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The influence of secreted factors and extracellular vesicles in ovarian cancer metastasis

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ABSTRACT

Ovarian cancer cells mainly metastasise within the peritoneal cavity, the lethal consequence of tumour progression in this cancer type. Classically, changes in tumour cells, such as epithelial to mesenchymal transition, involve the down-regulatinon of E-cadherin, activation of extracellular proteases and integrin-mediated adhesion. However, our current understanding of ovarian tumour progression suggests the implication of both intrinsic and extrinsic factors. It has been proposed that ovarian cancer metastases are a consequence of the crosstalk between cancer cells and the tumour microenvironment by soluble factors and extracellular vesicles. Characterisation of the alterations in both the tumour cells and the surrounding microenvironment has emerged as a new research field to understand ovarian cancer metastasis. In this mini review, we will summarise the most recent findings, focusing our attention on the role of secreted factors and extracellular vesicles in ovarian cancer metastasis.

1. Introduction

Ovarian cancer metastases differ from the classical pattern of hematogenous or lymphatic metastasis found in most cancer types. Tumour types such as breast cancer often disseminate hematogenously, establishing metastases organotropically in other organs (e.g. bone, liver, brain) [1]. Other tumours such as melanoma have a considerable biological heterogeneity and metastasise in multiple organs distally by both lymphatic and hematogenous dissemination [2]. During ovarian cancer metastasis, tumour cells normally metastasise in the peritoneal cavity and the omentum by a somewhat passive mechanism, accumulating in the peritoneal fluid [3]. This process indicates that ovarian tumour cells hold, somehow, a preference to stay in the peritoneal cavity and metastasise in the mesothelium as primarily 'soil' for metastatic ovarian cancer 'seeds'.

The seed-and-soil hypothesis proposed by Stephen Paget in 1889 stated that the organ-preference patterns of tumour metastasis are the product of favourable interactions between metastatic tumour cells (the 'seed') and their organ microenvironment (the 'soil') [4,5]. Indeed, our current knowledge of tumour progression supports this theory, as tumour microenvironment became a crucial factor regulating metastatic outcome of multiple tumour types [6]. During this process there is an active crosstalk that exists between primary tumour and distant organs. Various stimuli released by cancer cells, including soluble factors and extracellular vesicles (EVs), are involved in the generation of suitable microenvironments for metastasis, also known as pre-metastatic niches (PMNs) [7,8]. PMNs are formed by the recruitment of non-resident

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cells, such as bone-marrow-derived cells (BMDCs), subsequently attracting circulating tumour cells [6,9,10]. Although congruent with both Paget's and Ewing's theories, the concept of the PMN proposes that the tumour itself pre-conditions specific organ sites for *future* metastatic disease *via* tumour-derived factors reinforcing the crosstalk between tumour cells and their microenvironment.

Several secreted factors and proteins have been involved in early invasion of the mesothelium and mesothelium metastasis being a major mechanism involved in peritoneal metastasis of ovarian and other types of cancer [11,12]. The omentum is the most frequent place for ovarian cancer metastasis [13] and has been associated with the presence of adipose tissue-derived mesenchymal stem cells [14] as well as with the abundance of 'milky spots' [15,16]. These are organised aggregates of immune cells and a complex network of capillaries with a high vascular density, with omental adipocytes that seem to exert complementary action towards the promotion of intraperitoneal metastasis [3]. It has been described that MMP-2 expression by metastatic serous ovarian cancer (SOC) cells has been involved in their attachment to peritoneal surfaces. MMP-2 inhibition before intraperitoneal dissemination in mice significantly decreased tumour growth and metastasis and extended survival [17]. Similarly, tissue transglutaminase (TG2) is up-regulated in epithelial ovarian cancer (EOC) cells compared with normal ovarian epithelium, and its secretion in ascites fluid leads to intraperitoneal tumour dissemination by enhancing cell adhesion to the extracellular matrix and modulating β1 integrin subunit expression [18]. Importantly, secretion of several factors, such as fibronectin from mesothelium cells, has been related to metastatic behaviour of ovarian cancer cells [19]. Overall, these data support the importance of secreted factors and proteins from tumour and mesothelium cells in ovarian cancer metastasis.

Besides soluble factors, EVs secreted from ovarian cancer cells have been involved in metastasis. As an example, ovarian cancer-derived exosomes have been used in the generation of artificial PMNs using exosomes from ascites of different types of ovarian cancer cell lines in implantable devices called 'M-Trap', which efficiently attract ovarian cancer cells [20]. M-Trap acts as a 'bait' for metastatic ovarian cancer cells, trapping them; these results suggest that extrinsic signals from the tumour microenvironment are actively involved in ovarian cancer cell homing. However, this specific study was performed using immunocompromised mice and still needs to be tested in further models.

