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Role of cancer-associated fibroblasts in tumor structure, composition and the microenvironment in ovarian cancer (Review)

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Abstract. Ovarian cancer (OVAC) remains the most lethal gynecological malignancy; it is ranked fifth among the most common types of cancer that affect women worldwide. Several aspects of the disease, including molecular pathogenesis, epidemiology, histological subtypes, poor prognosis at early stages due to the absence of specific signs and symptoms, and curative treatments in the advanced stages are all responsible for the poor survival rate, which is evaluated to be at 5 years once the cancer is diagnosed and treatment begins. A better understanding of the pathogenesis of ovarian cancer is therefore crucial, even though unexplored pathways, in order to improve the prognosis of patients with OVAC and to develop novel therapeutic approaches. Accordingly, the tumor microenvironment, defined as the combination of proteins produced by all tumor cells and by non-cancerous cells or the stroma, and composed of several cells, including those from the immune, inflammatory and adipose systems, as well as the mesenchymal stem, endothelial and fibroblasts cells, has recently attracted attention. Of particular interest are fibroblasts, which can be activated into cancer-associated fibroblast (CAFs) to become a potent supporter of carcinogenesis, promoting the initiation of epithelial tumor formation, tumor growth, angiogenesis and metastasis, as well as therapeutic resistance and immunosuppression. Thus, the targeting of CAFs for early diagnosis and effective therapy warrants our attention. In this review, we discuss the mechanisms through which CAFs may affect the structure, composition and microenvironment of the ovarian tumor. We also aim to highlight important aspects of OVAC pathobiology involving CAFs, in an attempt to provide insight into novel diagnostic windows and provide new therapeutic perspectives.

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1. Introduction

Among the various types of gynecological cancers, ovarian cancer (OVAC) remains the most lethal type which affects women worldwide. Indeed, several aspects of OVAC render it a very challenging malignancy for diagnosis and treatment. The survival rates are estimated to be approximately 5 years, following diagnosis at an early stage of the disease, which very rarely occurs, as the symptoms are non-specific and can be summarized into ordinary abdominal pain or bloating (1). The survival of women with OVAC is often closely related to the outcome of surgical reduction (2) that arises when the cancer has already progressed to the stage of aggressive metastases to the adjacent abdominal organs (1,3).

Therefore, the acute forms of OVAC usually correspond to the advanced stages, when the cancer has eventually reached the level of intestinal obstruction and pleural effusion. In general, abdominal bloating or swelling, adnexal mass, changes in menstruation, rectal bleeding or atypical glandular cells on a cervical cytology examination, discomfort in the pelvic area, frequent urinary needs and changes in bowel habits refer to subacute manifestation (1,4). When a patient reaches such an advanced stage of the disease, the clinical management consists of performing a reduction surgery followed by adjuvant chemotherapy or vice versa (5). Subsequently, depending on the cases encountered, following the initial response, tumor recurrence is mostly observed due to residual disease in women who will eventually succumb to the disease due to progressive chemoresistance (5).

Moreover, the stimulation of immune system through the use of immunotherapy, which has recently been used to effectively eliminate tumors in various type of cancer, has been limited in some patients, which have become unresponsive or

have developed resistance (6,7). In general, despite progress being made in the treatment of OVAC, a large number of women (70-80%) who have first responded to treatment eventually suffer a relapse and succumb to the disease (8). Thus, it is mandatory to improve the early diagnosis of OVAC and to develop effective treatments in order to cope with current surgical limitations and reduce resistance to treatment that often occurs in response to chemotherapy (5) or recently in response to immunotherapy (6,7). Therefore, taking all early observations into consideration, and as suggested by the data, therapies targeting only cancer cells alone seem to be insufficient for the treatment of such a malignant cancer and arguments are in favor of co-targeting cancer cells and their microenvironment (9).

