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Cancer-Associated Fibroblasts and Their Putative Role in Potentiating the Initiation and Development of Epithelial Ovarian Cancer[1]

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Abstract

The progression of ovarian cancer, from cell transformation through invasion of normal tissue, relies on communication between tumor cells and their adjacent stromal microenvironment. Through a natural selection process, an autocrine-paracrine communication loop establishes reciprocal reinforcement of growth and migration signals. Thus, the cancer-activated stromal response is similar to an off-switch-defective form of the normal, universal response needed to survive insult or injury. It is becoming clearer within the cancer literature base that tumor stroma plays a bimodal role in cancer development: it impedes neoplastic growth in normal tissue while encouraging migration and tumor growth in a co-opted desmoplastic response during tumor progression. In this review, we discuss this reciprocal influence that ovarian cancer epithelial cells may have on ovarian stromal cell–reactive phenotype, stromal cell behavior, disrupted signaling networks, and tumor suppressor status in the stroma, within the context of cancer fibroblast studies from alternate cancer tissue settings. We focus on the exchange of secreted factors, in particular interleukin 1β and SDF-1α, between activated fibroblasts and cancer cells as a key area for future investigation and therapeutic development. A better understanding of the bidirectional reliance of early epithelial cancer cells on activated stromal cells could lead to the identification of novel diagnostic stromal markers and targets for therapy.

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Introduction

Epithelial ovarian cancer is the most lethal gynecologic malignancy among women in the United States and other industrialized nations, resulting in approximately 15,000 deaths and nearly 22,000 new cases in 2009 [1]. Survival rates approaching 90% are achievable among ovarian cancer patients diagnosed at an early stage. Nonetheless, early detection is challenging because nonspecific symptoms of early ovarian lesions go unnoticed until the patient presents with an abdominal distension due to late-stage tumor growth and accumulation of ascites fluid. Despite extensive surgical debulking followed by an aggressive platinum/taxane–based chemotherapy and radiotherapy regimen, recurrence and dissemination occurs frequently. Late-stage high-grade ovarian cancer metastasizes rapidly to the omentum and surrounding abdominal organ surfaces [2]. Several studies have noted that the defined histological categories of ovarian carcinoma tend to associate with particular underlying molecular mechanisms, including genetic mutations (e.g. *KRAS*, *p53*, *BRCA1/2*), allelic amplification, and carcinogens [3–6]. Thus, specific genetic mutations among diverse histomorphologic ovarian cancer subtypes allow pathologists to identify and diagnose tumor specimens by microscopy [7,8]. However, the origin and causes of

Abbreviations: CAFs, cancer-associated fibroblasts; CLIC4, chloride intracellular channel 4; CXCR, CXC chemokine receptor; CXCL, chemokine (CXC motif) ligand; ECM, extracellular matrix; FAP-1α, fibroblast activation protein-1α; GRO-α, growth-regulated oncogene α; IL, interleukin; NF-κB, nuclear factor κB; OSCC, oral squamous cell carcinoma; RBPJκ, recombination binding protein Jκ; SDF-1α, stromal-derived factor 1α

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ovarian carcinoma, particularly the cooperative interaction with an activated stromal tumor microenvironment, remain to be elucidated.

Ovarian tumorigenesis is initiated by the malignant transformation of epithelial cells derived from the pelvic müllerian duct, likely originating either from the continuous outer ovarian surface epithelial cell layer or from fallopian tube epithelial cells [9]. Of note, there is accumulating evidence implicating fallopian tube epithelial cells, especially those derived from the fimbriated ends, as the likely origin for high-grade serous carcinoma [10]. In contrast to the dedifferentiation observed after transformation in most epithelial cancers, ovarian cancer progression results in distinct histological subtypes (or histotypes) that are reminiscent of the differentiated morphology of the surrounding gynecological anatomy: high-grade and low-grade serous, endometrioid, clear cell, mucinous carcinoma, and tumors of low malignant potential [11]. These distinct histotypes allow clinicians to monitor for increased levels of serum markers for early detection of ovarian cancer, such as CA125 [11]. Monitoring secreted communication signals sent by ovarian epithelial cancer cells is a mainstay of ovarian cancer patient follow-up; however, these signals are only a part of the epithelial-stroma communications network.

The histomorphologic complexion of ovarian cancer varies according to the histotype and grade of the developing carcinoma. Low-grade serous carcinoma displays a consistent, differentiated papillary growth architecture, with a key feature being uniform nuclei and numerous psammoma bodies [12] and a comparatively high proportional contribution of cancer stromal cells and expansive tumor microenvironment (Figure 1, *A* and *B*, fibroblasts indicated by *black arrows*), highlighted by the reactive stroma marker α-smooth muscle actin (α-SMA) (Figure 1, *C* and *D*). Low-grade and high-grade serous carcinomas likely initiate by means of independent genetic pathways, as evidenced by molecular analysis, clinical appearance, and morphologic features [13]. High-grade serous carcinoma is characterized by a distinctive growth pattern of highly stratified and fenestrated epithelium, a very high mitotic rate, a threefold variation in nuclear size and pleomorphism [12], and a relatively low proportion of cancer-associated stromal cells (Figure 1, *E-H*, fibroblasts highlighted by *black arrows* and positive staining for α-SMA immunohistochemical staining; nuclear atypia highlighted by *red arrows*). Endometrioid carcinoma, so named for a resemblance to the cribriform morphology of the endometrium, exhibits clusters of tube-shaped glandular lumens that are lined by stratified, potentially squamous (in the case of morules), epithelium lacking mucinous deposits [12], and displays a moderate to a low proportion of cancer-associated stroma. Mucinous carcinoma is a heterogeneous designation, ranging in morphology from expansile glandular to multilobular, pilus-like, papillary, to a solid infiltrative epithelial phenotype, with a key unifying feature being extensive mucus-like deposition from goblet cells [14], and a relatively large proportional contribution of stromal cells. Clear cell carcinoma displays characteristic microscopic features that include multiple complex papillae with densely hyaline basal lamina cores and occasional hyaline bodies, with epithelial cells displaying enlarged, clear cytosolic bodies [12], and a relatively dense extracellular matrix (ECM) that lacks the high fibroblastic concentration of other histotypes. Overall, the relative proportion of stroma in advanced ovarian cancer ranges from 7% to 83% of tissue composition, with a median of 50% contribution, and this estimation does not vary significantly according to histotype, International Federation of Gynecology and Obstetrics stage, or grade [15].