Secreted factors, including exosomes, have been studied to improve our understanding of ovarian cancer. Nonetheless, some of the limitations of reported studies include: i) they are mostly based in cell lines, ii) Sample size and analysis of biological meaning of molecules secreted in exosomes remain to be uncovered. Exosomes from plasma of ovarian cancer patients, patients with benign disease and healthy controls were evaluated in several studies [21,22]; finding that plasma from ovarian cancer patients contained higher levels of exosomal proteins compared to plasma from patients with benign tumours or healthy controls. Nevertheless, we are far from finding the use in the clinic of these parameters, studies so far are descriptive of the observations, but further insights are needed for the application of liquid biopsies in ovarian cancer management. iii) While the majority of ovarian malignant tumours are epithelial in nature, several histological subtypes of these cancers are well described (EOC, SOC, endometrioid, etc.), and studies on exosomes and ovarian cancer studies are sometimes mixed on their histological subtypes. Indeed, proteomic comparisons of cancer EVs have demonstrated differences in vesicle cargo even among various ovarian cancer adenocarcinoma models *in vitro* [23,24]; it is likely that secreted factors and EV cargo differ between various tumour subtypes, therefore a better description of the works is needed.

Accumulating data demonstrate the importance of tumour microenvironment in ovarian cancer progression and the involvement of stromal-derived induction of the malignant progression [3,25].

1.1. *Ovarian cancer – adipocyte crosstalk*

Soluble factors secreted from the adipose tissue play an active role in ovarian cancer cell dissemination. Metastatic seeding of the omentum by the SOC cell line SKOV3 is partially attributed to the secretion of chemokines from omental adipocytes such as interleukin (IL)-6, IL-8, TIMP metallopeptidase inhibitor 1 (TIMP1) and monocyte chemotactic protein 1 (MCP1/CCL2) by omental adipocytes (Fig. 1). In this model, IL-6 and IL-8 receptors, as well as their ligands, IL-6 and IL-8, are crucial in SKOV3 cell metastasis since their inhibition reduced adhesion of SKOV3 cells [26]. Importantly, antibody-mediated inhibition of IL-6, IL-8, MCP-1 and TIMP-1 resulted in a reduction of *in vitro* ovarian cancer cell atraction towards adipocytes by at least 50% [26]. Interestingly, intraperitoneal injection of the ovarian cancer cell lines confirmed an increase in tumour burden in obese mice using the syngeneic EOC cell line ID8 and the SOC cell line SKOV3 *in vivo* by enhancing vascularity, diminishing M1/M2 macrophage ratio and altering lipid regulatory factors (fatty acid binding protein 4 (FABP4) (Fig. 1) [27]. In this work, enhanced intraperitoneal tumour burden was observed in overweight or obese animals. Histological analyses suggested that alterations in lipid regulatory factors, enhanced vascularity and decreased M1/M2 macrophage ratios. In addition, leptin (hypersecreted molecule in obese subjects) secreted by adipose tissue increases ovarian cancer cell migration, invasion and epithelial to mesenchymal transition (EMT) due to the activation of JAK/STAT3, PI3/AKT and RhoA/ROCK signalling downstream of the leptin receptor (Ob-Rb) using several SOC cell lines (Fig. 1) [28]. Furthermore, Ob-Rb is increased in metastatic lesions more than in primary tumours, which was associated with worse survival in overweight patients in high-grade SOCs [28]. Leptin also contributed to the maintenance of stemness and the mesenchymal phenotype in ovarian cancer cells [28]. These studies show that obesity impacts on ovarian cancer metastatic success by the influence of adipocyte-secreted factors in tumour cell behaviour [27,28].

Along tumour progression, tumour cells induce changes in the surrounding adipocytes, including delipidation and conversion towards a cancer-associated adipocyte (CAA) phenotype, which is characterised by a lower lipid content, fewer late adipose markers and overexpression of inflammatory

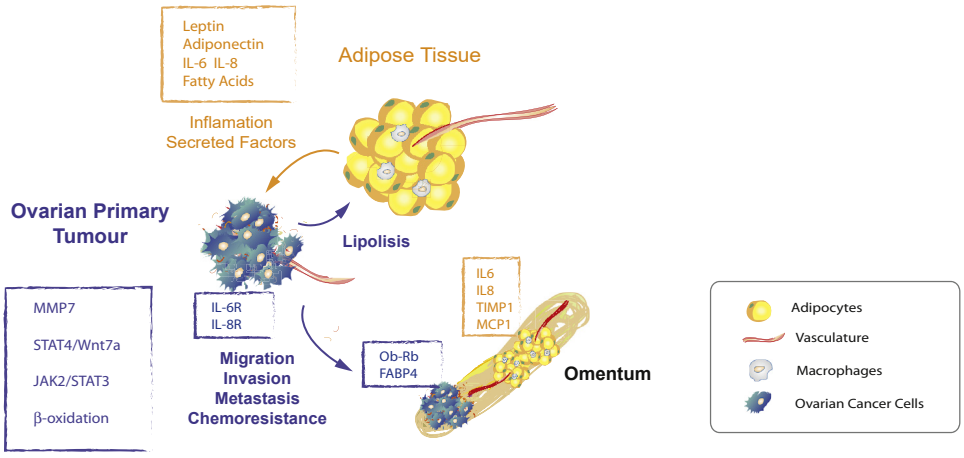

Fig. 1 – Main mechanisms involved in ovarian cancer – adipose tissue communication during metastasis. Schematic representation of how the secretion of specific adipokines and pro-inflammatory cytokines from adipose tissue (leptin, adiponectin, IL-6, IL-8, fatty acids) influences ovarian cancer migration and metastasis. Soluble factors act as local players that mediate in primary tumour growth and metastasis (see text for more details). In ovarian cancer, obesity influences metastasis to the omentum due to the increase in cytokines (IL-6, IL-8, TIMP1, MCP1) and over-expression of specific markers (Ob-Rb, FABP4) in ovarian cancer cells. Specific pathways are activated in ovarian cancer cells due to the action of these factors (see text for more details). Tumour cells induce phenotypical changes in the surrounding adipocytes including delipidation and conversion towards cancer-associated adipocytes (CAA). In turn, mature adipocytes secrete free-fatty acids. Factors modulated are shown in yellow (adipocytes) and blue (ovarian cancer).

