Cancer-associated fibroblasts in nonsmall cell lung cancer: From molecular mechanisms to clinical implications

Kit Yee Wong1,2,3 | Alvin Ho-Kwan Cheung1,2,3 | Bonan Chen1,2,3 | Wai Nok Chan1,2,3 | Jun Yu2,4 | Kwok Wai Lo1,2,3 | Wei Kang1,2,3 | Ka Fai To1,2,3

1Department of Anatomical and Cellular Pathology, State Key Laboratory of Translational Oncology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China
2Institute of Digestive Disease, State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Hong Kong SAR, China
3Li Ka Shing Institute of Health Science, Sir Y.K. Pao Cancer Center, The Chinese University of Hong Kong, Hong Kong SAR, China
4Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR, China

Correspondence
Wei Kang and Ka Fai To, Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China. Email: weikang@cuhk.edu.hk (W. K) and kfto@cuhk.edu.hk (K. F. T.)

Funding Information
Chinese University of Hong Kong, Grant/Award Number: 2020.001; Research Grants Council, University Grants Committee, Grant/Award Numbers: CUHK 14100019, CUHK 14118518

Abstract
Lung cancer is the common and leading cause of cancer death worldwide. The tumor microenvironment has been recognized to be instrumental in tumorigenesis. To have a deep understanding of the molecular mechanism of nonsmall cell lung carcinoma (NSCLC), cancer-associated fibroblasts (CAFs) have gained increasing research interests. CAFs belong to the crucial and dominant cell population in the tumor microenvironment to support the cancer cells. The interplay and partnership between cancer cells and CAFs contribute to each stage of tumorigenesis. CAFs exhibit prominent heterogeneity and secrete different kinds of cytokines and chemokines, growth factors and extracellular matrix proteins involved in cancer cell proliferation, invasion, metastasis and chemoresistance. Many studies focused on the protumorogenic functions of CAFs, yet many challenges about the heterogeneity of CAFs remain unresolved. This review comprehensively summarized the tumor-promoting role and molecular mechanisms of CAFs in NSCLC, including their origin, phenotypic changes and heterogeneity and their functional roles in carcinogenesis. Meanwhile, we also highlighted the updated molecular classifications based on the molecular features and functional roles of CAFs.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited. © 2022 The Authors. International Journal of Cancer published by John Wiley & Sons Ltd on behalf of UICC.
INTRODUCTION

Lung cancer is one of the most common malignancies worldwide, particularly in men. The American Cancer Society reported approximately 2.2 million new cases of lung cancer and approximately 1.8 million new deaths in 2020. Lung cancer is histologically classified as small-cell lung carcinoma (SCLC) and nonsmall-cell lung carcinoma (NSCLC). NSCLC represents approximately 80% of all lung cancer and is mainly divided into squamous cell carcinoma, adenocarcinoma and large cell carcinoma. These subtypes have unique histological and biological features. Enhancing insight into the genome alterations revealed various oncogenic driver mutations in NSCLC.

To understand the biological perspectives of lung cancer, researchers have mainly focused on malignant cells, such as various signaling pathways. However, these just represent one of the hallmarks of cancer. Cancers are not simply composed of cells with deranged signaling pathways but include a complex tumor microenvironment (TME). Like the theory of “Seed and Soil” which was proposed by Dr Stephen Paget, cancers cells seed in congenial soil, the TME, where they grow and expand. The TME is an ecosystem composed of multicellular and noncellular components. Four major components of the TME are: (1) the tumor immune microenvironment (TIME) consists of immune cells such as natural killer (NK) and T cells; (2) vascular components include lymphatic endothelial cells (LECs) and pericytes; (3) the extracellular matrix (ECM) is comprised of diverse collagen, glycoproteins and proteoglycans; (4) stromal components consist of mesenchymal STEM cells (MSCs) and cancer-associated fibroblasts (CAFs). These cells in the TME interact with the malignant cells closely, which promote the whole tumorigenesis process, from tumor initiation to progression.

CAFs are one of the well-known and critical components in the tumor stroma. CAFs are worthy of mention since they are conducive to all aspects of tumorigenesis in different stages and many cancer types, including tumor proliferation, tumor invasion and metastasis and interfering with the immune system. Given the multifaceted functions of CAFs, many studies attempted to “switch off” the function of CAFs to target tumors more effectively. Controversially, some investigations have demonstrated that some CAFs have an antitumorigenic role. More importantly, how can CAFs transit from a tumor defender into a tumor supporter? For example, tumor-associated exosomes have been identified recently as an essential cellular interchange mechanism between tumor cells and CAFs. Isolated exosomes from tumor cells and CAFs are implicated in multiple steps of CAFs evolution, such as normal fibroblasts (NFs) differentiation into CAFs, CAF-like state maintenance and promotion of CAFs’ oncogenic properties. Extracellular vesicles produced by tumor cells can activate normal fibroblasts to a CAF-like state, which in turn produces a secretome to modulate the tumor microenvironment. In this review, we summarized recent studies on the roles of CAFs and, particularly in NSCLC, where scar formation and fibrosis are common phenomena.

