). A consequence of increased oxygen flow thought the ETC is superoxide production ($O_2^{\cdot-}$) which can lead to mitochondrial redox signaling used by tumor cells.

Table 1

Published reports of growth factor, cytokine, and chemokine levels in ovarian cancer

Growth factors and cytokines	Levels in circulation (Healthy) [pg/ml]	Levels in circulation (Ovarian Cancer) [pg/ml] *Unless indicated*	Levels in OC ascites fluid (AF) [pg/ml] *Unless indicated*	Additional information
IL6	5.186[156]	29.97[43]	14845.28[43] 391.09[157] 2955(median)[40] 6419[38]	Increased in malignant AF compared with benign[9,157]
IL8	10.9 (median)[158] 29.3 (median)[159]	28.05[43] 100.4 (median)[160]	1408[38] 202.17 in HGSOC and 456.03 in Mucinous and endometroid[157] 48.7 (median)[160]	Increased in non-serous OC AF compared with benign peritoneal effusion[157] Increased in malignant AF compared with benign[160]
IL10	12.6 (median)[159]	17.60[43] 17.3 (median)[160]	24 (median)[40] 137.85[43] 23.5 (median)[160]	Increased in AF compared with plasma[43,161] No change in malignant AF compared with benign[160]
IL2	14 (median)[159]	14.97[43]	3.74[43]	Reduced in AF of OC compared with plasma level. No difference between AF from malignant vs benign[162]
VEGF	61.6 (median)[159]	96.65[43] 9470[163]	10670.35[43] 10250[163] 2330 (median)[164]	Increased in AF compared with plasma[43] Increased in malignant AF compared with benign[163]
TGF β1	1.985 **ng/ml**[165] 1.000 **ng/mL**[166]	4–20 **ng/ml**[28,167,168]	14.435 **ng/ml**[162] 3.5 – 47.734 **ng/mL**[57] 388 pg/mL[31]	Blood level higher in all OC tumors (benign and malignant) compared with healthy controls[162] Elevated in AF from serous and endometroid OC compared with benign fluid[31]
TGF β2	<0.2 **ng/mL**[168]	8.1 **ng/mL**[169]	<0.1–4 **ng/mL**[57]	Higher serum level was reported to corelate with improved survival[169]
TNF-α	0.06[170]	11.01[43] 8.5[170]	16.63[43] 210[170]	Increased serum level in OC patients compared with healthy individuals[170] Increased in AF of OC compared with peritoneal lavage of healthy individuals[170]
IFN-γ	12.99[171]	284.51[43]	91.55[43] 2.76 (median)[172]	No difference between AF of OC and benign tumors[162] Decreased in AF of OC compared to plasma level[43]
PDGF-BB	8473 (median)[173]	960.19[43] 11422(median)[173]	31.31[43]	Reduced level in OC, AF compared with plasma[43] Increased level in serum OC compared to normal individuals[173]
FGF2	41.7 (median)[159] 33 (median)[173]	53 (median)[173]		Increased in serum of OC compared with normal individuals
RANTES	5839 (median)[159]	13269.13[43]	228.55[43]	Decreased in OC AF compared with serum[43] Elevated serum level in OC compared with benign ovarian cyst[174]
CCL2 (MCP-1)	41.5 (median)[159] 230[175]	490[175] 108.45[43]	4280[175] 1456.21[43]	Increased serum level in OC compared with healthy individuals[43,175]
CCL3 (MIP-1α)	7.1 (median)[159]	13.40[43]	15.55[43]	No change detected between OC serum and AF[43]
CCL4 (MIP-1β)	70[176]	145.07[43] 24.1 (median)[177]	729.78[43] 22.5 (median)[177]	Elevated in OC AF compared with serum[43] No changes reported in[177]

Growth factors and cytokines	Levels in circulation (Healthy) [pg/ml]	Levels in circulation (Ovarian Cancer) [pg/ml] *Unless indicated*	Levels in OC ascites fluid (AF) [pg/ml] *Unless indicated*	Additional information
CCL11 (Eotaxin)	242[178]	243.8 (median)[177]	104.1 (median)[177]	Reduced level in AF compared with blood
CXCL1	58.6[179]		321[31]	Elevated in OC AF compared with benign fluid[31]
CXCL5	162 (median)[180]		913[31]	Elevated in OC AF compared with benign fluid[31]
CXCL10	576.2 (median)[159]	726.2 (median)[177]	10,001 (median)[177]	Elevated in AF compared with blood from OC[177]