cytokines and proteases [29]. The *in vitro* co-culture of SOC cancer cells line with adipocytes induces lipolysis and the transfer of lipids to tumour cells, fuelling tumour growth (Fig. 1) [26]. In turn, mature adipocytes secrete free-fatty acids (FFAs) that are transferred to tumour cells. The transfer of FFAs induces β-oxidation and stimulates the up-regulation of FABP4 in omental metastases using the SOC cell line SKOV3 [26]. Consistent with the role of lipids as an energy source for ovarian cancer cell growth, metastatic burden and the adipocyte content in the omentum are inversely correlated *in vivo* using multiple cell lines of ovarian cancer [16].

Adipose-derived mesenchymal stem cells (AD-MSCs) have received more attention for their roles in the development of cancer, promoting tumour proliferation and invasive properties. Interestingly, the omentum is considered a rich source of AD-MSCs. Moreover, AD-MSCs from the omentum promote proliferation, migration and chemoresistance of serous and adenocarcinoma ovarian cancer cell lines [14]. Although these authors did not define specifically the mechanism involved, they postulated that AD-MSCs may influence ovarian cancer metabolism consistent with the proposed 'reverse Warburg effect' in which stromal-derived lactate is consumed by adjacent cancer cells to fuel oxidative phosphorylation. However, future studies are needed to determine this hypothesis [14]. In addition to promote cancer, AD-MSCs can also secrete paracrine cytokines to enhance cancer cell proliferation and metastasis [30]. These authors showed that AD-MSCs enhance sphere formation and *in vivo* tumour initiation of breast and colon cancer cells. Interaction of AD-MSCs and cancer cells stimulated secretion of IL-6 in AD-MSCs, which in turn acted in a paracrine manner on cancer cells to enhance their malignant properties through activation of JAK2/STAT3 pathway in cancer cells [30].

Clinically, data is still developing, and more agreement is needed in defining the parameters influencing ovarian cancer, the relevance of ovarian cancer subtype and the factors associated with obese individuals. Data suggest that higher body mass index (BMI) increases risk of non-high-grade SOCs, but not the more common and aggressive non-high-grade SOCs subtype [31]. Other reports found that high BMI is associated with an increased risk of type I ovarian cancer (e.g. serous borderline and low-grade serous invasive tumours) [32]. However, no association was found in this study with type II or high-grade SOCs. Similarly, high BMI is a detrimental factor for survival in low-grade serous [33] and epithelial ovarian cancers [34]. Nevertheless, reading all these studies, it is not clear whether the fact of being overweight at the time of diagnosis or before affects survival. Several factors may explain these differences (e.g. the inclusion criteria), determining the intra- or inter-study heterogeneity is a must when analysing the impact of obesity on ovarian cancer survival. Overall, obesity seems to act as a paracrine variable, leading to remodelling of the tumour micro-environment and secretion of factors that reinforce metastatic behaviour.

1.2. *Cancer-associated fibroblasts and ovarian cancer crosstalk*

Cancer-associated fibroblasts (CAFs) often represent the majority of stromal cells in various types of human carcinoma,

including ovarian cancer [3]. Analysis of ovarian cancer stromal signatures using micro-dissected tumour samples obtained from advanced high-grade SOC patients identified that stromal microfibrillar-associated protein 5 (MFAP5) is a prognostic marker for poor survival [35]. This protein stimulates ovarian cancer motility and metastatic potential via the Ca2+-dependent focal adhesion kinase/cAMP response element-binding protein/troponin C type 1 signalling pathway (FAK/CREB/TNNC1) (Fig. 2). Interestingly, targeting MFAP5 decreased tumour growth and metastasis [35]. Similarly, analysis of gene expression in micro-dissected stromal and epithelial components of high-grade serous ovarian tumours identified versican (VCAN) as a key gene in CAFs that promotes the motility and invasion of ovarian cancer cells [36]. In this mechanism, versican expression was modulated by the activation of TGFβ signalling in CAFs induced by TGFβ ligands secreted by tumour cells [36]. This key molecule promotes the activation of the nuclear factor-κB signalling pathway up-regulating CD44, MMP-9 particularly at the stroma–cancer interface in a panel of several ovarian cancer cells. This suggests that CAF-derived *VCAN* modulates ovarian cancer cell motility and invasion potential via activation of *CD44* and *MMP9* [36].