Indeed, the heterogeneity of OVAC is likely related to epigenetic and genetic factors (10), which has led to the failure of effective eradication due to the complexity of signaling networks that are themselves interconnected in a complex manner. The microenvironment of the tumor, distinct from the peritoneal tumor (11), also plays a role in effective treatment, and is composed by all the proteins that will be produced by various tumor cells, including several types of immune cells, such as macrophages, natural killer cells and T cells, as well as a large number of chemokines and cytokines at the level of extracellular matrix (ECM) (12). All these components will actively interact together for the promotion of OVAC cell growth and metastasis (13). The tumor microenvironment (TME) also contains non-cancerous cells, termed the stroma, which is composed of several types of cells, including endothelial cells, mesenchymal stem cells, fibroblasts, endothelial cells, adipocytes, pericytes, and inflammatory and immune systems cells (14). It has been shown that fibroblasts, the major component of the stroma, can be stimulated by a variety of proliferative signals to become activated fibroblast, termed cancer-associated fibroblasts (CAFs). Over time, CAFs have been identified as an essential component of tumor progression (15) that maintain the microenvironment optimal for cancer cell survival and proliferation (15,16) through reciprocal crosstalk between cancer cells and fibroblasts. Data suggest that CAFs are involved in epithelial progression, cancer invasion, metastasis and therapeutic resistance, since following their activation, they provide, in turn, a favorable and adequate microenvironment owing to the various soluble factors produced (17). In general, the Data suggest that, together with cancer cells, CAFs may be one of the essential components of the TME that could represent molecular therapeutic targets for the treatment of cancer (18). Hence, the pathological understanding of OVAC through the close involvement of CAFs in malignant tumors is crucial for developing novel strategies for early diagnosis and/or therapies (19).

2. Role of ovarian fibroblasts

Anatomically, ovaries are retro-peritoneal organs, ovoid in shape, presenting two main regions including the cortex and the medulla. The cortical region, also termed the stroma, is full of connective tissues, low in collagen fibers, but rich in fibroblasts and myofibroblasts and contains the ovarian organelles (follicles). Fibroblasts play important roles in this environment, including guiding inflammation, ECM deposition, epithelial differentiation regulation (20) and wound healing (20,21). Indeed, many components of the fibrillar ECM, including collagens type I, III and V and fibronectin are synthesized by fibroblasts (21,22) that also play a role in the creation of basement membranes via the excretion of type IV collagen and laminin (23). In addition, fibroblasts also secrete matrix metalloproteinases (MMPs), ECM-degrading proteases, that emphasize the crucial role of fibroblasts in maintaining ECM homeostasis through the regulation of its turnover (23,24). Therefore, fibroblasts are considered as the main origin of ECM components and the principal mediators of scarring and tissue fibrosis. Accordingly, in the stroma compartment, fibroblasts are the element that tightly control the maintenance of ovaries or tissue homeostasis through different interactions between cells and the production of ECM components, the result of which is to provide the elements necessary for the proper architecture and function of tissues. That said, changes in the characteristics of the stroma are indeed an initial attempt to 'repair the damage' by inducing a transformation of the epithelium and, interestingly, fibroblasts can 'detect' signals between cells and the ECM through their adhesion's integrin-dependent cell-matrix, and subsequent changes occur in the dynamics and composition of the stroma. The reciprocal complex interactions between the stroma and epithelium result in changes in the ECM and tumor stroma (25). This control is crucial to preserve normal organ or tissue morphology and for fibroblasts to have a proper function, as the function of normal fibroblasts is typically to suppress tumor formation (26).

However, it well known that compared to fibroblasts isolated from healthy organs or tissues, those involved in the cicatrization or are derived from fibrotic tissue secrete significantly higher levels of normal constituents of the ECM (27,28). Such activated fibroblasts decrease their production once the wound is repaired and the resting phenotype is then supposedly restored (21). However, it is unknown whether these activated fibroblasts recover or rest in a resting phenotype followed by restocking of this particular tissue region, leaving fibroblasts at rest from the adjacent tissue (29). This is relevant since as regards organic fibrosis, fibroblasts located on the tumor site remain activated and experimental data have suggested a leading role for fibroblasts in defining the degree and magnitude of the tumor evolution (30). Thus, the mechanisms underlying the unmitigated activation of fibroblasts remain largely unknown.