THE DEFINITION AND BIOLOGICAL PROPERTIES OF CAFs

Fibroblasts were first identified in the 1850s as connective tissue cells responsible for synthesizing collagen. Fibroblasts in normal tissue are generally considered quiescent, that is, in a resting state. Fibroblasts can be challenging to define because of a lack of unique markers expressed exclusively and by all fibroblasts. Some markers such as vimentin, platelet-derived growth factor receptor-α (PDGFR-α) and fibroblast specific protein 1 (FSP1) can be used as markers for quiescent fibroblasts. However, these markers are not only expressed in fibroblasts. Thus, the tissue location and morphology are always required for their identification.

Quiescent fibroblasts are the major component of ECM under physiological conditions. They are activated by tissue repair and regeneration in response to tissue damage. As observed in wound healing, fibroblasts accumulate at the damaged site and transform into myofibroblasts, and subsequently promote angiogenesis and deposition of ECM. Myofibroblasts produce many kinds of cytokines and chemokines. They are also a significant source of ECM-degrading proteases, maintaining ECM homeostasis by regulation of ECM turnover, and promoting angiogenesis with increased production of vascular endothelial growth factor A (VEGFA). Myofibroblasts secrete transforming growth factor-beta (TGF-β) and express α-smooth muscle actin (α-SMA) at closing wounds. and are critical for maintaining the homeostasis of adjacent epithelial cells by growth factors (GFs) secretions and by direct mesenchymal-epithelial cell interactions. When the wound is healed, myofibroblasts are restored to their quiescent status or are removed by apoptosis. Such reversibility is a hallmark feature of fibroblasts associated with tissue repair.
2.2 Activation of fibroblasts into CAFs

Tumors may be considered as “wounds that do not heal.” In a normal situation, fibroblasts have an antitumorigenic activity that suppresses tumor growth. For example, fibroblasts in lymph nodes transport potential antigens and contribute to leukocytes’ migration, resulting in effective immune responses. However, cancer is an advancing and unabated injurious stimulus which initiates fibroblast activation. Fibroblasts are then transformed into irreversible cancer-associated fibroblasts (CAFs), which behave like myofibroblasts in some aspects. They are not removed by apoptosis. This process is called cancer fibrosis.

To acquire tumor-promoting phenotypes, the quiescent fibroblasts are activated via diverse mechanisms (Figure 1A). First, epithelial cancer cells secrete growth factors into the surrounding microenvironment, stimulating the recruitment and activation of fibroblasts. Among these factors, transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are critical regulators. In lung cancer, TGF-β facilitates invasion of cancer cells through tumor-stromal interactions. TGF-β orchestrates tumor stroma development and promotes angiogenesis, immune evasion and remodeling of the ECM. In microarray gene expression analysis, the gene signatures related to TGF-β signaling are enriched in CAFs isolated from NSCLC tissues compared to the normal tissue. PDGF is one of the profibrotic growth factors secreted by cancer and stromal cells, inducing CAFs activation. Cancer cells secrete PDGF to act on the stromal cells, especially endothelial cells and fibroblasts in vivo. In contrast to TGF-β, the primary functions of PDGF are enhancing fibroblasts’ growth and proliferation through MAPK downstream signaling pathways without causing their differentiation into myofibroblasts. PDGF is also a crucial factor in neo-angiogenesis and establishing protumorigenic stroma. FGF, an angiogenic endothelial cell mitogen, is a pleiotropic molecule that functions on epithelial and mesenchymal cells in an intracrine, autocrine and paracrine manner. Most studies focused on FGF-2, which describes how it changes the phenotype of fibroblasts, leading to cell activation.

Besides growth factors, lung cancer cells produce different inflammatory modulators such as the interleukin family (IL-6, IL-8, IL-17, IL-22), tumor necrosis factor-α (TNF-α) and VEGF to promote their progression, invasion and angiogenesis. Many studies found that these inflammatory cytokines are related to fibroblast activation in lung cancer. Leukemia inhibitory factor (LIF), an IL-6 class proinflammatory cytokine, is an example. It mediates ECM remodeling and TGF-β-dependent actomyosin-contractility via crosstalk between the JAK1/STAT3 and RhoA/ROCK/MLC2 signaling pathways, which results in carcinoma cell invasion in vitro and in vivo. Actomyosin contractility generates mechanical force to remodel the ECM for cell migration, which is caused by CAFs. The roles of STAT3 and SMAD are also implicated in lung fibrosis.

In NSCLC, the oxygen level is deficient (0.7-46 mm Hg), thus hypoxia is a characteristic of the lung cancer microenvironment. This remodels the composition of TME, and induces the expression of HIF-1α in fibroblasts. The expression of HIF-1α in fibroblasts also induces the conversion of normal fibroblasts into CAFs, and CAFs activation can be inhibited effectively by HIF-1α-specific inhibitors or HIF-1α knock-out. Moreover, p62, an autophagy regulator, is highly expressed in NSCLC under hypoxia. This induces autophagy, the nuclear factor erythroid 2-related factor 2 (Nrf2)-related antioxidant signaling, and the activating transcription factor 6 (ATF6)-related ER-stress response, causing

![Figure 1](Color figure can be viewed at wileyonlinelibrary.com)
CAF activation. Autophagy inhibitors such as 3MA and HCQ can block CAF activation and tumor progression, supporting the critical role of p62-dependent autophagy in CAFs activation.66 While Nrf2 is persistently elevated, fibroblasts are induced into a state of increased activity and acquire the CAF phenotype, leading to tumor growth.67