STAT4 was found over-expressed in EOC cells associated with poor outcome in ovarian cancer patients [37]. This factor induced EMT of EOC cells *in vivo*, but not *in vitro*, suggesting that tumour microenvironment could play a role in this process. Indeed, functional analysis revealed that STAT4 over-expression in ovarian cancer cells secretes Wnt7a and induces the release of CXCL12, IL-6 and vascular endothelial growth factor-A (VEGFA) by CAFs within the tumour microenvironment that could be responsible for *in vivo* EMT (Fig. 2) [37].

Recently, it has been reported that AD-MSCs from omentum of EOC patients express CAF markers, including α-SMA and fibroblast activation protein (FAP), via the TGF-β1 signalling pathway. These results suggest that AD-MSCs may be a novel source of CAFs and that they participate in the interaction between tumour cells and the omental micro-environment may be favouring the formation of metastatic niches [38].

But fibroblasts not only provide the primitive matrix for attachment of the tumourigenic cells, they also may influence the interaction of tumour cells with the micro-environment by integrins. For instance, activated fibroblast secretes EGF-inducing integrin α5 expression on SOC ascitic cells, thus promoting exacerbation of ovarian cancer [39]. Vascular cell adhesion molecule 1 (VCAM-1, an integrin ligand) expressed on mesothelium cells has been reported to interact with α4β1 on SOC and adenocarcinoma cell lines playing an important role in ovarian tumour growth, and it may be used as a prognostic factor and novel therapeutic target for ovarian cancer. Importantly, the inhibition of VCAM1 or its ligand α4β1 abolished dissemination and colonisation in an ovarian cancer xenograft model [40].

Clinically, a number of the members of the integrin family, including α5β1 and αvβ3 or αvβ5 integrins, are markedly elevated in aggressive ovarian tumours [17,41–43]. In EOC, several studies have focused on the role of α5β1, which is expressed in 40% of patients with advanced ovarian carcinoma and is involved in dissemination of ovarian cancer [44,45]. The median survival of the patients with α5β1-integrin over-expression was significantly worse [42]. But other integrins have also a role in ovarian cancer, for example, α6β1, α2β1 and α3β1 have been implicated in spheroid adhesion of ovarian cancer cells to peritoneal cells through binding to laminin and type IV collagen, respectively [45].

Integrins on exosomes have been also related to cancer progression, in particular, the amount of integrins α6, αv and β1 correlates with tumour stage across a variety of epithelial cancer cells including ovarian cancer [46]. Importantly, in a seminal study published by Hoshino and colleagues in 2015 [47], tumour-derived EVs were demonstrated to harbour

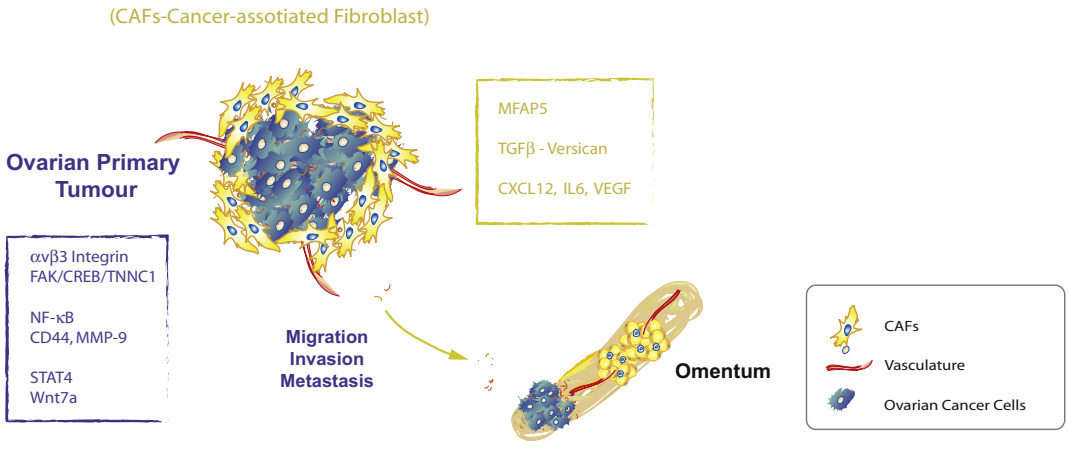

Fig. 2 – Communication between CAFs and ovarian cancer cell during metastasis. Schematic representation of the main factors secreted by cancer-associated fibroblasts (MFAP5, TGF-β b, Versican, CXCL12, etc.). Secretion of these factors influences metastasis to the omentum due to signalling activation (integrins, Nf-κβ, FAK, STAT4, see text for more details). Factors modulated are shown in yellow (CAFs) and blue (ovarian cancer).

particular integrin patterns, namely α6β1, α6β4, αvβ5 and αvβ3 that determine and predispose the formation of PMNs in different organs, and guided organ-specific metastasis, unfortunately none of them related to ovarian cancer [47].

