The Role of Tumor Stroma in Cancer Progression and Prognosis

Emphasis on Carcinoma-Associated Fibroblasts and Non-small Cell Lung Cancer

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Abstract: Maintenance of both normal epithelial tissues and their malignant counterparts is supported by the host tissue stroma. The tumor stroma mainly consists of the basement membrane, fibroblasts, extracellular matrix, immune cells, and vasculature. Although most host cells in the stroma possess certain tumor-suppressing abilities, the stroma will change during malignancy and eventually promote growth, invasion, and metastasis. Stromal changes at the invasion front include the appearance of carcinoma-associated fibroblasts (CAFs). CAFs constitute a major portion of the reactive tumor stroma and play a crucial role in tumor progression. The main precursors of CAFs are normal fibroblasts, and the transdifferentiation of fibroblasts to CAFs is driven to a great extent by cancer-derived cytokines such as transforming growth factor-β. During recent years, the crosstalk between the cancer cells and the tumor stroma, highly responsible for the progression of tumors and their metastasis, has been increasingly unveiled. A better understanding of the host stroma contribution to cancer progression will increase our knowledge about the growth promoting signaling pathways and hopefully lead to novel therapeutic interventions targeting the tumor stroma. This review reports novel data on the essential crosstalk between cancer cells and cells of the tumor stroma, with an emphasis on the role played by CAFs. Furthermore, it presents recent literature on relevant tumor stroma- and CAF-related research in non-small cell lung cancer.

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Disclosure: The author declares no conflicts of interest.

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ISSN: 1556-0864/11/0601-0209

Key Words: Tumor, Stroma, Lung cancer, NSCLC, Carcinoma-associated fibroblasts, CAFs, TGF-β, PDGF, FGF2.

(*J Thorac Oncol.* 2011;6: 209–217)

Lung cancer mortality is high, and annual lung cancer deaths equal prostate, breast, colon, and rectum cancers combined.[1,2] Despite the advancement in knowledge on molecular mechanisms and the introduction of multiple new therapeutic lung cancer agents, the dismal 5-year survival rate (11–15%) remains relatively unaltered.[1,3] This reflects the limited available knowledge on factors promoting oncogenic transformation to and proliferation of malignant cells.

Until recent years, the principal focus in cancer research has mostly been the malignant cell itself. As a consequence, today, there is a significant discrepancy between the vast knowledge about cancer biology generated in experimental settings and the translation of this knowledge into information that can be used in clinical decision making. Understanding the nature of the tumor environment today may be equally important for future cancer therapies as understanding cancer genetics per se. Cancers are not simply autonomous neoplastic cells but also composed of fibroblasts, immune cells, endothelial cells, and specialized mesenchymal cells. These different cell types in the stromal environment can be recruited by malignant cells to support tumor growth and facilitate metastatic dissemination.

Although the "seed and soil" hypothesis was presented more than a century ago by Stephen Paget,[4] we are now starting to comprehend the complex crosstalk between the tumor cells (the "seeds") and the tumor-growing microenvironment (the "soil"). We now know that tumor growth is not determined only by malignant cells, because interactions between cancer cells and the stromal compartment have major impacts on cancer growth and progression.[5] Aggressive malignant cells are clever at exploiting the tumor microenvironment: tumor cells can (1) reside in the stroma and transform it, (2) alter the surrounding connective tissue, and (3) modify the metabolism of resi-

dent cells, thus yielding a stroma, which is permissive rather than defensive.[6]

Beyond overcoming the microenvironmental control by the host, key characteristics of cancer cells is their ability to invade the tissue and metastasize distantly.[7,8] For invasion and metastasis, the concerted interactions between fibroblasts, immune cells, and angiogenic cells and factors are essential.[7,9–17]

In the search for new successful targets for anticancer therapies, a better understanding of differences between normal and tumor stroma will be imperative. This review will focus the interplay between malignant cells, the stromal environment, and carcinoma-associated fibroblasts (CAFs) in particular and present non-small cell lung cancer (NSCLC)–specific research within this field.