MicroRNAs (miRNAs) are small endogenous noncoding RNAs that mainly downregulate target gene expression68-70 and are potential biomarkers in cancer patients.71-73 miRNAs also contribute to the activation of CAFs during cancer progression. Previous studies demonstrated that some miRNAs are highly expressed in lung adenocarcinoma and promote CAFs activation, such as miR-196a,74 miR-21075 and miR-21.76 Moreover, some miRNAs are highly expressed in NF in lung fibrosis and alter the phenotype of primary fibroblasts, such as miRNA-15577 and Let-7d miRNAs.78 These findings suggest that miRNAs can have a regulatory role in transforming NFs to CAFs in lung adenocarcinoma.

Besides posttranscriptional control, epigenetic mechanisms are also implicated in CAF activation.68,69,79,80 A study showed that the proinflammatory cytokine leukemia inhibitory factor (LIF) enhanced the invasive potential of CAFs, by mediating the acetylation of STAT3 by histone acetyltransferase p300, and subsequently the activation of JAK1 by promoter methylation of SHP1.79 Recently, an interesting study highlighted that CAFs transactivated the lncRNA HOX transcript antisense RNA (HOTAIR) expression in breast cancer cell via the secretion of TGF-β1, and also histone H3K27 trimethylation to activate the CDK5 pathway, contributing to cancer metastasis and EMT.80 More epigenetic control modes in CAFs activation are expected to be unraveled.

Many studies reported exosomes as the messengers participating in the crosstalk between cancer cells and fibroblasts in promoting CAFs activation.24,81 Exosomes are lipid-bilayer extracellular nanovesicles carrying microRNAs,25,75,82 IncRNAs,27 proteins,21,84 metabolites and other substances.85,86 Zhang et al determined the protein secretome of fibroblasts treated with or without cancer cell-derived exosomes. They found that cancer cells and fibroblasts have a bidirectional interaction; Cancer cell-derived exosomes activate fibroblasts into CAFs while CAFs secreted proteins enhances proliferation and migration of NSCLC cells.87

3 | THE HETEROGENEITY OF CAFs

3.1 | The origin of CAFs

Emerging evidence suggests that CAFs are a highly heterogeneous population of cells.16 Such heterogeneity might be due to the numerous potential cellular sources and precursors of CAFs (Figure 1B).88,89 NFs can be activated by the TME stimuli, such as local hypoxia, oxidative stress and GFs released from the neighboring tumor cells and infiltrating immune cells. This theory suggests that CAFs can be derived from resident fibroblasts activated by adjacent tumor cells and are the primary source of CAFs.90 TGF-β, secreted by the stromal and cancer cells, promotes the migration of resident fibroblasts and their transformation into CAFs. In some organs, such as the pancreas and liver, the resident fibroblasts are known as quiescent pancreatic stellate cells (PSCs) and hepatic stellate cells (HSCs), respectively.91,92 They can acquire a myofibroblast-like phenotype such as α-smooth muscle actin (α-SMA) expression upon TGF-β and PDGF activation. Furthermore, some CAFs can transdifferentiate from mesenchymal stem cells (MSCs),93,94 CXCL-12 and TGF-β, which are secreted by tumor cells, stimulate the recruitment of MSCs and their activation into CAFs.16,95,96 Bone marrow derived (BM)-MSCs show upregulation in Calponin 1, α-SMA and collagens by myocardin-related transcription factors (MRTF) to induce differentiation into CAFs.88,97,98

Studies also suggested that pericytes,99,100 smooth muscle cells surrounding small border vessels can transdifferentiate into CAFs. Pericytes have been considered an essential source of myofibroblasts.101,102 The process starts with pericycle detachment from endothelial cells, followed by migration into the lung interstitium and then activation to become myofibroblast via TGF-β/Smad2/3 and PDGF/Erk signaling pathways.103-105 Here, the transforming growth factor-β receptor (TGF-βR) and platelet-derived growth factor-β receptor (PDGF-βR) are modified by core fucosylation (CF). α1,6-fucosyltransferase (FUT8) is the only known enzyme that catalyzes CF.106,107 In FUT8 knockdown cells, CF is out of function, and this inhibits TGF-β/Smad2/3 and PDGF/Erk signaling pathways.108 Some studies suggested that Sonic Hedgehog (Shh) is also involved in CAFs activation.109,110 Shh contributes to branching morphogenesis lung specification in the developing lung.111 In normal conditions, Hedgehog (Hh) activity is low. In the context of bleomycin injury, lung damage induces Hh pathway activity, and Shh overexpression increases fibrotic collagen deposition.112 In idiopathic pulmonary fibrosis, Hh activity can promote multiple profibrotic processes, including enhanced sensitivity to TGFβ and PDGF, leading to increased migration, contractility and survival in human lung fibroblasts.113,114