3. Origin of ovarian CAFs

Although the origin of activated fibroblasts remains enigmatic, it has long been thought that their main source was resident fibroblasts or mesenchymal stem cells (31), as observed with the conversion of mesothelial cells into myofibroblasts by mesothelial-to-mesenchymal transition (MMT) (32), becoming the most important source of CAFs in inflammatory and fibrotic peritoneal pathologies (33). However, CAFs appear at first as the host the response caused by tumor growth in response to epithelial damage (34,35). Initially, the recruitment of CAFs in the nascent neoplastic region may reflect the antitumor early response (35,36). The accumulation of CAFs in the wound will facilitate a series of cascades of repair and tissue remodeling, in addition to controlling repairs and

preventing possible tissue damage (35,37). However, organic fibrosis, which is a condition associated with the continuous activation of fibroblasts, results in chronic inflammation and altered lesions, and in the generation of the functional renewal of the affected tissue CAFs. This may bring about a fundamental program, seemingly enhanced by other cells, to border the unnecessary scar response, as the latter may result in fibrosis (38-41). In addition, biological aging or fibroblast senescence can also be related to the numerous excretions of pro-tumorigenic factors and may result in fibroblast activation during oncogenesis. The related downregulation of NOTCH CSL effector proteins, as well as p53 protein effectors can overcome the intrinsic safety mechanism of senescence, and allow CAF activation and proliferation (42).

However, it is known that injured epithelial and immune cells are recruited by cytokines released at the site of injury into normal tissues, including molecular patterns associated with damage, secreted growth factors, such as transforming growth factor (TGF)-β proteins, platelet-derived growth factors (PDGFs), inflammatory cytokines and chemokines [interferon (INFγ; IFNG), tumor necrosis factor (TNF)α; TNF), interleukins (ILs)] (29,43), reactive oxygen species (ROS), matrix metalloproteases (MMPs) (44) and the production of extracellular collagelike proteins, such as laminins, elastin, etc. (45). These mediators induce CAFs from resident fibroblasts and suggest that the activation of fibroblasts into CAFs results from numerous pathways activated by factors from OVAC cells. For example, the upregulation of TNFα vs. TGF-α, subsequently regulated by an inflammatory process, activates nuclear factor-κB (NF-κB) (19) and may activate CAFs. In turn, TGFα resulting from the activation by CAFs may induce epidermal growth factor receptor (EGFR) signaling in OVAC, thereby stimulating cancer cell growth (46). The activation of fibroblasts is in general promoted *in vivo* via the TGF-β-Smad-dependent MMT program (47). Additionally, a minor source of CAFs is omental adipocytes, which undergo the loss of lipids or lipid derivatives and differentiate into fibroblastoids or pre-adipocytes at the adipose stage (48-50), or a variation of adipocytes into fibroblasts, as observed in type 2 diabetes or obesity, during inflammatory fibrotic edifications due to dysfunctional adipose tissue (51). The source of CAFs has been also suggested to derive from vasculopathies and atherosclerotic plaques (52), in which the transition from an endothelial to a mesenchymal phenotype has been observed (53).

4. Mechanisms through which CAFs affect tumor structure, composition and the microenvironment in ovarian cancer

Once activated, CAFs induces the signalization of EGFR in cancer cells and promote the growth of cancer cells (46) and epithelial-mesenchymal transition (EMT) following activation by the increased expression of the progranulin peptide (PGRN), known to stimulate EMT, positively regulating α-smooth muscle actin (α-SMA) in fibroblasts. More importantly, molecular interference between CAFs and the OVAC TME, upregulated by the TGF-β/TGF-βRs/Smad pathway in CAFs, leads to overexpression and subsequent gene secretion targets in the form of versican (54) involved in migration and invasion by CD44 binding subsequently activated by the NF-κB and JNK signaling pathways. This offers the possibility for OVAC cells to further support a pro-inflammatory TME and tumor evolution (54). It should also be noted that increased levels of PGRN and α-SMA, as well as low levels of the cell adhesin molecule (CAM, E-cadherin, during CAF activation promote a poor prognosis (14,55).