THE TUMOR STROMA

The tumor stroma basically consists of (1) the nonmalignant cells of the tumor such as CAFs, specialized mesenchymal cell types distinctive to each tissue environment, innate and adaptive immune cells,[13,18] and vasculature with endothelial cells and pericytes[19,20] and (2) the extracellular matrix (ECM) consisting of structural proteins (collagen and elastin), specialized proteins (fibrilin, fibronectin, and elastin), and proteoglycans (Table 1).[21] Angiogenesis is central for cancer cell growth and survival and has hitherto been the most successful among stromal targets in anticancer therapy. Initiation of angiogenesis requires matrix metalloproteinase (MMP) induction leading to degradation of the basement membrane, sprouting of endothelial cells, and regulation of pericyte attachment. However, CAFs play an important role in synchronizing these events through the expression of numerous ECM molecules and growth factors, including transforming growth factor (TGF)-β, vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) 2.[19]

From Normal Stroma to Tumor Stroma

The normal tissue stroma is essential for maintenance and integrity of epithelial tissues and contains a multitude of cells that collaborate to sustain normal tissue homeostasis. There is a continuous and bilateral molecular crosstalk between normal epithelial cells and cells of the stromal compartment, mediated through direct cell-cell contacts or by secreted molecules, as depicted in Figure 1.[22] Thus, minor changes in one compartment may cause dramatic alterations in the whole system.

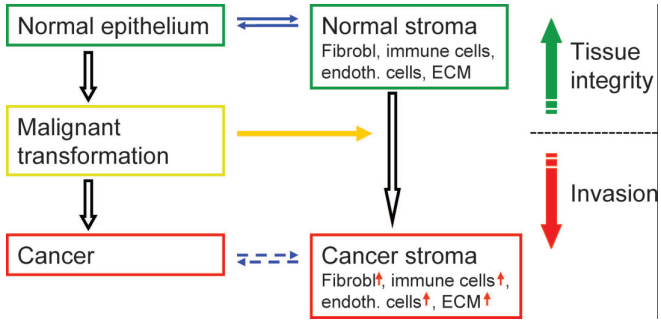

FIGURE 1. Crosstalk (efferent and afferent signaling) between the normal epithelial cells and the stromal cells (blue arrows) maintains tissue equilibrium and integrity (green arrow). During epithelial carcinogenesis, the efferent signaling changes (orange arrow). This leads to alterations in the stroma. The new established crosstalk (dashed blue arrow) between tumor cells and cells of the tumor stroma leads to invasion and subsequently to metastasis (red arrow). Adapted from *J Pathol.* 2003;200:429–447.

In the mid 1980s, Dvorak[23] observed the similarity between stroma from wounds and tumors, because both entities had active angiogenesis and numerous proliferating fibroblasts secreting a complex ECM, all on a background of fibrin deposition. Consequently, the tumor stroma has been commonly referred to as activated or reactive stroma.

A genetic alteration during cancer development, leading to a malignant cell, will consequently change the stromal host compartment to establish a permissive and supportive environment for the cancer cell.[24] During early stages of tumor development and invasion, the basement membrane is degraded, and the activated stroma, containing fibroblasts, inflammatory infiltrates, and newly formed capillaries, comes into direct contact with the tumor cells. The basement membrane matrix also modifies cytokine interactions between cancer cells and fibroblasts.[25] These cancer-induced alterations in the stroma will contribute to cancer invasion.[26] Animal studies have shown that both wounding and activated stroma provides oncogenic signals to facilitate tumorigenesis.[27,28] Although normal stroma in most organs contains a minimal number of fibroblasts in association with physiologic ECM, the activated stroma is associated with more ECM-producing fibroblasts, enhanced vascularity, and increased ECM production (Figure 1).[21] This formation of a specific tumor stroma type at sites of active tumor cell invasion is considered an integral part of the tumor invasion and has been termed as tumor stromatogenesis by Giatromanolaki et al.[29]

The expansion of the tumor stroma with a proliferation of fibroblasts and dense deposition of ECM is termed a desmoplastic reaction.[30] It is secondary to malignant growth and can be separated from alveolar collapse, which do not show neither activated fibroblasts nor the dense collagen/ECM. Morphologically this is termed desmoplasia and was initially conceived as a