CAFs can also derive from epithelial cells (ECs).115 ECs differentiate into functional CAFs, which express FSP-1 and αFAP via TGF-β-mediated epithelial to mesenchymal transition (EMT).116,117 Endothelial cells contribute to the pool of CAFs through endothelial-to-mesenchymal transition (EndMT) in cancer, mainly via TGF-β and SMAD signaling.118,119 Several groups have also reported adipocyte conversion into CAFs. Mature adipocytes can activate the Wnt/β-catenin pathway, leading to adipocyte “dedifferentiation” to acquire a fibroblast-like morphology.99,120,122 It is suggested that using lineage tracing method with single cell spatial analysis can find out the main role and function of each cell type in tumor development, thus accounting for CAFs heterogeneity.123

3.2 | Subpopulation of CAFs in NSCLC and other cancers

Determination of subtypes of CAFs has met significant obstacles due to the heterogeneity of their origin, phenotype and function among different individuals in different tumor types. Based on different classification methods, there are different names for different subtypes of CAFs, as shown in Table 1 and Figure 1C. Different classifications have been proposed in relation to the different analysis approaches, for example the
In pancreatic cancer, the LD-CAFs are associated with tumor areas with lower cel-

The proposed classification of CAFs in breast, pancreatic and lung cancer

Clusters 5 and 7 were highly similar because of lower myogenesis and high mTOR expression signature. The differences between them were predominantly related to the expression of glycolysis genes, demonstrating metabolic differences between various CAF subsets. Su et al searched for cell-surface markers to identify clinically-important subtypes and found CD10\(^*\)GPR77\(^+\) lung CAFs. They are related to chemoresistance and poor survival in breast and lung cancer patients.\(^1\) Hu et al also found three functional subtypes identified by lung cancer therapeutic profiling.\(^2\)

### TABLE 1 The proposed classification of CAFs in breast, pancreatic and lung cancer

| Classification methods | Cancer type | Origin or function | Proposed CAF subtype | References |
|------------------------|-------------|--------------------|---------------------|------------|
| Spatial distributions  | Breast cancer | Originate from peripheral blood vessels | Vascular CAFs | 124 |
|                        |             | Originate from resident fibroblasts in local tissues | Matrix CAFs | |
|                        |             | Proliferation section of vascular CAFs | Cycling CAFs | |
|                        |             | Similar in phenotype to tumor epithelial cells | Developmental CAFs | |
| Biomarkers             | Breast cancer | Highly express basement membrane protein, Highly express products that promote tumor invasiveness | CD146\(^-\) CAFs | 125 |
|                        |             |                     | CD146\(^-\) CAFs | |
| Phenotypes             | Pancreatic cancer | Myofibroblastic phenotypes | myCAFs | 126 |
|                        |             | Inflammatory phenotypes | iCAFs | |
| Functions              | Pancreatic cancer | Epithelial-to-mesenchymal transition (EMT) | EMT-CAFs | 127 |
|                        |             | Proliferation | PRO-CAFs | |
| Histological features  | Lung cancer | High desmoplastic CAFs | HD-CAFs | 128 |
|                        |             | Low desmoplastic CAFs | LD-CAFs | |
| Single-cell RNA        | Lung cancer | A strong signature of EMT and clustering with tumor cells | Cluster 1 | 129 |
| sequencing technique   |             | A high level of \(\alpha\)-SMA and cocluster with pericytes | Cluster 2 | |
|                        |             | Enriched in the leading edge of the tumor | Cluster 4 | |
|                        |             | Lower myogenesis and high mTOR expression signature | Clusters 5 and 7 | |
| Cell-surface markers   | Lung cancer | Chemoresistance and poor survival | CD10\(^*\)GPR77\(^+\) CAFs | 130 |
| Therapeutic profiling  | Lung cancer | HGF\(^\text{High}\) and FGF7\(^\text{High/Low}\) | Subtype 1 | 131 |
|                        |             | HGF\(^\text{Low}\) and FGF7\(^\text{High}\) | Subtype 2 | |
|                        |             | HGF\(^\text{Low}\) and FGF7\(^\text{Low}\) | Subtype 3 | |

Note: Based on the characteristic and functional studies of CAFs, CAFs are divided into different subtypes and exert diverse phenotypes and functions. Importantly, the previous studies identified four main categories of lung CAFs which are characterized by microarray technology, single-cell RNA sequencing technique, cell-surface markers and therapeutic profiling.

immunophenotype, RNA expression profile and histologic findings. In breast cancer, based on spatial distribution, CAFs can be classified as vascular CAFs, matrix CAFs, cycling CAFs and developmental CAFs. These subtypes of CAFs have discrete gene expression profiles. The gene sets detected for vascular CAFs were related to vascular development and angiogenesis, while matrix-related genes dominated in matrix CAFs. Cycling CAFs are the proliferating section of vascular CAFs and are enriched for gene sets of the cell cycle. Lastly, differentiation-related genes were hallmarks of developmental CAFs.\(^1\)

In breast cancer, CAFs subtypes can also be defined by their biomarkers, such as CD146\(^-\) CAFs and CD146\(^-\) CAFs. Compared to CD146\(^-\) CAFs, CD146\(^-\) CAFs have higher metastasis and invasion ability and lead to a poorer prognosis.\(^2\) In pancreatic cancer, the subtypes of CAFs can be characterized by their phenotypes, namely the myofibroblastic phenotype (myCAFs) and inflammatory phenotype (iCAFs).\(^3\) MyCAFs are highly expressed in \(\alpha\)-SMA and located adjacent to cancer cells, while iCAFs secrete inflammatory mediators such as interleukin-6 (IL-6) and are located far away from cancer cells.\(^3\) Based on the heterogeneity features of CAFs, CAFs can also be divided into EMT(epithelial-to-mesenchymal transition)-CAFs and PRO (proliferative)-CAFs. These subtypes are correlated with activation of MAPK pathway and STAT3 pathway.\(^4\)