The progression of ovarian cancer tumor cell populations, from cell transformation through invasion of normal tissue and eventual metastasis, likely relies on a critical secretory reciprocal communication with their adjacent stromal microenvironment. A component that is vital to our understanding is how synergistic communication signals sent by cancer epithelial cells are interpreted and translated into a non–cell-autonomous secretion- and growth-activating response in cancer-associated fibroblasts (CAFs). Deciphering the role of this paracrine and reciprocal cancer-stromal communication network in the early initiatory stages of ovarian cancer is fundamental to understanding abnormal acute and chronic fibroblast activation. Although multiple cell types are present in the stromal ECM compartment of the various ovarian cancer histotypes, CAFs have been shown to play a critical role in determining overall clinical outcome of cancers throughout the body. Gene expression profiling of CAFs in multiple cancers has identified genes that are differentially expressed in comparison to normal fibroblasts, and these genes may shed light on malignant epithelium-activated fibroblast secretory interaction and cooperative cellular behavior. Further, molecular indicators of an activated ovarian CAF state may enable the development of non–cancer cell markers for early-stage detection of the extent of aggressive growth promotion and may thus yield additional candidates for therapeutic intervention. Therefore, in this review, we focus on putative intracellular and intercellular signaling activators and pathways in CAFs, including ovarian, that affect communication with cancer cells and their normal neighbors. First, we give a brief perspective on the role of the stromal microenvironment in cancer. Then, we discuss the influence that ovarian cancer epithelial cells have on stromal cell behavior, disrupted pathway signaling in CAFs of various cancer types that may be involved in ovarian CAFs, and tumor suppressor status in CAFs of ovarian, breast, and prostate carcinomas. Finally, we summarize the status of identifying ovarian CAF contribution to ovarian carcinoma and discuss future hypotheses. Overall, our focus is on the exchange of secreted factors between activated fibroblasts and cancer cells as a key area for future investigation and therapeutic development in ovarian cancer treatment.

A Historical Perspective: Selective Activation in the Tumor Stromal Microenvironment

Although some cancers develop stromal independence through epithelial-mesenchymal transition (EMT) before metastasis, successful tumorigenic initiation likely requires a coevolutionary stimulus of the stromal microenvironment whereby cancer-associated stromal cells receive acute, prolonged activation signals to form a reactive, secretory phenotype [16–18]. Activated stromal response in cancer was first described in 1979, when Seemayer et al. [19] observed that myofibroblasts, or activated fibroblasts, played a critical role in the desmoplastic response to neoplastic mammary carcinoma. In 1983, an initial distinction was made between the migratory behavior of CAFs and that of normal fibroblasts, when Mensing et al. [20] noted that dermal tumor-associated fibroblasts displayed a more differential chemotactic response to fibronectin than did normal fibroblasts. Shortly thereafter, Strauli et al. [21] and Haemmerli et al. [22] used the rabbit V2 carcinoma model and observed that V2 cancer cell invasion into rabbit mesentery was mediated, in part, by a multiplication of co-opted connective tissue cells and enhanced ECM deposition, characterized by a transdifferentiation of recruited fibrocytes (circulating fibroblast progenitor cells) into myofibroblasts. In the late 1980s, studies showed that interactions between lung tumor cells and lung tumor–associated fibroblasts are likely to play a critical role in ECM degradation, as well as in the selection of tumor cells that eventually metastasize [23], and that activated immune cells and tumor cells increased the ECM-degrading capacity of

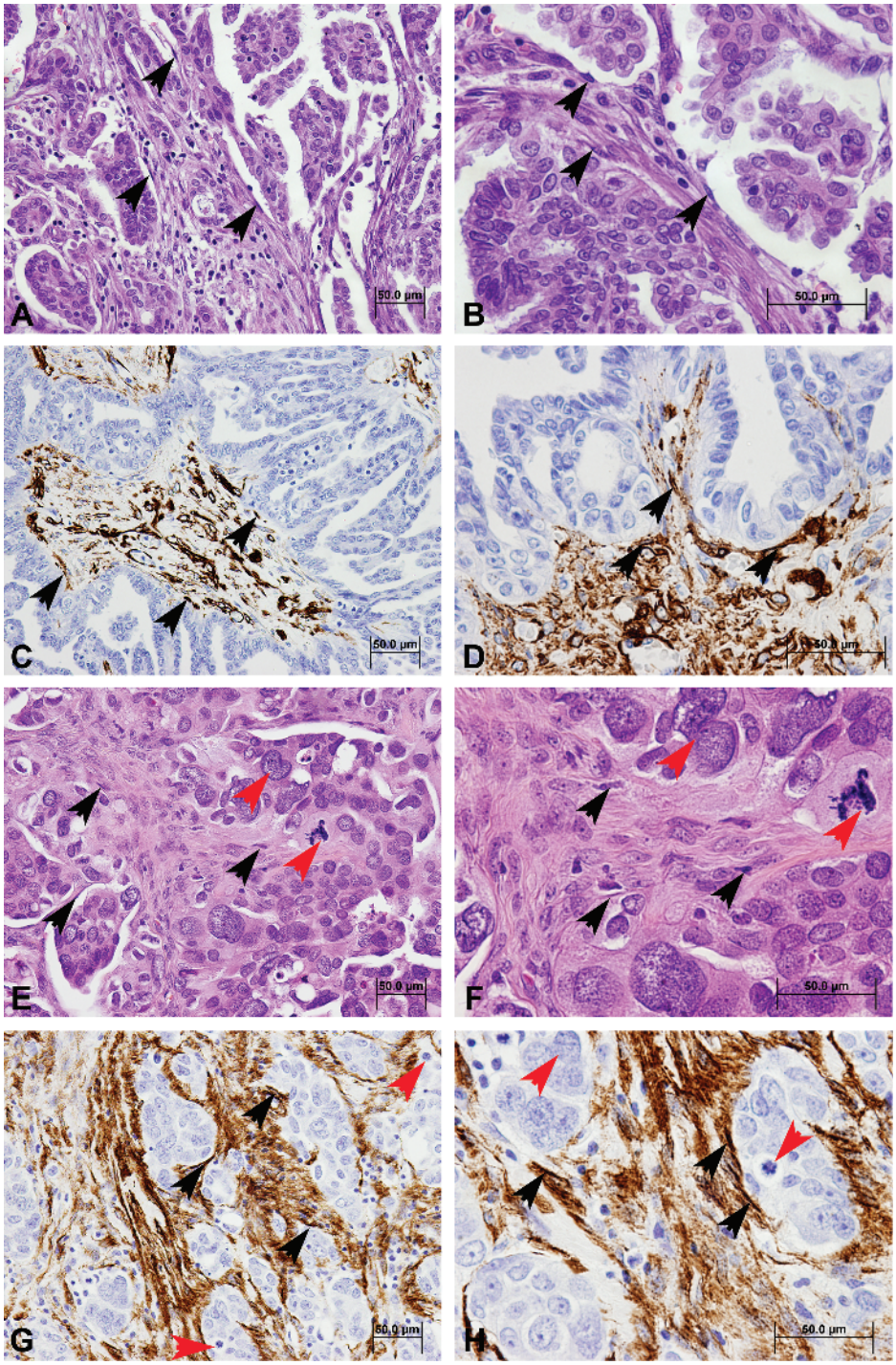

Figure 1. Histomorphology and interaction of ovarian cancer epithelial cells and CAFs. The morphologic characteristics of low-grade serous ovarian carcinoma include a papillary growth architecture and uniform nuclei (A and B; hematoxylin and eosin stain), with a comparatively high proportional contribution of cancer stromal cells identified by immunohistochemistry for α-SMA (C and D), a marker that highlights activated reactive CAFs (indicated by black arrows). The altered histomorphology of high-grade serous carcinoma is characterized by a distinctive growth pattern of stratified epithelium with high mitotic rate and a threefold variation in nuclear size and pleomorphism (E and F; hematoxylin and eosin stain). Nuclear atypia are highlighted by red arrows. High-grade serous carcinoma displays a high proportion of CAFs (G and H) highlighted here by immunohistochemistry for α-SMA (black arrows). Scale bars, 50 μm. Magnification, ×200 (A, C, E, and G); ×400 (B, D, F, and H).