TABLE 1. Summary of Stromal Components Related to Carcinoma-Associated Fibroblast-Mediated Tumorigenesis

Stromal Components	Constituents/Function
ECM	Collagen, elastin, fibrilin, fibronectin, laminin, and proteoglycans. Major components are fibronectin and collagen type I.
	Modulates cell differentiation, morphology, and proliferation.
MMPs	ECM-degrading endopeptidases (proteases), main substrates are collagens. More than 21 forms.
Fibronectin	Glycoprotein. Binds ECM components and integrins. Control the activity of growth factors, proteases, protease inhibitors.
Tenascin-C	ECM glycoprotein. Interacts with fibronectin. Involved in regulating morphogenetic cell migration and organogenesis.

ECM, extracellular matrix; MMP, matrix metalloproteinase.

defense mechanism to prevent tumor growth,[31–34] but data have shown that in established tumors, this process, quite oppositely, participates in several aspects of tumor progression, such as angiogenesis, migration, invasion, and metastasis.[21,35,36] The latter studies show that fibroblasts and tumor cells can enhance local tissue growth and cancer progression through secreting ECM and degrading components of ECM within the tumor stroma.[21,35] This is in part related to the release of substances sequestered in the ECM, such as VEGF (Table 2), and cleavage of products from ECM proteins as a response to secretion of carcinoma-associated MMPs.[5,37,38]

Cancer Cell-Derived Profibrotic Growth Factors, Angiogenic Factors, and the Tumor Stroma

Profibrotic growth factors, released by cancer cells, such as TGF-β, platelet-derived growth factor (PDGF), and FGF2 govern the volume and composition of the tumor stroma as they are all key mediators of fibroblast activation and tissue fibrosis (Table 2).[9,23] PDGF and FGF2 play significant roles in angiogenesis as well.

Angiogenic factors, such as the VEGF family, are essential in the emergence of the activated stroma.[39] Although VEGF can be released by malignant cells, the fibroblasts and inflammatory cells are the principal source of host-derived VEGF.[40] VEGF induces neovascularization and microvascular permeability, leading to extravasation of plasma proteins such as fibrin, which subsequently attracts fibroblasts, inflammatory cells, and endothelial cells.[39,41,42] These cells produce ECM yielding desmoplasia, which again enhance tumor angiogenesis.[39,43,44]

TABLE 2. Summary of Major Growth Factors and Chemokines Related to Carcinoma-Associated Fibroblast-Mediated Tumorigenesis

Factor	Function
TGF-α	Considered important in wound healing. Induces epithelial development. Closely related to EGF.
TGF-β	TGF-β is the most frequent. Normally controls proliferation and cellular differentiation. Role in immunity and cancer. In cancer, it may lead to progression and metastasis.
PDGF	Promotes proliferation of connective tissue. Regulate cell growth and division. Role in angiogenesis. Regulates interstitial fluid pressure.
FGF2	Present in BM and in the subendothelial ECM of vessels. Promotes proliferation of different cells. Role in angiogenesis.
EGF	Binds to its receptor EGFR, leads to cellular proliferation, differentiation, and survival.
VEGF	Increase vascular permeability. Role in early stages of desmoplasia. Important role in angiogenesis.
HGF	Paracrine cellular growth, motility, and morphogenic factor. Secreted by mesenchymal cells, acts on epithelial or endothelial cells.
IGF-1	Growth factor. Has growth-promoting effect on almost every cell in the body.
CTGF	Can promote endothelial cell growth, migration, adhesion, and survival. It is implicated in endothelial cell function and angiogenesis.
CXCLs and CCLs	Chemokines of the CXC and CL types. Attractants of leukocytes. Important in angiogenesis, carcinogenesis, tumor progression, and metastasis.
SFRP1	Act as soluble modulators of Wnt signaling.
SPARC	Associated with cancer cell migration and invasion.
ILs	Cytokines. Inflammatory response against infection. Enables transmigration of lymphocytes. IL-1 and -6 may contribute to cancer progression.