During the progression of OVAC, the stromal cells that surround the tumor appear to be a distinct 'innocent'; microenvironment, which in reality, hides various complex interactions between tumor and stromal cells and particularly CAFs, leading to an increase in the expression of vascular endothelial growth factor (VEGF), with CAFs being the main source of VEGF (56). VEGF can be activated by PDGF, also produced by CAFs. PDGF acts by its receptor to induce angiogenesis by indirectly recruiting stromal fibroblasts secreting VEGF (57). PDGF also recruits and induces bone marrow-derived cells (BMCs) to form endothelial or smooth muscle cells, and consequently to promote endothelial and smooth muscle cells proliferation and migration (57). In addition, endothelial cells produce the PDGF B subunit that can induce pericyte recruitment to the vascular wall and maintain endothelial stability, leading to tumor angiogenesis (58). Moreover, in OVAC, VEGF-A, another VEGF family member, stimulates cancer stem cells (CSCs) in OVAC via its activation of the VEGFR2-dependent receptor in order to upregulate the integration site 1 of B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi1) (59). CAFs are also involved in creating and maintaining CSCs through insulin-like growth factor 1 receptor (IGF-IR) activation. This activation induces Nanog expression, the main transcription factor that confers the pluripotency of autonomous renewal and ground state to the phenotype of embryonic stem cells and reprogramming into cancer cells (60). IGF signaling is associated with OVAC chemoresistance and tumor development (61); IGF-1R-AKT signaling activation through the actions of chemotherapeutics agents increase rise the production of genes involved in self-renewal (Oct4/Sox2/Nanog), promoting heterogeneous functioning in ovarian CSCs during the acquisition of chemoresistance (62). However, metastasis to other organs occurs when cancer cells recruit normal fibroblasts into the tumor mass to be activated by various self-regulated genetic and epigenetic alterations regulated by cancer cells, for which the prolonged activated state may be related to epigenetic reprogramming (63-65). Indeed, the global hypomethylation of CAF genomes has been reported (66), as is the case of the promoter of the hypermethylation of the RAS protein activator like-1 (RASAL1), leading to the suppression of its transcription, the augmented activity of Ras-GTP and the perpetuation of fibroblast activation in renal fibrosis (64). This global hypermethylation suggests the possibility of stimulating the upregulation of genes associated with CAF secretome. As discussed in the previous section, fibroblast activation in CAFs is multifactorial (29,43-45) and activators include epidermal PDGF, fibroblast growth factor (FGF)2, EGF and C-X-C motif chemokine ligand (CXCL)12 (43). In addition, communication between cells through adhesion molecules include CAFs, intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (67). Subsequently, tumor growth is dependent on the irregular and uncontrollable proliferation of cancer cells and CAFs that are undeniably inducers of tumorigenic activation signals (68), producing autocrine and/or paracrine cytokines promoting the biologically features of the tumors.

However, tumor-associated macrophages (TAMs), another major component of the stroma are also affected by CAFs (69). TAMs were at first classified through two distinct cell types, including the 'classically' activated (M1 or type I) and the 'alternatively' activated (M2 or type II) macrophages.

M1 macrophages produce a large part of ROS and can orchestrate an anti-tumor TH1 immune response, while M2 macrophages play an important role in tumor progression, promoting the reparation of tissues, as well as angiogenesis. M2 macrophages also produce immunosuppressive factors, including IL-10, arginase, indoleamine 2,3-dioxygenase (IDO) and TGF-β (69). The enrolment of monocytes into the TME and their differentiation into M2 macrophages are promoted by CAFs (70). Particularly, CAFs induce the secretion of CXCL12/stromal cell-derived factor-1 (SDF-1), the macrophage colony-stimulating factor (M-CSF or CSF-1), IL-6 and CCL2/MCP-1 dynamically encouraging the enrolment of monocytes to the TME and their differentiation into a M2 immunosuppressive phenotype (70-72). Thus, CAFs and OVAC are associated at all levels of cancer development and progression. In addition, CAFs may be a determinant of malignant cancer progression and at the same time are an important target for cancer treatments, since they produce factors that facilitate the angiogenic recruitment of endothelial cells and pericytes, such as growth factors, chemokines and the ECM.