normal lung fibroblasts [24]. In 1989, Carnemolla et al. [25] observed an oncogenesis-specific secretion from activated stroma: the alternatively spliced fibronectin isoform B was expressed only by transformed lung fibroblasts. In a seminal publication, Nagy et al. [26] noted that generation of generic fibrinolytic tumor stroma is *perpetual* and *critical* to successful tumorigenesis and, therefore, cancer resembles a wound that is unable to heal. Thus, stromal fibroblasts possess a bimodal functional role in tissue biology. Normal stromal fibroblasts can impede the

abnormal growth of preneoplastic epithelial cells in a variety of normal tissues (Figure 2). However, stromal fibroblasts can also be protumorigenic, responding in a co-opted desmoplastic response where they encourage both migration and invasion of epithelial cancer cells [27–31] (Figure 2, *last panel*).

These seminal publications have led to the viewpoint that aggressive malignancies selectively perpetuate within stromal microenvironments that are richly populated with activated and reactive cells (CAFs, myofibroblasts, angiogenic precursors, immune cells, and others), which can be collectively reprogrammed to support overall tumor growth [32]. A functional, reciprocal interaction is now currently acknowledged between tumor cells and the tumor microenvironment, wherein tumor stromal cells very likely facilitate a critical role during both tumor cell initiation and growth progression [33]. Thus, epithelial cancers are no longer considered as isolated clusters of transformed epithelial cells that invade passive, uninvolved neighboring regions, namely, stroma. An alternate perspective has acquired increased stature within the past few decades: a tumor-promoting stromal microenvironment selectively facilitates, through reciprocal juxtacrine communication, the proliferation and invasion of epithelial cancer cells. Therefore, cancer cells that activate and maintain a protumorigenic, anti-immunogenic niche would retain a selective growth advantage [32]. This natural selection process is strikingly similar both to Dvorak's wound healing analogy and to some processes in embryogenesis [34], where an autocrine-paracrine communication loop establishes reciprocal reinforcement of growth and migration signals, thereby limiting healing or development (Figure 2). This perspective, which has been pioneered by Dvorak [36], Rowley [18], and others, implies that the cancer-activated reactive stromal response is an off-switch-defective form of the normal, universal biologic response needed to survive tissue insult or injury [35].

Cancer Epithelial Cell Activation of the Reactive Stromal Phenotype in Ovarian and Other Cancers

Activation of fibroblasts to a myofibroblast phenotype through reciprocal paracrine interaction with neoplastic and transformed epithelial cells has been observed in several *in vitro* studies using ovarian cells. For example, medium conditioned by SKOV3 cells, an established, malignant ovarian cancer cell line, induced transdifferentiation of normal ovarian fibroblast to a myofibroblast phenotype characterized by elevated reactive stroma marker α-smooth muscle actin (α-SMA) [37]. Further, the authors identified chloride intracellular channel-like 4 (CLIC4) and various reactive oxygen species as potential SKOV3-secreted factors that could yield the same ovarian myofibroblast stromal phenotype *in vitro* [37]. Analogously, breast carcinoma progression