TGF, transforming growth factor; PDGF, platelet-derived growth factor; FGF2, fibroblast growth factor-2; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; CTGF, connective tissue growth factor; CXCL and CLL, chemokines; SFRP1, secreted frizzled-related protein 1; SPARC, secreted protein acidic and rich in cysteine; IL, interleukin; BM, bone marrow; ECM, extracellular matrix; EGFR, epidermal growth factor receptor.

NORMAL FIBROBLASTS AND CAFS

Fibroblasts were originally described more than 100 years ago and are still for the most part defined by their location and what they are not—nonsmooth muscle cells, nonendothelial cells, and nonepithelial cells of the stroma. The lack of specific markers has historically prevented more thorough investigations of fibroblasts. Gene analyses have recently revealed that fibroblasts are quite dissimilar cells and dependent on the tissue from which they derive and the specific roles they are called to play.[45]

From Normal Fibroblasts to CAFs

In tissue homeostasis, normal fibroblasts are in an inactive quiescent state, embedded within the fibrillar ECM primarily consisting of collagen type I, laminin, fibronectin, and proteoglucans and interact with their surroundings through cell receptors called integrins.[36] Fibroblasts become activated in wound healing and fibrosis, and these cells, also called myofibroblasts,[46] differ morphologically and functionally from quiescent fibroblasts. On activation, these cells are capable of producing relevant signal mediators, such as growth factors, cytokines, chemokines, and other immune modulators.[47] However, as soon as the wound healing is completed, most of these activated fibroblasts are removed from the granulation tissue by apoptosis.[48] Cancer has been considered "a wound that never heal," because the activated fibroblasts are not removed by apoptosis as in normal wound healing.[21] Instead, these cells are prominent contributors in carcinogenesis.[49]

CAFs

In tumors, activated fibroblasts are termed as peritumoral fibroblasts or CAFs.[11,50,51] CAFs, like activated fibroblasts, are highly heterogeneous and believed to derive from the same sources as activated fibroblasts. The main progenitor seems to be the locally residing fibroblast, but they may also derive from pericytes and smooth muscle cells from the vasculature, from bone marrow-derived mesenchymal cells, or by epithelial or endothelial mesenchymal transition.[52–54] The term CAF is rather ambiguous because of the various origins from which these cells are derived, as is the difference between activated fibroblasts and CAFs. There are increasing evidence for epigenetic and possibly genetic distinctions between CAFs and normal fibroblasts.[55,56] CAFs can be recognized by their expression of α-smooth

muscle actin, but due to heterogeneity α-smooth muscle actin expression alone will not identify all CAFs.[57,58] Hence, other used CAF markers are fibroblast-specific protein 1, fibroblast activation protein (FAP), and PDGF receptor (PDGFR) α/β.[36,59]

In response to tumor growth, fibroblasts are activated mainly by TGF-β, chemokines such as monocyte chemotactic protein 1, and ECM-degrading agents such as MMPs. Although normal fibroblasts in several in vitro studies have demonstrated an inhibitory effect on cancer progression, today, there is solid evidence for a cancer-promoting role of CAFs. In breast carcinomas, as much as 80% of stromal fibroblasts are considered to have this activated phenotype (CAFs).[11,60]

The Role of CAFs in Tumor Progression and Metastasis

CAFs promote malignant growth, angiogenesis, invasion, and metastasis.[36,61–63] The roles of CAFS and their potential as targets for cancer therapy have been studied in xenografts models, and evidence from translational studies has revealed a prognostic significance of CAFs in several carcinoma types.[54]

In the setting of tumor growth, CAFs are activated and highly synthetic, secreting, for example, collagen type I and IV, extra domain A-fibronectin, heparin sulfate proteoglucans, secreted protein acidic and rich in cysteine, tenascin-C, connective tissue growth factors, MMPs, and plasminogen activators.[62,64] In addition to secreting growth factors and cytokines, which affect cell motility, CAFs are an important source for ECM-degrading proteases such as MMPs that play several important roles in tumorigenesis.[11,65] Through degradation of ECM, MMPs can, depending on substrate, promote tumor growth, invasion, angiogenesis, recruitment of inflammatory cells, and metastasis.[65,66] Besides, a number of proinflammatory cytokines seem to be activated by MMPs.[67,68] In a recent review, Kessenbrock et al.[66] report on the multiple functions of MMPs in the tumor stroma and have categorized the proteases according to roles in (1) tissue invasion and intravasation, (2) angiogenesis, (3) regulation of inflammation, and (4) preparation of the metastatic niche.