Meanwhile, there is no standard naming for lung CAFs and the above naming in different cancer types are not translatable to lung CAFs. In lung cancer, according to histological features, Hao et al discovered two CAF subtypes from 28 NSCLC patients characterized by proliferating CAFs, namely high desmoplastic CAFs (HD-CAF) and low desmoplastic CAFs (LD-CAF).\(^5\) HD-CAF showed a sharp rate of collagen matrix remodeling, invasion and tumor growth compared to LD-CAFs.\(^6\) LD-CAFs are associated with tumor areas with lower cellularity and less desmoplastic stromal reaction, and its predominance appears to portend a better prognosis than HDCAFs cases. Moreover, Lambrechts’ group used single-cell RNA sequencing technique to divide lung CAFs into Clusters 1, 2, 4, 5 and 7.\(^7\) For example, Clusters 1 and 4 were similar, but Cluster 1 showed a strong signature of EMT, an extensive repertoire of extracellular matrix proteins, and TGF-\(\beta\)-associated genes. Also, Cluster 1 was enriched within the tumor cells while Cluster 4 was enriched in the leading edge of the tumor. Cluster 2 exhibited a high level of \(\alpha\)-SMA and coclustered with pericytes.\(^8\) Clusters 5 and 7 were highly similar because of lower myogenesis and high mTOR expression signature. The differences between them were predominantly related to the expression of glycolysis genes, demonstrating metabolic differences between various CAF subsets. Su et al searched for cell-surface markers to identify clinically-important subtypes and found CD10\(^*\)GPR77\(^+\) lung CAFs. They are related to chemoresistance and poor survival in breast and lung cancer patients.\(^9\) Hu et al also found three functional subtypes identified by lung cancer therapeutic profiling.\(^10\) Subtypes 1 and
2 CAFs have high HFG and FGF7 expression, protecting lung cancer cells by chemoresistance. HGF is a MET ligand that mediates EGFR-inhibitor resistance via AKT and MAPK signaling. Subtype 3 CAFs have low HGF and FGF7 but express chemokines with chemoattractant properties for T lymphocytes and monocytes. Thus, Subtype 3 CAFs are associated with better clinical responses.

### 3.3 | The main molecular markers of CAFs

Due to the heterogeneity of CAFs, no marker can be used as a universal and specific marker for all CAFs in different types of cancers. In addition, there are different subsets of CAFs in the tumor, increasing the difficulty in defining the appropriate markers for CAFs. In lung cancer, the most used CAF markers include, but are not limited to, alpha-smooth muscle actin (α-SMA) and fibroblast activation protein-1 (FAP-1). The reported markers are summarized in Table 2, although none of these markers are CAF-specific, and can be expressed in other cells. Some highly expressed markers have been demonstrated to associate with advanced stages and unfavorable survival outcomes, such as FGFR1, FGF2, FAP, FSCN1 and LOXL1 (Figure 2).

α-SMA is widely considered as the most frequently used CAF marker. CAFs which show α-SMA expression have high collagen gel contractility (a measure of matrix remodeling capacity) and migration capacity compared to NFs. They are associated with a high tumor Ki-67 labeling index, lymph node metastasis, the poor 5-year overall survival rate of the patients, and aggressive biological behavior in NSCLC.

Fibroblast-activation protein (FAP), a cell-surface serine protease, is a promising drug target to inhibit CAFs. FAP-1 is selectively expressed by stromal mesenchymal cells and functions in

| TABLE 2 | The potential biomarkers of CAFs in lung cancer |
| --- |
| **Potential biomarker** | **Biological functions** | **Promoting roles in tumors** | **References** |
| αSMA | Cell contractility, structure and integrity | Tumor proliferation, immunosuppressive and impeding drug delivery | 138-140 |
| FAP-1 | ECM remodeling, fibrogenesis, serine protease activity | Tumor progression and metastasis and shaping the immunosuppressive TME | 141,142 |
| FGFs/FGFRs | Cell proliferation, migration, differentiation and angiogenesis | Tumorigenesis | 143 |
| PDGFRβ | Receptor tyrosine kinase activity | Immunomodulation, M2 polarization and angiogenesis | 142 |
| LOXL1 | Elastin, homeostasis and matrix remodeling during injury, fibrosis and cancer development | Tumorigenesis | 144 |
| VCAM1 | Endothelial cell adhesion, leukocytes and mediates adhesion, signal transduction and immune responses | Growth and invasion | 145 |
| Podoplanin | Cell migration and adhesion, a specific marker of lymphatic endothelium and lymph angiogenesis | Resistance to EGFR-TKIs, invasion, tumorigenesis and metastasis | 146-152 |
| Vimentin | Cell motility, structure and integrity | Metastasis and invasion | 140,153 |
| GFPT2 | Controls the flux of glucose into the hexosamine pathway | Metabolic reprogramming | 154 |
| MMP-2 | Degradation of ECM proteins and glycoprotein | Angiogenesis, tumor invasion and cell mobility | 155,156 |
| CD99 | Cell adhesion, migration, death, differentiation and inflammation | Migration, invasion and metastasis | 157 |
| CD34 | Cell-cell adhesion factor | Tumor vascularization | 158 |
| CD10 *GPR77* | Inflammatory and enzymatic functions | Tumor formation and tumor chemosensitivity [https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chemosensitivity](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chemosensitivity) | 130 |
| CD200 | Promote the protection of neurons | Promoting cancer formation and chemoresistance | 159 |
| Fascin | Regulators of the cytoskeleton | Epithelial-to-mesenchymal transition and cellular invasion | 160 |