1.3. *Macrophages*

Analysis of early metastasis to the omentum indicates that ID8 EOC cell line relies on the interaction with immune cell aggregates in syngeneic C57BL/6 mouse models [15]. Both local and systemic host inflammatory responses accompany ovarian tumour progression, normally by the secretion of soluble factors (Fig. 3) [48]. Interestingly, depletion of peritoneal macrophages (but not neutrophils or natural killer cells) has been reported to reduce ES-2 ovarian clear cell carcinoma cell metastasis, as measured by ascites formation and peritoneal metastasis using immunodeficient mouse models [49]. In this experimental setting, VEGF expression was reduced in macrophage-depleted mice, suggesting that pro-vasculogenic factors secreted by tumour-associated macrophages (TAMs) could play a role in ovarian cancer metastasis (Fig. 3) [49]. Although the macrophage products MMP-9 and VEGF have previously been implicated in ovarian tumour progression [50,51], these authors did not observe differences between macrophage-depleted and control mice in MMP-9 production or activity [49]. Several other secreted soluble factors have also been involved in ovarian metastasis; VEGF-C, VEGF-D and VEGF-A secreted from CD11b(+) macrophages are responsible for producing dysfunctional lymphangiogenesis (Fig. 3). Accordingly, the combined blockade of VEGF-C/D and VEGF-A signalling with soluble VEGF receptor-3 and VEGF-Trap has been proposed to inhibit ovarian ascites formation using different cell lines of ovarian cancer [52].

In addition, TAMs also secrete the proinflammatory cytokine IL-6, an important interleukin that has been involved in ovarian cancer [53–55]. It has been demonstrated that IL-6 increases anchorage-independent growth, proliferation, adhesion and invasion, while it leads to depletion of endogenous IL-6 expression in IL-6-over-expressing ovarian cancer SKOV-3 cells [56]. IL-6 released from TAM stimulated the expression of PD-L1 at the surface of HO8910 and SKOV3 ovarian cancer cells suggesting a potential mechanism involved in immune cells evasion [57]. Interestingly, CAFs are the main source of IL-6 in ovarian cancer tissue promoting the activation of JAK/STAT3 in the SOC cell line OVCAR3-enhancing proliferation, invasion and epithelial to mesenchymal transition (EMT). EMT led to chemotherapy resistance [58], indicating that this pathway may be a potential target to prevent ovarian cancer progression as previously noted [53,55]. On the other hand, elevated IL-6 by several ovarian cancer cell lines is induced by platinum-based chemotherapy promoting the polarisation of macrophages towards

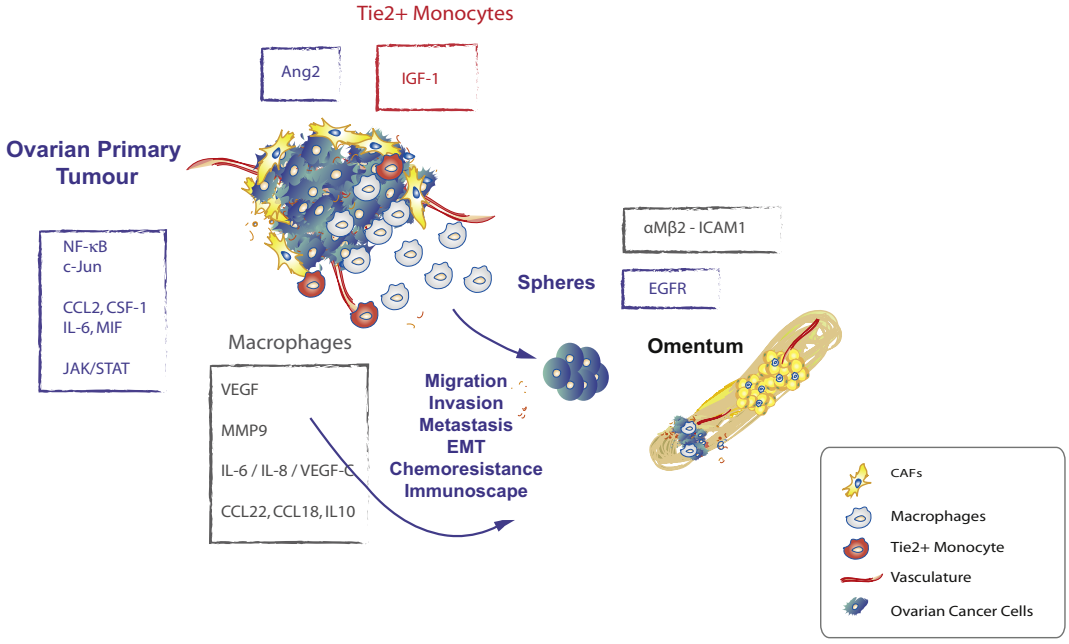

Fig. 3 – Macrophages and other immune cells reinforce ovarian cancer metastasis. Schematic representation of how macrophages secreted soluble factors (e.g. VEGF, IL-6, IL-8) during ovarian cancer progression favouring invasion and metastasis. These factors promote the activation of specific pathways (e.g. NF-κβ, c-Jun) and the secretion of different extracellular factors (e.g. CCL2, CSF-1, IL-6, MIF, EMPRINN). Tie2-expressing monocytes secrete IGF-1 in response to Ang2 secreted by ovarian cancer cells. The formation of ovarian cancer cell spheroids is reinforced by interaction with macrophages that promote EGFR expression by specific integrins and adhesion molecules on their surface (ICAM, αMβ2 integrin). Cancer cell-related changes are shown in boxes. Factors modulated are shown in grey (macrophages), red (Tie2-expressing monocytes) and blue (ovarian cancer).

immunosuppressive TAMs [59]. Intra-cellular levels of IL-6 are higher in immunosuppressive macrophages (PD-L1(+) CD68(+)) and are also increased during ovarian cancer progression in different types of ovarian cancer patients. These macrophages may be considered as a novel cell population contributing to immune escape of ovarian cancer [60].