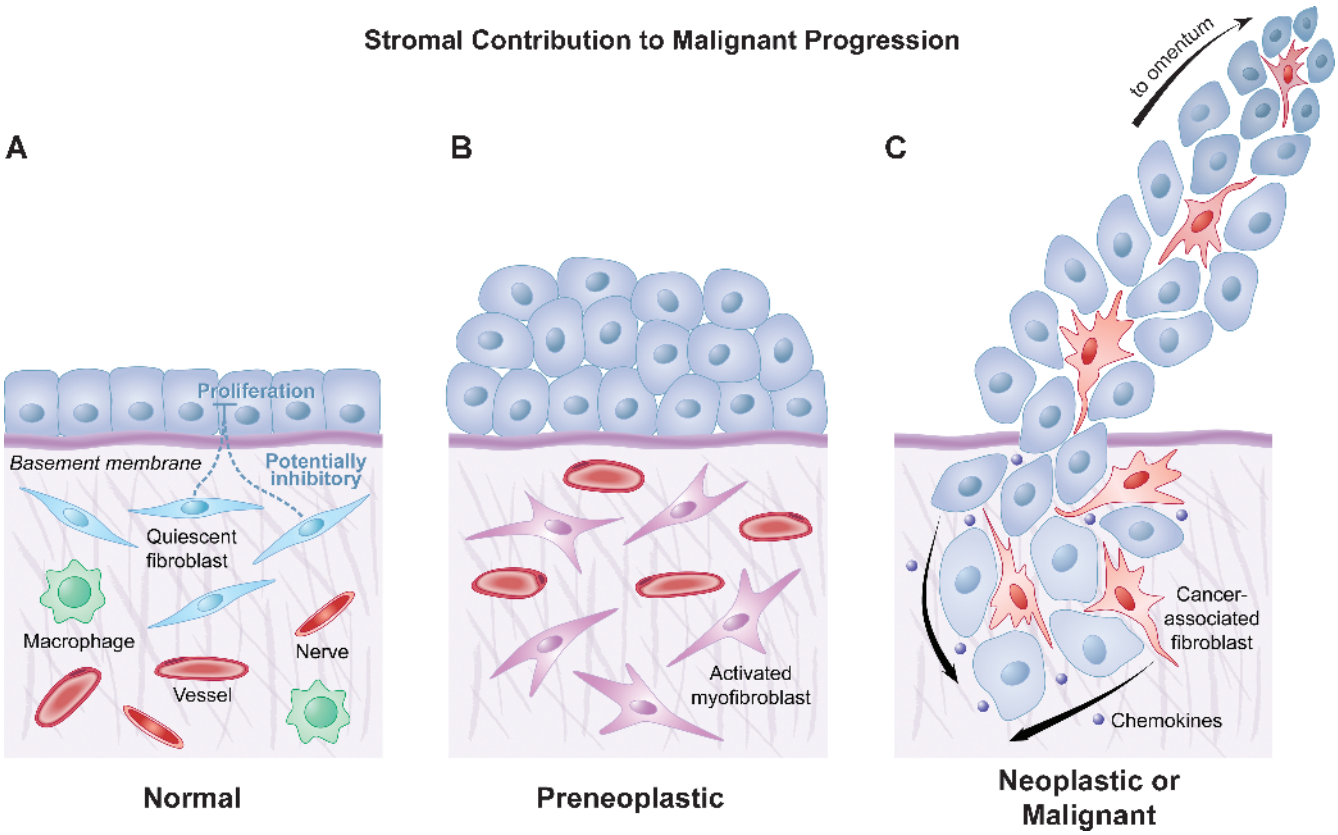

Figure 2. The contribution of CAFs to malignant progression of ovarian cancer. (A) Normal ovarian tissue is composed of ovarian or fallopian tubal epithelial cells and an extracellular microenvironment consisting of quiescent fibroblasts and other supportive mesenchymal cell types that collectively inhibit inappropriate or preneoplastic epithelial proliferation. (B) Transition from normal to preneoplastic, or neoplastic, ovarian or fallopian tubal epithelial cells involves reciprocal secretory communication with activated myofibroblasts, whether tissue resident or recruited from circulation. (C) Progression to malignant ovarian cancer relies, at least initially, on a secretory co-opting of ovarian CAFs through exchange of intercellular secreted factors (e.g., chemokines like IL-1β and GRO-α) with CAFs to facilitate dissemination to the omentum. CAFs may directly facilitate this metastatic movement, although it is likely that EMT of ovarian or fallopian tumor cells provides a reservoir of secretory, ECM-digesting, migratory CAF-like cells.

was associated with increased expression of CLIC4 in breast cancer–associated stromal cells, relative to a reduced expression in normal breast epithelial cells [38]. Moreover, CLIC4 induced up-regulation of α-SMA in breast cancer CAFs *in vitro*, and CLIC4-overexpressing breast myofibroblasts stimulated xenograft tumorigenesis [38]. A recent study showed that normal human fibroblasts selectively inhibited proliferation of prostate and lung cancer–derived tumor cells in a direct cell contact–dependent manner [39]. Similarly, it was shown that normal human breast–associated fibroblast inhibition of tumorigenic breast cancer cells was significantly enhanced in direct coculture compared with indirect coculture, relative to breast CAF stimulation of breast tumor cell proliferation [40]. Ovarian stromal cell type is critical in determining whether metastasis occurs because it was observed that fibroblasts derived from the omentum, the richly vascularized fatty subperitoneal layer draping the ovaries, augmented ovarian cancer cell adhesion and invasive behavior, whereas omentum-derived mesothelial cells functionally inhibited ovarian cancer cell aggressiveness [41]. Very recently, a study used the reactive stroma markers α-SMA and fibroblast activation protein (FAP) to correlate the presence of ovarian CAFs to ovarian cancer patient clinical outcome, finding a significant association with the occurrence of lymph node and omentum metastases, as well as elevated lymphatic vessel density and microvessel density [42]. Moreover, the authors showed that CAFs isolated from ovarian cancer tissue induced ovarian cancer cell invasion and migration *in vitro* [42]. These data demonstrate that ovarian CAFs are directly related to ovarian cancer progression and metastasis. However, more work must be done in identifying specific ovarian cancer epithelial cell–secreted factors that directly facilitate ovarian fibroblast activation and protumorigenic and prodissemination secretory activation.

Activated CAFs, across most cancers, secrete a wide variety of growth factors, chemokines, collagens, and matrix-modifying enzymes, collectively supplying a communication network and altered three-dimensional ECM scaffold that governs the proliferation of cancer cells, tumor invasion, and metastasis across tissue types [43]. Therefore, it is of interest whether the proportion of tumor stroma cells in the tumor microenvironment within cancer reflects a mutual growth pattern. Interestingly, the impact of the proportional representation of the stromal compartment on tumor invasiveness and histological dedifferentiation has been studied in prostate disease [44], colorectal cancer [45], breast carcinoma [46], and pulmonary carcinoma [47]. Specifically, in ovarian cancer, component aspects of the tumor stromal compartment have been described as prognostic patient severity indicators, including blood vessel architecture [48], extent and type of inflammatory cells [49], and ECM-interacting, ovarian fibroblast–secreted factors, including the glycosaminoglycan hyaluronic acid [50] and a hyaluronan-partner glycoprotein versican [51]. In another study, conditioned medium from ovarian clear cell carcinoma (ES-2) cells included paracrine-acting cytokine signals, which upregulated ovarian fibroblast transcription of urokinase-type plasminogen activator, a key enzyme in cancer cell invasion and metastasis [52]. Moreover, parallel epithelial and stromal expression patterns were observed in tissue samples from patients with aggressive ovarian cancer for the paracrine-secreted markers cyclooxygenase 1 (COX-1), COX-2, microsomal prostaglandin E synthase-1 (mPGES-1), and EP1-2, factors that collectively promote angiogenesis and proliferation while simultaneously discouraging apoptosis [53]. Moreover, the relative stromal abundance and composition of the ovarian tumor microenvironment itself was found to have an independent, statistically significant prognostic value, particularly in late-stage epithelial ovarian cancer patients [15]. These data demonstrate that women with a high proportion of ovarian tumor stroma display decreased overall survival. Interestingly, this study did not identify a significant relationship between the proportion of ovarian tumor stroma and ovarian tumor histotype [15]. This suggests that the proportion or percentage of ovarian tumor stroma may functionally activate a universal ovarian stromal communication mechanism that is independent of the gynecological tissue or cell type of origin. The authors theorized that decreased survival of ovarian cancer patients with a high proportion of cancer stroma may potentially reflect an insufficient penetration, or altered resistance mechanism, to drug treatment based on cell adhesion [15]. Further, another study focusing on ovarian carcinosarcomas identified that patients with late-stage disease and a high percentage of ovarian cancer stroma displayed reduced survival and poor clinical outcome [54]. Therefore, it is becoming clear that ovarian cancer cell–secreted factors, within the context of altered interaction from cell-cell communication, directly promote the activation of stromal cell secretion, migration, and function (Figure 3*A*).

Critical Factors Mediating the Activated Response of CAFs from Diverse Cancers, Including Ovarian Carcinoma

Several recent reviews have focused on the therapeutic potential of targeting the activated cancer–associated stromal compartment [55,56]. Some putative CAF markers that have been correlated with cancer incidence or progression in other cancer types are also likely to play a critical role in ovarian tumorigenesis (Figure 3*B*). It has been shown that an oncogenic cancer epithelium results in a tumor microenvironment replete with inflammatory mediators, growth factors, matrix remodeling enzymes, and angiogenic factors [31,57,58]. The net result of this milieu leads to a recruitment of associated, activated fibroblasts, ostensibly due to reciprocal interaction through inflammatory factors.