After injection of B16M melanoma cells in mice, the formation of liver metastases was associated with an early activation of stellate cells (fibroblast-like) in the liver, as these seemed important for creating a metastatic niche and promoting angiogenesis.[69] MMPs have also been linked to tumor angiogenesis in various in vivo models.[70] CAFs, when coinjected into mice, facilitated the invasiveness of otherwise noninvasive cancer cells.[71] Furthermore, xenografts containing CAFs apparently grow faster than xenografts infused with normal fibroblasts.[72]

SIGNALING BETWEEN CAFS AND OTHER CELL TYPES IN THE TUMOR STROMA

At CAF recruitment and accumulation in the tumor stroma, these cells will actively communicate with cancer cells, epithelial cells, endothelial cells, pericytes, and inflammatory cells through secretion of several growth factors, cytokines, and chemokines, as shown in Figure 2 and Tables 1 and 2.[36] CAFs provide potent oncogenic molecules such as TGF-β and hepatocyte growth factor (HGF).

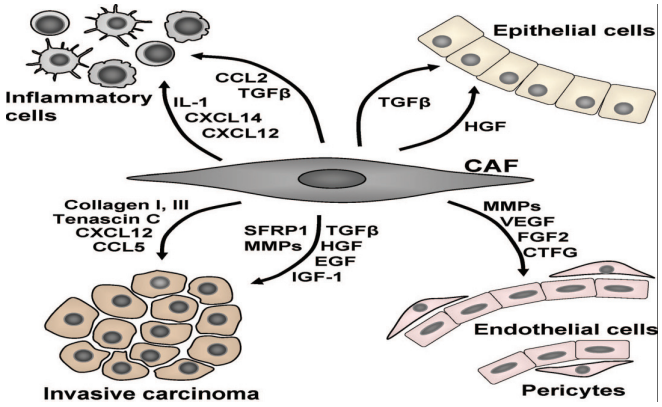

FIGURE 2. Efferent signaling from carcinoma-associated fibroblast in the tumor stroma. The fibroblasts communicate with malignant cells, epithelial cells, endothelial cells, pericytes, and inflammatory cells through the secretion of several growth factors and chemokines. Adapted from *Nat Rev Cancer*. 2006;6:392–401; *Curr Opin Genet Dev*. 2009;19:67–73; and *Semin Cell Dev Biol*. 2010;21:33–39. CCL, chemokine ligand; CXCL, chemokine ligand; CTGF, connective tissue growth factor; EGF, epithelial growth factor; FGF2, fibroblast growth factor 2; IGF-1, insulin-like growth factor-1; HGF, hepatocyte growth factor; IL, interleukin; MMPs, matrix metalloproteinases; SFRP1, secreted frizzled-related protein 1; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

TGF-β is a pleiotropic growth factor expressed by both cancer and stromal cells. TGF-β is, in the normal and premalignant cells, a suppressor of tumorigenesis,[73,74] but as cancer cells progress, the antiproliferative effect is lost, and instead, TGF-β promotes tumorigenesis by inducing differentiation into an invasive phenotype.[75,76] TGF-β may also instigate cancer progression through escape from immunosurveillance,[77] and increased expression of TGF-β correlate strongly with the accumulation of fibrotic desmoplastic tissue and cancer progression.[22] Recently, a small molecule inhibitor of TGF-β receptor type I was reported to inhibit the production of connective tissue growth factor by hepatocellular carcinoma (HCC) cells, resulting in reduced stromal component of the HCCs. Inhibition of the TGF-β receptor aborted the crosstalk between HCCs and CAFs and consequently avoided tumor proliferation, invasion, and metastasis.[78] HGF belongs to the plasminogen family and is tethered to ECM in a precursor form. It binds to the high-affinity receptor c-met, and overexpression or constant oncogenic c-Met signaling lead to proliferation, invasion, and metastasis.[54,79,80]