It is interesting to note that interactions between macrophages and ovarian cancer cells are bidirectional; macrophages increase tumour cell invasiveness in a TNF-α- and NF-κB/JNK-dependent mechanism involving downstream mediators such as extracellular matrix metalloproteinase inducer (EMPRINN/Basigin) and migratory inhibitor factor (MIF) (Fig. 3) [61]. In turn, ovarian cancer cells promote macrophage differentiation towards an alternatively activated TAM phenotype [62]. Indeed, the survival of ovarian cancer (mostly SOC patients in this work) is linked to the presence of TAMs with a transcriptional signature that is characterised by a low expression of protumourigenic and immunosuppressive markers and an up-regulation of genes linked to interferon signalling [62].

Ovarian cancer cells also induce macrophage accumulation by the secretion of the chemoattractant factor MCP-1; this factor is secreted mainly by tumours cells and can be detected *in vitro* and *in vivo* in several types of ovarian cancer [63]. MCP-1 protein may contribute to the accumulation of TAMs, which may afterwards influence tumour cell behaviour. In ovarian cancer patients, tumour cells and macrophages produce the chemokine CCL22, which mediates the trafficking of regulatory T cells (Tregs), may be also fostering tumour cell immunoescape (Fig. 3) [64]. TAMs have been described as promoting spheroid formation and enhanced metastasis in mouse ovarian cancer cell models. In this model, TAMs are localised in the middle of ovarian cancer cell spheroids and promote EGF secretion by EOC cells, interestingly therapeutic use of EGFR inhibitors prevented the early dissemination of SOC cells as well [65]. Regarding extracellular proteases, *in vitro* analysis of ovarian cancer cells invasion with TAMs suggests that they promote the up-regulation of MMP-2, MMP-9 and MMP-10 expression and enhanced ovarian cancer cells invasion via a toll-like receptor signalling pathway (Fig. 3) [66].

Specific subsets of monocytes such as Tie2-expressing monocytes (TEMs) have been recently linked to ovarian cancer progression. TEMs were significantly increased in tumour foci, peripheral blood and ascites from ovarian cancer patients showed diagnostic value and being positively correlated with the microvascular density tumour tissue (Fig. 3) [67]. In this model, TEMs promote angiogenesis via secretion of insulin growth factor 1 (IGF-1) both *in vivo* and *in vitro* after stimulation by angiopoietin 2 (Ang2) from different types of ovarian cancer cell lines. This study suggests that novel therapies targeting the Ang2-TEMs-IGF1 axis in ovarian carcinoma may represent a novel way to block tumour-microenvironment communication [67].

1.4. *Role of extracellular vesicles in ovarian cancer metastasis*

Secreted extracellular vesicles (EVs) have been described playing a role in PMN formation and metastasis in several cancer types, including ovarian cancer [20,47,68–70]. EVs are a heterogeneous population of vesicles formed by exosomes (40–100 nm diameter and originating in the multivesicular bodies) and microvesicles (MVs, 100–1000 nm diameter that bud directly from the plasma membrane [71]. Other types of vesicles have been described, such as cytoplasts [72] and large oncosomes [73]. EVs carry different molecules, including proteins, RNA, DNA, lipids and metabolites [74–76]. EV cargo is normally representative of the cell of origin. Studies on cancer models demonstrate that EVs are a major tumour-derived factor that promotes recruitment of bone-marrow-derived and other stromal cells to PMNs, reinforcing metastasis [68].

In ovarian cancer, AD-MSCs-derived exosomes reduce the viability of serous and adenocarcinoma ovarian cancer cell lines as well as impair their wound-repair and colony-forming ability [77]. In this model, exosomal miRNAs secreted from MSCs are the potential regulators of cell-cycle progression promoting anti-tumour effects. AD-MSCs-derived exosomes also induce the upregulation of pro-apoptotic molecules such as BAX, CASP9, CASP3 and the down-regulation of BCL2, thereby activating apoptotic signalling (Fig. 4) [77]. Exosomes derived from macrophages can also influence SOC cell lines and inhibit metastasis [78]. These authors identified 19 miRNAs that are differentially expressed in exosomes derived from macrophages, treated with or without the tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK). They found that microRNA-7 (miR-7) in macrophages and its secreted exosomes shuttled to ovarian cancer cells, reducing the activity of the EGFR/AKT/ERK1/2 axis reducing ovarian metastasis in a xenograft mouse model (Fig. 4) [78]. It has been reported that exosomes from other stromal cell types such as CAAs and CAFs also shuttle miRNAs to different types of ovarian cancer cell lines, favouring tumour progression. Mechanistically, miR-21 suppresses ovarian cancer apoptosis and confers chemoresistance by binding to its direct novel target, apoptosis protease-activating factor-1 (APAF1) [79]. It has been also shown that exosomes derived from two models of SOC cell lines induce both the phenotypic and functional transformation of AD-MSCs into a tumour-associated myofibroblastic cell phenotype in tumour stroma (Fig. 4) [80].