Note: The CAF markers in lung cancer are divided into growth factors, cytokines, ligands of immune cells, components in the extracellular matrix and other soluble factors. Each CAF marker exerts its biological and protumorigenic functions. Abbreviations: FAP-1, Fas-associated phosphatase 1; FGF, fibroblast growth factors; GFPT2, GFPT2; LOXL1, lysyl oxidase-like 1; MMP-2, matrix metalloproteinase-2; VCAM1, vascular cell adhesion molecule-1; αSMA, alpha-smooth muscle actin.
FIGURE 2   Legend on next page.
wound healing, fibrotic reactions, inflammatory conditions and tumor development. Several studies reported that FAP-1 positive CAFs exerts immunoadjuvant roles in NSCLC and FAP-1 is considered as a molecular target in anti-CAFs therapy. For example, a phase I dose-escalation study with sibrotuzumab, an antibody to human FAP, in patients with advanced NSCLC, showed that sibrotuzumab explicitly binds to the tumor sites without apparent side effects.

4 | THE PROMOTING ROLE OF CAFs ON CANCER CELLS

CAFs play a pivotal role in tumorigenesis and are involved in different oncogenic pathways (Figure 3). Increasing evidence has supported the protumorigenic roles of CAFs, which are summarized in Table 3.

4.1 | Proliferation and growth

In two-dimensional and three-dimensional (3D) coculture models with lung CAFs, cancer cells grow faster than without coculture. CAFs produce cytokines and growth factors that promote tumor proliferation in lung cancer cells in autocrine and paracrine manners, such as C-X-C motif chemokine ligands including CXCL8, CXCL2, CXCL12, TGF-β, and PDGF (Figure 3A). In addition, lung CAFs secrete Cardiotrophin-like cytokine factor 1 (CLCF1) and IL6, which stimulate the growth of cancer cells via the JAK/STAT signaling pathway. Li et al demonstrated that CAF-secreted IL-22 significantly enhanced the proliferation, migration and invasion of lung cancer cells via the...
| Proteins involved in CAFs | Associated pathways in cancer cells | Coculture model/drug used | References |
|--------------------------|-----------------------------------|--------------------------|------------|
| IL-6                     | JAK2/STAT3                        | CAFs/NFs: NSCLC clinical samples | 168,170    |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023) and SK-MES-1 (RRID:CVCL_0630) | In vivo    |
| IL-22                    | PI3K-Akt-mTOR, IL-6-IL-6R         | CAFs/NFs: NSCLC clinical samples | 171        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023) and NCI-H1650 (RRID:CVCL_1483) | In vivo    |
| CXCL12                   | ERK                               | CAFs/NFs: NSCLC clinical samples | 172        |
|                          |                                   | Cell line: NSCLC-derived neoplastic cell lines and A-549 (RRID:CVCL_0023) | In vivo    |
| CLCF1                    | Proposed: JAK-STAT and MAPK pathway | CAFs/NFs: Mouse fibroblast and lung adenocarcinomas clinical samples | 170        |
|                          |                                   | Cell line: LKR10 and LKR13 cells from Kras<sup>LAl</sup> mouse, LSZ2 cells were derived through xenograft passages from Kras<sup>SLG12D</sup> mice, A-549 (RRID:CVCL_0023) and NCI-H1299 (RRID:CVCL_0060) | In vivo    |
| VCAM-1                   | AKT and MAPK pathway              | CAFs/NFs: Lung cancer clinical samples | 145        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023) and NCI-H358 (RRID:CVCL_1559) | In vivo    |
| GGT5                     | N.A.                              | CAFs/NFs: LUAD clinical tissue | 173        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023) and ACC212102 (RRID:CVCL_D074) | In vivo    |
| TGF-β                    | TGF-β pathway                     | CAFs/NFs: Human fetal lung fibroblast 1 (HFL1) (RRID: CVCL_0298) | 174        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023) | In vivo    |
| HIF-1α                   | NF-kB signaling                   | CAFs/NFs: Mouse spontaneous LC model (TetO-EGFRL858R; CCSP-rtTA) and lung adenocarcinoma clinical tissues | 64         |
|                          |                                   | Cell line: LL/2 (LLC1) (RRID:CVCL_4358), MRC-5 (RRID:CVCL_0440), Mouse embryonic fibroblast (MEF) cells were isolated from C57BL/6J wild-type mice embryonic and A-549 (RRID:CVCL_0023) | In vivo    |
| FGF2                     | FGF/FGFR pathway                  | CAFs/NFs: WT and Fgf9-DT mice | 143        |
|                          |                                   | Cell line: TAMs and endothelial cells from Fgf9-DT mice | In vivo    |
| SDF-1                    | CXCR4-mediated signaling pathway which involved NF-κB and Bcl-xL | CAFs/NFs: Lung cancer clinical samples | 175        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023) and PLA-801D (RRID:CVCL_7110) | In vivo    |
| Fut8                     | EGFR signaling                    | CAFs/NFs: Lung adenocarcinoma clinical sample | 176        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023), NCI-H322 (RRID: CVCL_1556), human lung fibroblast (HLF) cells, MRC-5 (RRID:CVCL_0440) and HFL1 (RRID:CVCL_0298) | In vivo    |
| miR224                   | Inhibiting SIRT3/AMPK and activating mTOR/HIF-1α | CAFs/NFs: NSCLC clinical samples | 177        |
|                          |                                   | Cell line: A-549 (RRID:CVCL_0023), NCI-H1299 (RRID:CVCL_0060) and HUVEC-C (RRID:CVCL_2959) | In vivo    |
| p53                      | N.A.                              | CAFs/NFs: Lung cancer clinical samples | 178        |
|                          |                                   | Cell line: Calu-1 (RRID:CVCL_0608), NCI-H460 (RRID: CVCL_0459), NCI-H1299 (RRID:CVCL_0060) | In vivo    |