Chemokines and Cytokines

Generalized inflammation of the female peritoneum, typically associated with ovarian micrometastases and stromal invasion, has been tied to elevated expression of IL-8 [59,60]. Moreover, up-regulation of tumor-regulated IL-6, IL-8, and IL-1β is associated with the inflammatory network, promoting tumorigenesis, angiogenesis, and metastasis in various cancers, including those of the breast, prostate, and pancreas [61]. Our laboratory has identified several stromal-activating chemokines, including IL-6, IL-8, and growth-regulated oncogene α (GRO-α), which are significantly elevated in transformed, neoplastic ovarian surface epithelial cells [62]. Moreover, we have demonstrated that GRO-α–overexpressing ovarian fibroblasts with inhibited p53 significantly increased ovarian cancer epithelial proliferation and tumorigenic growth in a mouse xenograft model [63]. Furthermore, our laboratory demonstrated that IL-1β is significantly elevated in Ras-transformed ovarian carcinoma cells [62], and we have evidence that ovarian cancer cell–secreted IL-1β attenuates p53 in neighboring ovarian fibroblasts (unpublished observations). As a comprehensive signaling axis, the IL-1β/IL-1 receptor 1 (IL-1R1) activates a defined set of downstream signaling effectors and binding partners [64]. IL-1R1 axis effectors include several isoforms of the IL-1 receptor–associated kinases (IRAK proteins), as well as the protein-protein interaction effector tumor necrosis factor-α receptor–associated factor 6 [65–67]. Nuclear localization of IRAK-1 has been correlated with enhanced malignancy in lung cancer [68] and prostate cancer [69] and has been shown to bind directly to the IκBα promoter and enhance binding of nuclear factor-κB (NF-κB) p65

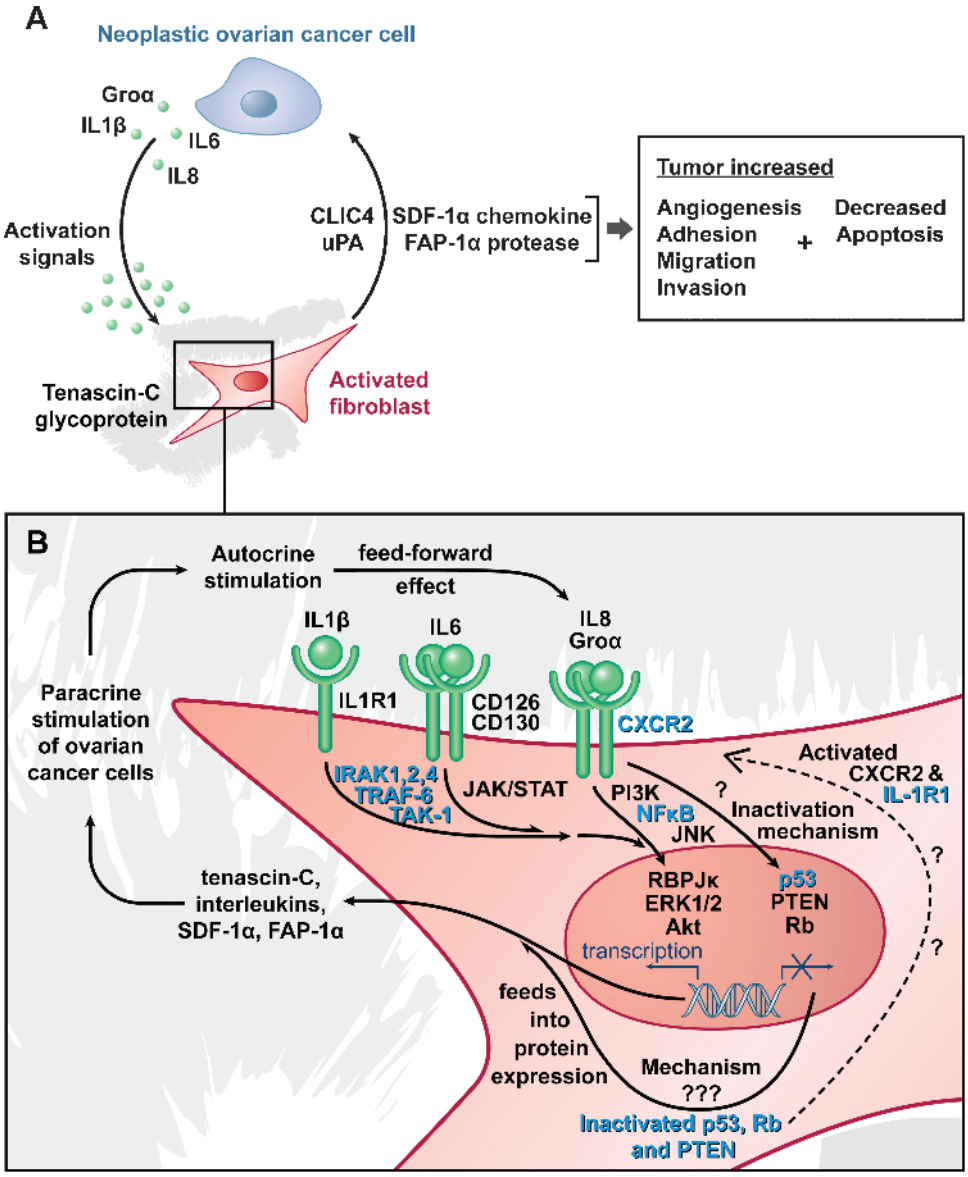

Figure 3. Model of critical pathways and mechanisms in the activation of ovarian fibroblasts by secretory interaction with neoplastic ovarian cancer cells. (A) Secreted activation signals, such as IL-1β, IL-6, and IL-8 or GRO-α, from neoplastic ovarian or fallopian tubal cancer epithelial cells stimulate a stromal phenotypic shift from quiescent to activated ovarian or omental fibroblasts. Activated CAFs are proliferative, are migratory, and secrete a variety of ECM-restructuring factors (e.g., FAP-1α, urokinase-type plasminogen activator), soluble cancer-activating chemokines (e.g., SDF-1α), and cell surface proteins (e.g., CLIC4). Cancer cell–mediated fibroblast activation selectively promotes tumor angiogenesis, adhesion, migration, and invasion while reducing apoptotic inhibition. (B) Activation of chemokine receptors on ovarian CAFs, including IL-1 receptor 1 (IL-1R1), CD126/CD130, and CXCR2, by ovarian cancer cells likely activates intracellular signaling cascade mediators in ovarian CAFs including IL-1R–associated kinases (IRAK) 1, 2, and 4; tumor necrosis factor α receptor–associated factor 6 (TRAF-6); IL-1–induced activation of c-Jun N-terminal kinase and NF-κB; as well as the c-Jun N-terminal kinase/Janus kinase (JAK)/STAT family members. In ovarian CAFs, these signaling mediators may activate AKT/extracellular signal–regulated kinase 1/2 (ERK1/2)/RBPJκ–mediated transcriptional up-regulation of ovarian CAF-secreted factors that impact epithelial ovarian cancer tumor cell aggressiveness, including the glycoprotein tenascin-C, protease FAP-1α, and myriad interleukins, especially IL-8. In parallel, downstream signals in ovarian CAFs from chemokine-activated receptors facilitate transcriptional or translational inactivation of tumor suppressors, like p53, by yet uncharacterized mechanisms. Once tumor suppressors like p53, PTEN, or Rb are inhibited/inactivated, two intracellular pathways are initiated that are not understood at all: 1) increased cellular production of chemokines, including IL-1β, IL-8, IL-6, and SDF-1α, and 2) chemokine receptors, like IL-1R1, are up-regulated. Collectively, ovarian fibroblast activation leads to paracrine ovarian cancer cell stimulation and autocrine stimulation, co-opting a desmoplastic-like stromal response for tumor cell initiation, survival, and inappropriate growth.