Ovarian-cancer-derived exosomes also influence metastatic progression [29]. Ovarian cancer-derived exosomes carry and transfer molecules that directly regulate tumour cell migration in recipient cells, including CD24, EpCAM [81]. CD24 is released by ovarian cancer cells at different levels depending on the model, but it is extensively secreted in exosomes isolated from malignant ascites fluid of ovarian carcinoma patients. However, no correlation with the expression of CD24 in tumour tissue sections was detected. Similarly, the epithelial cell adhesion molecule (EpCAM), known to be over-expressed in ovarian carcinomas, is secreted in exosomes, but no function was deciphered from this study. Activated matrix metalloproteinase including (MMP)-2, MMP-9, uPA have been also found in ovarian-cancer-derived EVs from malignant ascites [82], suggesting that EV secretion from ovarian cancer cells may lead to increased extracellular matrix degradation facilitating tumour cell invasion and metastasis [82]. Interestingly, exosomes can also discard tumour suppressor miRNAs favouring ovarian cancer progression [83]. In this work, the authors found that miR-6126 is ubiquitously released in

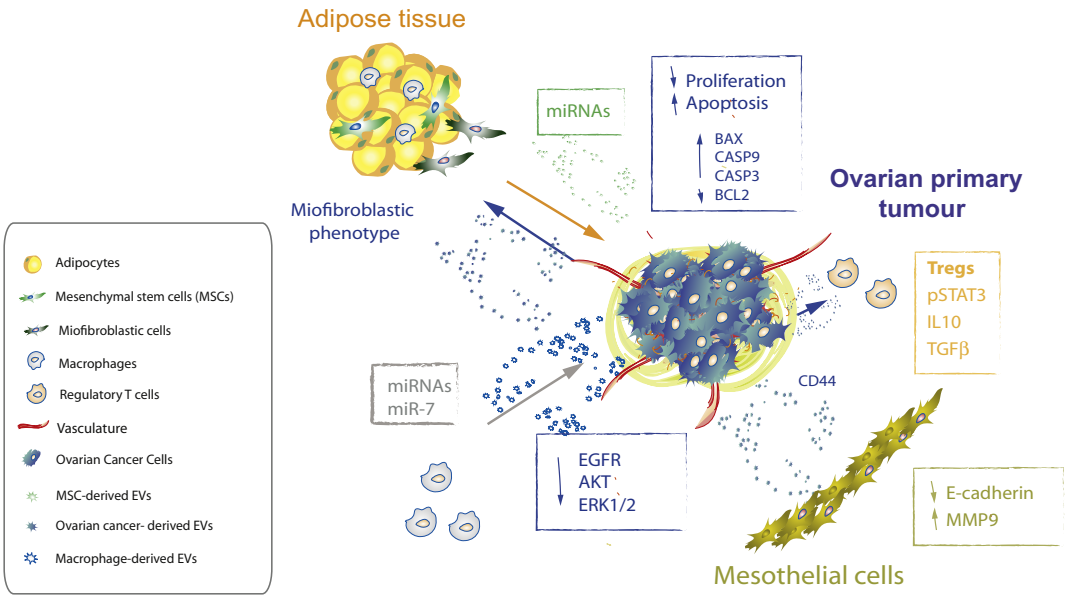

Fig. 4 – Communication between micro-environment and ovarian cancer cells through secreted vesicles. Exosomes from AD-MSCs reduce tumour cell proliferation activating apoptosis signalling. In turn, exosomes derived from ovarian cancer cells induce a myofibroblastic cell phenotype in MSCs. Macrophages secrete miR-7 promoting down-regulation of EGFR, AKT and ERK1/2. CD44 is secreted in ovarian cancer exosomes reinforcing mesenchymal transition in mesothelial cells. Similarly, exosomes from ovarian cancer cells induce Treg and the secretion of immunomodulatory chemokines (IL10). Secreted exosomes are represented by asterisks in grey (macrophages), blue (ovarian cancer cells), green (AD-MSCs). Cancer-cell-related changes are shown in boxes.

high abundance from both chemosensitive and chemo-resistant SOC cells via exosomes suggesting that release of miR-6126 favours ovarian cancer progression. Indeed, they found that naturally miR-6126 targets integrin β1, reduces invasion and migration of ovarian cancer cells *in vitro* and tumour growth *in vivo* acting as a tumour suppressor; its secretion in exosomes is therefore a potential mechanism of tumour progression to discard tumour suppressor molecules [83].

Interestingly, treatment of ovarian cancer cells with chemotherapeutic agents such as cisplatin led to the release of EV secretion, increasing invasion and resistance by a potential bystander effect (BE), a phenomenon that occurs when naïve cells exposed to signals (e.g. from stressed cells) can display the effects of stress [84]. BE is induced, for example, by ionising radiation and is mainly mediated by EVs. For example, naïve cells treated with media conditioned by heat-shocked cells showed higher levels of DNA damage and apoptosis than cells treated with media from control cells [85]. These results support that cancer cells stressed by the addition of cytotoxic chemotherapeutics could release EVs into the tumour micro-environment, which could then be taken up by other cells (including other cancer cells), leading to potential effects on tumour progression. The same authors reported that BE is also important in ovarian cisplatin resistance to chemotherapy using endometrioid adenocarcinoma cell lines; in this model treatment of ovarian cancer cells with cisplatin led to the release of EVs that could induce invasion and increased resistance when taken up by bystander cells. This was concomitant with changes in p38 and JNK signalling, suggesting that these pathways may be involved in mediating cisplatin resistance in ovarian cancer [84]. These results support that preventing EV release during chemotherapy is a potential therapeutic target by preventing BE.