PDGFs are regulators of fibroblasts and pericytes and play important roles in tumor progression.[81,82] It is a chemotactic and growth factor for mesenchymal and endothelial cells. It has a limited autocrine role in tumor cell replication, but is a potential player, in a paracrine fashion, and in tumor stroma development.[83,84] It induces the proliferation of acti-

vated fibroblasts and possibly recruits CAFs indirectly by stimulation of TGF-β release from macrophages.[85]

FGF2 has a fundamental role in fibroblast growth and tissue fibrosis/desmoplasia in tumors.[86] FGF2 has been shown to exert its effect on endothelial cells in both a paracrine and autocrine fashion as a consequence of secretion by tumor and stromal cells.[87–89]

THE ROLE OF TUMOR STROMA AND CAFS IN NSCLC

Taking into account the high frequency of new lung cancer cases and especially the poor prognosis in NSCLC, there has been a search for biologic markers that correlate with development and prognosis of this disease. Hitherto, no such marker has been established as a valid clinical predictor for diagnosis, therapy, or prognosis of NSCLC.[90]

By using short-term fibroblast cell cultures, Nakamura et al.[91] were able to demonstrate that proliferating fibroblasts from pulmonary adenocarcinomas are phenotypically different from fibroblasts of normal bronchus tissue. Besides, there are metabolism-related differences between lung cancer cells and tumor-associated stroma because cancer cells use more anaerobe metabolism compared with CAFs.[92]

To be able to target stromal components in the treatment of NSCLC, we need further knowledge on mechanisms involved in the cancer-stromal crosstalk, which drives cancer progression and metastasis. In the following, we will review NSCLC-specific research data on the interplay between malignancy and stromal cells.

Tumor Stroma, CAFs, and NSCLC Cells

The impact of crosstalk between NSCLC cells and normal fibroblasts was reported by Fromigue et al.[93] In a coculture model of NSCLC tumor cells and normal pulmonary fibroblasts, exposure to NSCLC cells rendered the fibroblasts with a set of modulated genes, which potentially would affect the regulation of matrix degradation, angiogenesis, invasion, cell growth, and survival.

Several translational studies on resected NSCLC tumors have recently uncovered new prognostic attributes by CAFs. In adenocarcinomas, carbonic anhydrase IX expression was a better prognostic predictor in CAFs than in cancer cells.[94] In a combined study, using cell cultures and resected adenocarcinomas, HGF and its receptor c-MET constitute an autocrine activation loop in CAFs, and this system possibly play a role in invasion and progression of adenocarcinomas.[95] In adenocarcinomas, podoplanin (lymphatic endothelial cell marker) expression was examined in cancer cells and CAFs.[96] Data showed that podoplanin was significantly associated with shorter survival, but this was related to CAFs rather than cancer cells.

MMPs are associated with multiple human cancers and were early considered as drug targets to treat cancer.[66] The first drug development programs started more than 2 centuries ago, and several small molecule antineoplastic broad-spectrum MMP inhibitors were tested against lung, prostate, and pancreas cancers in randomized phase III trials. The effect of these inhibitors turned out disappointing as they failed to show any survival benefit. Possible reasons for this has been discussed extensively.[97] Mounting evidence supports a dominant role for MMP-14 in migration and invasion of metastatic tumor cells.[98] The current strategy focus on development of more selective MMP inhibitors to be used in earlier stages of cancer.[99] In earlier studies, MMP expressions were in general only assessed in tumors. In resected NSCLC tumors, Ishikawa et al.[100] found that MMP-2 expression was a significant unfavorable prognostic factor for those with squamous cell carcinomas, but based on its expression in CAFs rather than tumor cells. In the light of novel results suggesting the importance of CAF-derived MMPs in tumorigenesis, their role will be taken into consideration along with the tumor cell-derived MMP targets. Tissue factor pathway inhibitor (TFPI)-2 is an inhibitor of plasmin and thus inhibits MMP activation. Silencing TFPI-2 in lung cancer cells lead to increased expression of MMP-1, -3, and -7 in pulmonary fibroblasts when grown in conditioned medium from TFPI-2-silenced cells.[101]