subunit in skin fibroblasts [70]. In breast cancer, CAFs were found to initiate and mediate tumorigenesis through a macrophage-recruitment inflammatory signature that was highly dependent on breast CAF NF-κB signaling [71]. Thus, as pathway effectors activated by the IL-1β/IL-1R1 signaling axis function both in the nucleus and cytoplasm of fibroblasts, these IL-1R1 pathway mediators may perhaps be responsible for eliciting IL-1β–mediated transcriptional or translational attenuation of tumor suppressors in ovarian CAFs (Figure 2*B*).

Stromal-derived factor-1α (SDF-1α), also called chemokine (CXC motif) ligand 12 (CXCL12), is a glutamic acid–leucine–arginine motif-negative chemokine lacking chemotactic influence on immune cells [71]. SDF-1α signals primarily through the CXC chemokine receptor 4 (CXCR4) [58], and the SDF-1α/CXCR4 signaling axis is deregulated in multiple malignancies, playing a critical role in promoting cancer cell migration and metastasis of many tumor types, including leukemia [72] where there is a preponderance of SDF-1α publications, ovarian and cervical cancer [73–77], prostate [78], breast [79], liver [80,81], colorectal [82], pancreatic [83], lung [84], and multiple myeloma [85]. Moreover, a few recent articles address the potential for CAF expression of SDF-1α contributing to cancer progression [86–89]. Several reports have discussed the involvement of the SDF-1α/CXCR4 signaling axis in epithelial ovarian cancer cell lines *in vitro*, or using tissue immunohistochemistry quantitation to determine predictive ability [77,90–94]. One recent publication showed elevated peritoneal dissemination *in vivo* after intraperitoneal nude mouse injection of 5 million ES-2 cells, a clear cell ovarian cancer line, with daily injection of systemic, exogenous high-concentration SDF-1α [95]. Moreover, a recent review of the role of the SDF-1α/CXCR4 signaling axis in ovarian cancer cell stated that SDF-1α overexpression coincides with ovarian cancer cell proliferation and metastasis, and identified several recently developed therapies that target either SDF-1α or CXCR4 [96]. CXCR4 is expressed not only in cancer cells; it can also be upregulated in fibroblasts through PDGF, IL-1β, and HIF-1α and can be attenuated in fibroblasts by activation of phosphatidylinositol 3-kinase and mammalian target of rapamycin [97,98]. Both exogenous IL-1α and oral squamous cell carcinoma (OSCC)–conditioned medium stimulated SDF-1α expression in CAFs, whereas CAF-conditioned medium stimulated OSCC cell invasion *in vitro* [99]. However, when exogenous SDF-1α was applied to OSCC cells in the absence of CAFs, the pattern of invasion in culture was different from that with CAF-conditioned medium, implying a complex multifactor intracellular communication [99]. Relating this chemokine pathway to tumor suppressor function, p53 can repress the expression of SDF-1α in embryonic lung fibroblasts [100], likely resulting in a microenvironment less conducive to tumor cell migration and survival. p53 activation directly attenuated invasion and migration of breast cancer cells through repressed *SDF-1α* gene transcription [100]. Thus, loss of p53 expression in CAFs may contribute to inappropriate activation of the CXCR2/4 signaling axis in tumorigenesis (Figure 2*B*). However, the functional role of the SDF-1α/CXCR4 signaling axis in ovarian CAFs, and whether this directly impacts ovarian epithelial tumorigenesis, migration, and metastasis, has not been completely addressed and warrants further investigation.

Activation-Associated Fibroblast Factors

Fibroblast activation protein-1α (FAP-1α), a cell surface protease with dipeptidyl peptidase and endopeptidase activity, is expressed by stromal cells in several different cancers [57] and has been used as a clinical therapeutic target by multiple immunoconjugate clinical studies in multiple cancer types [101–104]. FAP-1α is induced in ovarian fibroblasts by exposure to conditioned medium from a metastatic ovarian cancer cell line, HO-8910PM, or to exogenous factors TGF-β1 and IL-1β [105]. Once elevated, FAP-1α promotes proliferation, adhesion, and migration of metastatic ovarian cancer cells [105]. Similarly, a novel category of activated stromal response was identified in OSCC, termed *nemosis*, that correlates stromal expression of progrowth and proinflammation factors (α-SMA, FAP-1α, and fibroblast-specific protein-1α [FSP-1α]) with *in vitro* tumor cell function [106]. Thus, FAP-1α presents an interesting opportunity for further study in ovarian tumor stromal biology.

Tenascin-C is a secreted glycoprotein that is elevated in the stromal microenvironment of epithelial cancers, is likely to act by decreasing the formation of cell adhesion complexes thereby promoting proliferation and migration, and is considered a potential oncogene (reviewed in Hsia and Schwarzbauer [107] and Orend [108]). In a powerful multivariate index assay using serum from patients with ovarian cancer and controls, tenascin-C was included as 1 of 11 analytes (selected from 104 candidates) that could distinguish benign from malignant ovarian conditions with sensitivity and specificity of up to 90% [109]. Similarly, tenascin-C was identified as one of four distinguishing factors in an immunohistochemistry-based survival tree model of intrahepatic cholangiocarcinoma [110]. Furthermore, our data show that intense stromal expression of tenascin-C correlates with shorter survival duration in patients with all ovarian cancer histotypes, and specifically in those with high-grade serous tumors (unpublished observations).