Recently, CD44, a cell surface glycoprotein, was found secreted in SOC cell lines–derived exosomes, transferred and internalised in human peritoneal mesothelium cells (HPMCs). Upon exosome uptake, HPMCs underwent a mesenchymal transition characterised by the secretion of MMP-9 and down-regulation of E-cadherin. The inhibition of exosome release from cancer cells blocked CD44 internalisation in HPMCs and suppressed ovarian cancer invasion (Fig. 4) [86]. Ovarian-tumour-secreted microvesicles from different models of ovarian SOC and endometrioid cancer cell lines have been found to promote Treg expansion, suppressor function and resistance to apoptosis. In this model, microvesicle-treated Treg increased expression levels of phospho-STAT3, phospho-SMAD2/3, IL-10 and TGF-β1 as well as production and may be responsible for attenuating anti-tumour immune responses (Fig. 4) [87].

Overall, these data support that EVs secreted from the cells in the microenvironment such as macrophages and MSCs initially impair metastatic properties of ovarian cancer cells; on the other hand, EVs secreted from tumour cells seem to promote changes in the tumour microenvironment favouring

metastatic progression, and the secretion of proteases and factors involved in immunosuppression.

1.5. *Concluding remarks*

Ovarian cancer remains the most lethal disease among gynaecological malignancies, due to the majority of patients diagnosed in an advanced stage [88], with very low survival rates in 5 years. This could be attributed to the lack of effective tumour biomarkers for the early detection of this disease [88]. Circulating exosomes could serve as tumour biomarkers with the potential of providing information on early diagnosis, prognosis, response to therapy and development of chemo-resistance [89]. In ovarian cancer, as denoted in this review, several studies have demonstrated that secreted exosomes exist in ovarian cancer biological fluids that may be used to monitor the disease. Importantly, secreted exosomes play an active role in ovarian cancer progression together with soluble factors extrinsically involved in the communication with the micro-environment but also intrinsically facilitating chemo-resistance, invasion and metastasis.

Historically, mechanisms such as EMT have been considered as a hallmark during ovarian cancer progression. Several works also denoted that secreted exosomes may be involved in this mechanism. Recent studies revealed that LIN28, an RNA-binding protein, is secreted in IGROV1-derived exosomes inducing HEK293 cell migration and invasion increasing the expression of genes related to EMT [90]. On the other hand, TGFβ1 secreted in fibroblast-derived exosomes promotes EMT in SOC cell line models [91]. Similarly, in other models, EVs isolated from the highly malignant breast cancer cell line MDA-MB-231 stimulated with linoleic acid induce an EMT-like process in epithelial MCF10A cells [91]. However, finding the role of exosomes in initiating and establishing EMT *in vivo* will require intensive future investigation, since these studies are based in *in vitro* systems.

Importantly, as mentioned earlier, a role for exosomes has also been proposed in regulating the immune system and immune responses against ovarian tumours [60,87]. Indeed, ovarian carcinomas present a highly immunosuppressive tumour micro-environment through different mechanisms including down-regulation of tumour-associated antigens and antigen-presenting machinery [92], B7H4+-macrophages [93] and tolerance-inducing plasmacytoid dendritic cells, production of immunosuppressive cytokines, such as IL-10 and TGFβ [94] among others. Release of immunomodulatory factors such as CCL22, CCL18 and IL-10 may play a role in ovarian cancer cell immunoescape [94]. Novel players such as tumour EVs came recently to the scene – while EVs from the microenvironment have anti-tumour effects by horizontal transfer of miRNAs, EVs from ovarian cancer cells seem to influence stromal cells towards a pro-metastatic phenotype. Modulation of immune cells by ovarian-cancer-secreted EVs has been reported to suppres T-cell responses enhancing tumour growth by an arginase-1-dependent mechanism [95] or by GD3, a ganglioside expressed on the surface of tumour-derived exosomes [96]. Ovarian cancer ascites-derived exosomes have been found to suppress T-cell inducing apoptosis in a mechanism dependent on FasL [97]. The presence of immunosuppressive signal in EVs of ovarian tumours [98] represents a potential therapeutic target for patients with ovarian cancer. Nevertheless, there is a lack of specific therapies that could be applied to the clinical setting, and most of the studies are based on basic science; there is a need to apply all these results in the clinical field.

Circulating exosomes may constitute a novel biomarker in liquid biopies improving personalised medicine protocols in ovarian cancer patients. However, there are limited clinical studies that propose the use of specific biomarkers on circulating EVs to monitor and predict ovarian cancer outcome. Similarly, there is a limited number of studies applying the lessons learned from EV field to ovarian cancer treatment. Future research developing novel biomarkers detectable in circulation or by a simple test would impact improving patient survival, personalised treatments and reduce lethality.

Declaration of competing interest

The author declare no conflict of interest.

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