TGF

The clinical prognostic value of the TGF-β family has not been clarified. In three small previous studies on resected NSCLC tumors (range 53–91), the prognostic role of TGF-β expression emerged contradictory.[102–104] In a more recent study, the prognostic relevance of TGF-β expression was examined in both tumor and stromal compartments of 335 NSCLC samples.[105] There was no statistically significant association between tumor cell TGF-β expression and survival, although this marker was not expressed in stromal cells. This study showed, however, nuclear factor-κBp105 expression levels in both tumor epithelial and stromal cells to be favorable independent prognostic factors.[105] Nevertheless, TGF-β has been reported to enhance cell migration and up-regulate β1-integrin in cancer cells through PI3K/Akt, which again activate nuclear factor-κB.[106]

There are limited data on the prognostic role of TGF-α in NSCLC. In a coculture study of pulmonary fibroblasts from a healthy donor and adenocarcinoma cells, fibroblasts previously exposed to the cancer cells increased the proliferation of pulmonary adenocarcinoma cell lines.[107] This growth stimulation could, however, be blocked by antibodies against TGF-α and amphiregulin. Consistently, an examination of amphiregulin and TGF-α levels in pretreatment serum from nonsquamous NSCLC patients showed unfavorable prognostic impacts.[108]

PDGF

In NSCLC, the PDGFR-β is frequently expressed by CAFs but not on tumor cells that express the PDGF ligand.[109,110] In the early 1990s, abnormally high expression of PDGF was reported in NSCLC tumors, and this was associated with a potential role in neoplastic transformation and uncontrolled growth of lung cancer.[111] From multiple lung cancer microarray data, Tejada et al.[112] observed a significant expression of PDGF-A, PDGF-C, and PDGFR-α in lung carcinomas. They further noted that tumor-driven paracrine PDGFR-α signaling was a key determinant for stromal recruitment. Other in vitro data from endothelial cells exposed

to conditioned media from lung cancer cells support the notion that lung cancer and endothelial cells interact through various paracrine pathways for a reciprocal induction of PDGF-B and VEGF.[113]

In 92 resected NSCLC tumors, Kawai et al.[110] discovered that tumor cell PDGF-B expression predicted a poor survival. In a combined study of 128 NSCLC carcinomas, a lung cancer cell line, and animal implantation, PDGF-A was found to have a negative prognostic impact.[114] In a recent study of 335 NSCLC tumors, Dønnem et al.[115,116] examined the expression of PDGF-A, -B, -C, and -D and PDGFR-α and -β in both tumor and stromal compartments. Although tumor-associated PDGF-B, PDGF-C, and PDGFR-α were associated with a negative prognosis, stroma-associated PDGF-A, PDGF-B, PDGF-D, and PDGFR-α were favorable prognostic indicators. Further, stromal expression of PDGF-B, PDGF-D, and PDGFR-β was associated with less nodal metastasis.

FGF2

Previous data on FGF2's prognostic impact in NSCLC has been conflicting.[117,118] Some studies have reported high tumor cell FGF2 expression to correlate with poor survival,[119–121] whereas others find no such association.[122,123] Guddo et al.[118] examined the expression of FGF2 and FGF receptor (FGFR)-1 in tumor cells and stroma of 84 NSCLC tumors. The authors found that FGF2 and FGFR-1 expression in tumor cells, stromal cells, and vessels was directly correlated with host stromal response but not with angiogenic response. Besides, FGF2 expression in stroma was inversely correlated with lymph node metastasis. These findings corroborate our FGF2 and FGFR-1 expression data.[87] Here, the stromal FGF2 expression was a favorable prognosticator, whereas tumor cell FGF2 mediated an unfavorable prognosis.