Origin of the Ovarian Cancer Tumor Microenvironment

Although the origin of stroma in ovarian cancer is largely unknown, recent evidence from mouse xenograft models of multiple human cancer cell types (including ovarian) points to activation of tissue-resident fibroblasts, recruitment of hematopoietic precursors or mesenchymal stem cells (MSCs) [111,112], and promotion of senescent fibroblasts [63,113]. Using dynamic magnetic resonance imaging, it was observed that recruited fibroblasts formed a functional tumor neovasculature at the rim of ovarian cancer nodules, thereby identifying an activated ovarian fibroblast response with potential therapeutic implications [114]. In ovarian cancer, it was recently reported that ovarian cancer–derived lysophosphatidic acid stimulates differentiation of human adipose tissue–derived MSCs (hADMSC) to CAFs, elevating the expression of SDF-1α through a TGF-β1–mediated autocrine stimulation of Smad2 [115]. Moreover, the authors demonstrated that treating hADMSC with ovarian cancer patient ascites fluid, or with conditioned medium from ovarian cancer cells, induced expression of the reactive stroma marker α-SMA and phosphorylation of Smad2 and that this effect could be abrogated by pretreating with an lysophosphatidic acid receptor antagonist [115]. Also in ovarian cancer, the pro-inflammatory peptide, LL-37, a C-terminal peptide fragment of human cationic antimicrobial protein 18, was shown to be overexpressed in ovarian cancer tissue and to directly stimulate ovarian cancer cell migration *in vitro* [116]. This same group recently demonstrated that *in vivo* neutralization of LL-37 significantly inhibited xenograft tumor growth overall, likely through reduced engraftment of MSCs into ovarian tumor xenografts and disruption in the establishment of a tumor fibrovascular network [117]. Thus, LL-37 may facilitate ovarian tumor progression through recruitment of ovarian CAF progenitor cells that express proangiogenic factors. Pertaining to an alternate origin for ovarian cancer tumor microenvironment, senescent ovarian fibroblasts significantly increased *in vitro* migration of cMyc-mediated early neoplastic ovarian surface epithelial cells compared with coculturing with presenescent ovarian fibroblasts [118]. Further, this study demonstrated that senescent ovarian fibroblasts stimulated early neoplastic ovarian epithelial cell anchorage–independent colony growth, as well as increased proliferation and induced nuclear atypia in a three-dimensional spheroid *in vitro* model [118]. Thus, the etiology underlying the development of epithelial ovarian neoplasia may depend on the accumulation of senescent (or loss of presenescent) ovarian fibroblasts. Therefore, further work in identifying the likely multifactorial source of ovarian carcinoma–

associated stromal cells has therapeutic implications in recognizing and targeting stromal-mediated tumor activation in early-stage ovarian cancer patients.

Tumor Suppressor Status in CAFs

Of the many tumor suppressors characterized, p53 is the only one for which inactivation has been well substantiated in a variety of epithelial cancers and has been shown in a subset of cancer stromal cells [31,119]. The existence of genetic alterations in CAFs is controversial. Studies have identified distinct genetic alterations in breast and squamous CAFs, ranging from mutation of critical tumor-suppressor genes like phosphatase and tensin homolog (PTEN) and TP53, to loss of heterozygosity, or alterations in allelic copy number [120–125] (Figure 3*B*). Conversely, other studies have not identified similar changes in breast or ovarian CAFs, and no agreement on a unifying genetic alteration in all CAFs exists to date [126–128]. Several reports focusing on breast and prostate CAFs have identified epigenetic mechanisms, such as promoter methylation, that correlate with poor clinical factors [129–131], which need to be addressed in ovarian CAF biology. p53 function is intriguing because it activates non–cell-autonomous functions that likely contribute to tumor suppression through communication with normal fibroblasts. For example, p53-dependent secreted factors such as PTGF, a transforming growth factor-β (TGF-β) family member [132], IGF-BP3 [133], and other factors have been shown to facilitate stromal cell–mediated inhibition of prostate cancer cell growth [134]. Human breast cancer tumor cell injections using p53-null mice resulted in markedly increased tumor growth rates, relative to growth rates after injection into normal, p53-intact control mice [121], indicating that an activated host stroma with incapacitated p53 is tumor promoting. Indeed, p53 inactivation mutations were reported to occur in the fibroblastic stroma of both colon and breast cancers [122,124,135]. Moreover, *TP53* mutational status may be a predictor of CAF-mediated resistance to cytotoxic chemotherapeutics, although this response is highly variable across different tumor types [136,137]. Furthermore, breast carcinoma CAFs were recently shown to possess a nonmutated, but functionally deficient, form of p53 [138,139]. The question of whether genetic aberrations in CAFs could be the basis of the cancer-promoting phenotypes of ovarian CAFs remains to be resolved. We believe that irrespective of the tissue source, how close the stromal cell extraction/microcapture site is to the tumor determines whether one observes the genotypic mutational status of true CAFs, or instead normal fibroblasts.

Although there are very few publications addressing p53 regulation of secreted and membrane-bound factors in fibroblasts or CAFs, it is assumed that publications describing p53-mediated mechanisms in epithelial cells will likely translate into similar mechanisms in fibroblast p53 regulation. Elevated expression of both wild-type p53 and wild-type Rb in HeLa cells repressed promoter constructs for IL-6 and c-Fos [140]. Wild-type p53 mediated repression of the chemokine receptor 4 in breast cancer cells, which was negated by the expression of the p53 V143A dominant negative mutant, and cancer-specific p53 phospho-mutants R175H or R280K [141]. p53-mediated repression of EMMPRIN, a transmembrane glycoprotein that promotes survival, invasion, and metastasis through induced MMP expression, led to a decrease in MMP-9 in prostate cancer cells [142]. Specifically addressing p53 mutation in CAFs, colon CAFs overexpressing an alternate human p53 isoform, Δ133p53, displayed repressed miR-34a (a p53-activated microRNA that helps to facilitate senescence) and extended cellular replication *in vitro* [143]. Therefore, the cause and impact of p53 inhibition in ovarian CAFs, and whether this induces a reciprocal, intercellular communication with neoplastic ovarian epithelial cells to promote tumorigenesis and metastasis, have yet to be characterized.

Anecdotal evidence suggests that, in the absence of focal adhesion kinase (FAK), expression of Pyk2, an inhibitor of p53, prevented cisplatin-mediated apoptosis in human foreskin fibroblasts [144]. Further, a recent preclinical mouse xenograft study using an ATP-competitive reversible inhibitor of FAK and FAK2 (Pyk2) showed a potent inhibition of *in vivo* metastatic prostate cancer growth [145]. In another mouse model of prostate cancer, increased epithelial tumorigenesis led to a noticeable selection for p53-inhibited stromal cells [146], suggesting that tumor cell behavior may directly control the tumor suppressor status of stromal cells. Furthermore, it was shown recently that immortalized, nontumorigenic lung epithelial cells expressing mutant H-Ras and an siRNA against p53 could reduce p53 levels in human lung cancer CAFs more than in normal lung fibroblasts [147]. These data suggest a CAF-specific susceptibility to secreted factors from preneoplastic cells compared with normal fibroblasts, facilitating inhibition of the p53 non–cell-autonomous tumor suppressor function in CAFs. Similarly, our unpublished data indicate that conditioned medium from mutant H-Ras transformed ovarian surface epithelial cells selectively suppressed p53 in otherwise normal ovarian fibroblasts. Therefore, it can be stated that because tumor suppressor activity in the normal ovarian or fallopian tube stroma exerts an inhibitory influence on ovarian neoplastic initiation and progression, attenuation of p53 activity in the ovarian reactive stroma would strongly favor tumorigenic progression.