(Continues)
### Proteins involved in CAFs

**Table 3 (Continued)**

| Proteins involved in CAFs | Associated pathways in cancer cells | Coculture model/drug used | References |
|---------------------------|-----------------------------------|---------------------------|------------|
| FoxF1                     | Hedgehog signaling                | Cell line: Swiss 3 T3 (NIH 3 T3) (RRID:CVCL_0594), C3H/10 T1/2 clone 8 (RRID:CVCL_0190), A-549 (RRID:CVCL_0023), IMR-90 (RRID:CVCL_0347) and primary murine lung fibroblasts (MLFs) were isolated from the explant out-growth of lungs derived from wild-type or Foxf1 heterozygous mice. In vivo Conditioned medium collected from NIH 3 T3 (RRID:CVCL_0594) and Institute for Medical Research-90 (IMR-90) (RRID:CVCL_0347) | 179 |
| Migration, invasion, metastasis | Stimulation of EMT |  |
| IL-6                      | JAK2/STAT3 pathway, TGF-β pathway | Cell line: A-549 (RRID:CVCL_0023), NCI-H661 (RRID:CVCL_1577), SK-MES-1 (RRID:CVCL_0630) and NCI-H358 (RRID:CVCL_1559) In vivo | 168,180 |
| IL-22                     | PI3K-AKTmTOR pathway              | Cell line: A-549 (RRID:CVCL_0023) and NCI-H1650 (RRID:CVCL_1483) | 171 |
| Snail1 (transcription factor) | N.A.                             | Cell line: A-549 (RRID:CVCL_0023), NCI-H299 (RRID:CVCL_0060), SPC-A1 (HeLa derivative [endocervical adenocarcinoma], RRID:CVCL_6955) and LTEP-a2 (HeLa derivative [endocervical adenocarcinoma], RRID:CVCL_6929) | 181 |
| HGF                       | HGF/IGF-1/ANXA2 signaling         | Cell line: PC-9 (RRID:CVCL_B260) (del E746_A750) and HCC827 (RRID:CVCL_2063) (del E746_A750) | 182 |
| IGF-1                     | HGF/IGF-1/ANXA2 signaling         | Cell line: PC-9 (RRID:CVCL_B260) (del E746_A750) and HCC827 (RRID:CVCL_2063) (del E746_A750) | 182 |
| SRGN (a CD44-interacting factor) | CD44/NF-κB/claudin-1 (CLDN1) axis | Cell line: NCI-H299 (RRID:CVCL_0060), NCI-H322 (RRID:CVCL_1556), NCI-H358 (RRID:CVCL_1559), NCI-H23 (RRID:CVCL_1547), NCI-H460 (RRID:CVCL_0459) and A-549 (RRID:CVCL_0023) In vivo | 183 |
| PDGFBB                    | Inhibition of the PDGF-PDGFR signaling pathway | Cell line: A-549 (RRID:CVCL_0023) and PC-9 (RRID:CVCL_B260) and NCI-H1975 (RRID:CVCL_1511) | 184 |
| PDPPN                     | Rho-ROCK pathway                  | Cell line: A-549 (RRID:CVCL_0023) and PC-9 (RRID:CVCL_B260) | 185 |
| TIAM2                     | N.D.                              | Cell line: A-549 (RRID:CVCL_0023) and Medical Research Council cell strain-5 (MRC-5) (RRID:CVCL_0440) | 186 |
| Fascin                    | N.D.                              | Cell line: A-549 (RRID:CVCL_0023) and PC-9 (RRID:CVCL_B260) | 160 |
| HMGB1                     | TLR4/NF-κB pathway                | Cell line: A-549 (RRID:CVCL_0023) and NCI-H661 (RRID:CVCL_1577) | 187 |
| Gli1 (zinc finger transcription factor) | Hedgehog signaling               | Cell line: NCI-H358 (RRID:CVCL_1559) | 188 |
| SMAD3                     | N.A.                              | Cell line: NCI-H358 (RRID:CVCL_1559) | 188 |
| Proteins involved in CAFs | Associated pathways in cancer cells | Coculture model/drug used | References |
|--------------------------|-------------------------------------|---------------------------|------------|
| miR210                   | PTEN/PI3K/AKT pathway               | CAFs/NFs: Lung adenocarcinomas clinical samples Cell line: A-549 (RRID:CVCL_0023), NCI-H1975 (RRID:CVCL_1511) and Bronchial Epithelium transformed with Ad12-SV40 2B (BEAS-2B) (RRID:CVCL_0168) | 189        |
| miR224                   | SIRT3/AMPK/mTOR/HIF-1α axis         | CAFs/NFs: NSCLC clinical samples Cell line: A-549 (RRID:CVCL_0023) and NCI-H1299 (RRID:CVCL_0060) | 177        |