STROMAL INFLUENCE ON NSCLC THERAPY

As CAFs in many aspects differ from the cancer cells, one may speculate whether these activated fibroblasts may alter the responsiveness of tumor cells to chemotherapy. In primary CAF cultures from 37 breast and lung cancer tissues, CAF responses to chemotherapy were, as for cancer cells, highly variable.[124] Moreover, conditioned medium from lung fibroblasts (WI-38) impaired NSCLC H358 cell death induced by paclitaxel but not by cisplatin.[125] This may indicate a differential predictive treatment impact, mediated by fibroblasts.

In a recent study, NSCLC cells with epithelial growth factor receptor (EGFR)-activating mutations (PC-9, HCC827) were cocultured with fibroblasts and injected into severe combined immunodeficient mice to assess the effect of crosstalk on the susceptibility to EGFR tyrosine kinase inhibitors.[126] CAFs from lung cancer tissue produced HGF and, thus, activated the c-Met pathway. When cocultured with activated HGF-producing CAFs, the otherwise sensitive NSCLC cells actively recruited CAFs and became resistant to EGFR-tyrosine kinase inhibitors. Hence, crosstalk with stromal CAFs may play a role in development of treatment resistance.

TUMOR STROMA: A POSSIBLE TARGET FOR CANCER THERAPY

As described earlier, a tumor can not develop without the parallel expansion of a tumor stroma. Although we still do not comprehend the exact mechanisms regulating fibroblast activation and their accumulation in cancer, the available evidence points to the possibility that the tumor stroma or CAFs may be candidate targets for cancer treatment. This alley has, however, not been without pitfalls.

CAFs and MMPs have been considered two of the key regulators of epithelial-derived tumors representing potential new targets for integrative therapies, affecting both the transformed and nontransformed components of the tumor environment. As commented earlier, the experience with MMP inhibitors have so far been unsuccessful. Evidence that CAFs are epigenetically and possibly also genetically distinct from normal fibroblasts is beginning to define these cells as potential targets for anticancer therapy.[55] FAP, expressed in more than 90% of epithelial carcinomas, emerged early as a promising candidate for targeting CAFs,[127] and the potential therapeutic benefit of its inhibition was reviewed recently.[128] In preclinical studies, abrogation of FAP attenuates tumor growth and significantly enhance tumor tissue uptake of anticancer drugs.[129–131] In a phase I study, where patients with FAP-positive advanced carcinomas (colorectal cancer and NSCLC) were treated with FAP-antibody, the antibody bound specifically to tumor sites, but no objective responses were observed.[132]

The consistent and repeated findings of cancer cells that readily undergo invasion and metastasis in response to TGF-β have pointed to the need of novel anticancer agents targeting the oncogenic activities of TGF-β. A large number of anti-TGF-β antibodies and TGF-β-receptor I kinases have been tested preclinically during the past decade. Because of the lack of success, targeting of the TGF-β signaling system still remains elusive.[133] It should be noted that both protumoral and antitumoral effects have been assigned to TGF-β, and the multifunctional nature of TGF-β apparently represents the greatest barrier to effectively target this ligand, its receptor, or downstream effectors.

CONCLUSION

Advances in understanding the stromal contribution in cancer progression will enhance our awareness and knowledge on the reciprocal signaling that support and promote cancer growth, dedifferentiation, invasion, and survival. Unraveling of essential biologic and pathologic mechanisms involved has actualized the need of stroma-specific therapeutic strategies. Hopefully, it will be possible to selectively and successfully target oncogenic stromal activities beyond angiogenesis.

CAFs represent an important cell type in carcinogenesis and progression, and the recent findings presented herein have further actualized its role in proliferation, invasion, and metastasis. Besides, through interaction with other stromal cell types, CAFs also modulate angiogenesis and immunity. As a consequence, CAFs should be highly intriguing therapy targets. To succeed in establishing novel targeted therapy

against CAFs, high-quality, basic, and translational research will be necessary to further unveil the complex crosstalk between CAFs, cancer cell, and other cells of the tumor microenvironment.

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