Disruption of Stromal Intracellular Signaling Pathways by Cancer Cell Communication: Who Is Implicated?

Epigenetic modification of signaling pathways related to secretion in CAFs is a field in which many questions remain to be answered, but several target pathways in CAFs of various tumor tissue types point to a role for epigenetic modification in ovarian CAFs promoting ovarian cancer development. Within invasive and aggressive gliomas, tenascin-C is upregulated in stromal cells by recombination binding protein Jκ (RBPJκ), a Notch 2 cofactor for transcription in activated Notch signaling [148]. Thus, RBPJκ may facilitate intracellular signaling pathway interpretation of secreted epithelial-stromal cell communication (Figure 3*B*). In addition to signaling pathways directly activated by molecules secreted by CAFs, the mechanical stress generated during ECM modification by breast CAF-secreted factors may play a role in activating signaling pathways in mammary carcinoma cells, contributing to disease progression and compromised disease treatment [149]. Thus, the changing force exerted by the CAF-remodeled ECM on ovarian cancer cells needs to be considered to fully delineate the process of tumor progression. Interestingly, tenascin-C induction in lung fibroblasts depends on RhoA/RhoA–dependent kinase/integrin-linked kinase–mediated signaling in response to mechanical shear stress, although this pathway does seem to be bypassed through extracellular signal–regulated kinase 1/2 and PKB/Akt signaling [150].

Several recent publications have presented preliminary gene expression profiling data that have identified several secreted target proteins that control microenvironmental cross-talk, guidance, and remodeling (e.g., plasminogen activator inhibitor-2, dickkopf-related protein 1, t-type plasminogen activator) that were upregulated in breast CAFs, which likely to play a role in communicating with, and promoting the aggressiveness of, breast cancer cells [40,151]. It is highly likely that a

similar expression profiling study of ovarian CAFs would yield significant potential targets for therapeutic intervention.

Modeling Ovarian Cancer Epithelial-Stromal Cell-Cell Interaction

Despite our familiarity with characterized genetic alternations in ovarian cancer epithelial cells, we do not yet fully recognize how these genetic changes work together to not only transform normal ovarian surface epithelial cells into cancerous cells but also facilitate and recruit an activated stromal microenvironment. In addition, the inherent difficulties in accessing and deriving normal and cancer–associated ovarian fibroblast cell lines makes studying cross-talk between the stroma and epithelium in advanced ovarian cancer very challenging. Similarly, as ovarian carcinoma likely originates from heterogeneous origins, there is a paucity of research investigating stromal-epithelial interactions during tumor initiation in the ovary. Multiple seminal publications using murine model systems for studying ovarian carcinoma development have done so primarily through the introduction mutant K-Ras, c-Myc, or Akt onto a mutated p53 or PTEN background [152–154]. Developing models using human ovarian surface epithelial cells or human fallopian tube epithelial cells has proven a difficult task. Expression of SV40 large T and small t antigens genomic regions [155,156], or human papillomavirus type 16 *E6/E7* region [157], extended the cellular replication life of human ovarian surface epithelial cells, yielding marginal transformation and growth in anchorage-independent colony assays as well as nude mouse tumor growth. Recently, a promising *ex vivo* model was developed using human fallopian tube epithelial cells in the hopes of eventually building a model of late-stage serous ovarian carcinoma [158]. We have developed a genetically defined mouse xenograft model of ovarian carcinoma by expressing the catalytic subunit of human telomerase, oncogenic HRAS or KRAS mutants along with SV40 T/t antigens that yielded subcutaneous tumor formation and intraperitoneal ascites with corresponding CA125/mesothelin staining [62]. We refined this model further by replacing SV40 expression with inhibitory constructs knocking down p53 or Rb, allowing for stepwise delineation of oncogenic and transformative events in modeling ovarian carcinoma [159,160]. Therefore, existing models such as these allow for investigation into the underlying molecular mechanisms correlated with ovarian or fallopian epithelial cell specification, as well as stage-specific signaling mechanisms, in studying and modeling ovarian cancer.

In murine or *in vitro* models focusing on the role and contribution of stroma in ovarian cancer development, much work remains. A few existing publications directly address the ovarian stromal mechanisms promoting ovarian tumorigenesis. Recently, a murine ovarian carcinoma cell line stably overexpressing a fluorescent-tagged vascular endothelial growth factor 164 isoform was developed, which demonstrated significantly accelerated tumor growth with ascites formation, elevated tumor angiogenesis, and promotion of tumor cell survival relative to controls [161]. Thus, ovarian tumor cells within this model directly modulate their proximal tumor stromal microenvironment in promoting tumorigenesis and vascular support and may prove useful for future studies with therapeutic agents targeting the endothelial cell–specific stromal microenvironment contribution to tumor growth. Moreover, another recent study used mouse modeling to focus on ovarian stroma contribution and identified that the cell-cell interaction between ovarian epithelial cells and host stroma was an important factor in ovarian tumorigenesis [162]. Furthermore, we have used our Ras-mediated ovarian cancer mouse xenograft model to demonstrate that GRO-α–expressing senescent ovarian fibroblasts significantly promoted xenograft tumorigenesis of preneoplastic ovarian surface epithelial cells [63]. Thus, the scarcity of existing models allowing characterization of the role of ovarian CAFs in promoting ovarian cancer epithelial tumor growth indicates the real need for development in this field.

Conclusions and Future Work

Although progress has been made toward understanding the role of CAFs in ovarian tumorigenesis, many questions remain. Potential therapeutic targets of cancer- activated stromal signaling pathways that act as regulatory switches for tumor-promoting molecules have been identified—for example, IL-1β/IL-1R1, SDF-1α/CXCR4, GROα-1/CXCR-2, NF-κB p65, and tenascin-C—but detailed mechanisms remain to be worked out. An area of critical importance is deciphering the mechanism by which p53 or other tumor suppressors are inhibited in ovarian fibroblasts and the downstream mediators of this inhibition, which activate the expression of secretory tumor cell behavioral modulators like SDF-1α and FAP-1α (Figure 3*B*). Whether by genetic alteration of tumor suppressors in cancer stroma or by cross-signaling that upregulates key paracrine pathways that stimulate cell growth, successful tumor initiation and evasion of immune surveillance probably depend on proximal stromal activation. Thus, continuing to investigate the role of CAFs in ovarian cancer development should be a high priority for future work. Furthering our understanding of the contribution of activated stromal signaling pathways to ovarian tumorigenesis may yield specific intracellular signaling targets, which effectively suppress the contribution of cancer-associated stromal cells to malignancy, and also novel cancer stromal markers for early detection of ovarian cancer.

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