5. Targeting CAFs for diagnosis and cancer therapy

In preclinical and clinical *in vivo* studies, CAFs are identified by 4 types of markers, including: i) ECM components, including type I and II collagen, fibronectin, tenascin C (TN-C) and periostin, as well as remodeling enzymes, such as lysyl oxidase (LOX), LOXL1, MMP and tissue inhibitor of metalloproteinases (TIMP); ii) cytokines and growth factors, such as TGF-β, VEGF, PDGF, EGF, FGF, prostaglandin E2 (PGE2), connective tissue growth factor (CTGF), SDF-1 (CXCL12) and WNT; iii) ligands and receptors, such as PDGFRα/β, vascular cell adhesion molecule 1 (VCAM1), discoidin domain-containing receptor 2 (DDR2), TGF-βRI/II, EGFR, FGFR, bone morphogenetic protein receptor (BMPR)I (BMPR1A/B)/BMPRII, podoplanin and fibroblast activation protein alpha (FAP), as well as a decrease in caveolin 1 (CAV1) expression; iv) components of the cytoskeleton and cytoplasmic proteins, including desmin, vimentin, αSMA and FSP1/S100A4 (73).

Therefore, targeting CAF formation refers to defining the controlling pathways that lead to the activation or depletion of CAFs once they are formed. This may correspond to negatively interacting with complex pathways that can alter change the equilibrium of the OVAC microenvironment, and the risk of toxicity may exist. In this respect, currently, clinical treatments target the activation or modulation of CAF functions, but do not completely terminate their formation. This is the case for the gene encoding the serine-threonine protein kinase B-RAF (BRAF) that has been targeted in melanoma to activate CAFs. This remodels the tumor ECM and provides pro-tumorigenic signals supporting residual disease (74,75). In addition, Nagasaki *et al* (76) also reported that the neutralization of IL-6 receptors with antibody inhibited IL-6 signaling and tumor angiogenesis. This is carried out through the inhibition of interactions between the cancer and stroma, as cancer cells stimulate IL-6 secretion from fibroblasts and subsequently induce tumor angiogenesis, suggesting IL-6 as a novel anti-angiogenesis therapeutic target (77). In the same manner, poly(ADP-ribose) polymerase (PARP) inhibitors and VEGF/VEGFR inhibitors have been approved by the FDA as targeted therapies (14). In addition, inhibitors of angiogenesis FGFRs, PDGFRα/β, multi-target receptor tyrosine kinase (RTKi), cyclooxygenase (Cox)-2 and cytokines and their receptors are involved in clinical trials (78). The cell-surface serine protease, also termed FAP, for specifically targeting CAFs, is another class emerging as promising candidates. It has been shown that the depletion of FAP^+ CAFs in mice suffering from melanoma reduces the activity of immunosuppressive cells and improves the antitumoral activity of $CD8^+$ tumor-infiltrating T cells (79,80).

6. Conclusions and perspectives

Initially, the OVAC therapeutic options are chiefly reduction surgery surveyed by adjuvant chemotherapy or vice versa. With the understanding of the productive nature of the peritoneal cavity for carcinomatosis, of the mechanisms underlying the unmitigated activation of fibroblasts that remain largely unknown, as well as the complexity of receptor/ligand-mediated interactions between stromal cells and tumor cells, CAFs have been found to be a key part of the TME contributing primarily to the maintenance, progression and metastasis of OVAC and therapeutic resistance. Therefore, the development of novel early diagnostic tools and therapies targeting CAFs should be promising. Although several strategies against CAFs have been undertaken and have shown promising results, the effectiveness of most drugs targeting the stroma as single agents seems limited. Much remains to be done to identify, with minimal toxicity, the most effective combinations with anti-CAFs alone or in combination with other treatments.

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Authors' contributions

WS conceived and designed the study. SF was responsible for the collection and assembly of the articles/published data, inclusion criteria for the studies and interpretation. Both authors were involved in writing the manuscript. Both authors have read and approved the final manuscript.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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