| TGF-β                    | TGF-β pathway                       | CAFs/NFs: Normal human lung fibroblasts (NHFL) Cell line: A-549 (RRID:CVCL_0023) and NCI-NCI-H358 (RRID:CVCL_1559) In vivo | 180        |
| ECM remodeling           |                                     |                           |            |
| Vimentin                 | N.A.                                | CAFs/NFs: Lung adenocarcinomas clinical samples Transgenic mouse model | 190        |
| p53                      | N.A.                                | CAFs/NFs: Lung cancer clinical samples Cell line: Calu-1 (RRID:CVCL_0608), NCI-H460 (RRID:CVCL_0459) and NCI-H1299 (RRID:CVCL_0060) In vivo | 178        |
| MMP1, 3, 10              | N.A.                                | CAFs/NFs: Lung cancer clinical samples Cell line: NCI-H460 (RRID:CVCL_0459) | 178        |
| Integrin α11 β1          | N.A.                                | CAFs/NFs: NSCLC clinical samples Cell line: NCI-H460SM, A-549 (RRID:CVCL_0023) and primary human lung cancer cells In vivo | 191        |
| ST8SIA2 gene             | N.A.                                | CAFs/NFs: NSCLC clinical samples Cell line: A-549 (RRID:CVCL_0023) | 128        |
| Angiogenesis             |                                     |                           |            |
| CCL2/VEGFA               | N.A.                                | CAFs/NFs: adenocarcinomas, squamous cell carcinomas and larger cell carcinomas clinical samples Cell line: A-549 (RRID:CVCL_0023) NCI-H460 (RRID:CVCL_0459) in vivo | 192        |
| | miR-1/|miR-206/|miR-31 | FOXO3a/VEGF/CCL2 | CAFs/NFs: adenocarcinomas, squamous cell carcinomas and larger cell carcinomas clinical samples Cell line: A-549 (RRID:CVCL_0023) and NCI-H460 (RRID:CVCL_0459) in vivo | 192        |
| VEGF                     | JAK2/STAT3 pathway                 | CAFs/NFs: NSCLC clinical samples Cell line: A-549 (RRID:CVCL_0023) and NCI-H461 (RRID:CVCL_1577) and SK-MES-1 (RRID:CVCL_0630) | 168        |
| bFGF                     | JAK2/STAT3 pathway                 | Cell line: A-549 (RRID:CVCL_0023) and NCI-H292 (RRID:CVCL_0455) | 193        |
| SDF4                     | ERK1/2 and p38 pathways            | CAFs/NFs: HFL1 (RRID:CVCL_0298) Cell line: HUVEC-C (RRID:CVCL_2959) | 194        |
| miR210                   | JAK2/STAT3 pathway                 | Cell line: NCI-H1975 (RRID:CVCL_1511), A-549 (RRID:CVCL_0023), Swiss-3 T3 (NIH 3 T3) (RRID:CVCL_0594) and Ms-1 (RRID:CVCL_IQ55) In vivo | 75         |
activation of PI3K-Akt-mTOR signaling. These findings suggested that the role of CAFs in activating the AKT signaling pathway is crucial for cancer cell proliferation.

Some studies have highlighted the involvement of Hh signaling in the activation, proliferation and invasion of CAFs and cancer cells. The biological function of Hh signaling and the associated Gli transcription factors (Gli 1-3) promote organogenesis and lung branching morphogenesis. Olga et al reported that inhibition of Hh signaling induced a significant decrease in the proliferation of NSCLC cells by modulating cyclin D expression. In addition, NSCLC cells secreted Shh and activated Hedgehog signaling in CAFs. Lung CAFs remodel the ECM and deposit collagen, promoting cancer cells invasion and proliferation.

In lung adenocarcinoma cells (LUAD), high expression of GGT5 in CAFs contributed to tumor cell proliferation and drug resistance by increasing intracellular glutathione and reducing the intracellular reactive oxygen species (ROS) level. For other cancers, increasing...
In our study, IL-6 induces overexpression of EMT-related genes. However, the mechanism and role of CAFs in stimulating invasion and metastasis through two main aspects, which include EMT and ECM remodeling (Figure 3B). CAFs induce EMT by secreting soluble factors. In lung cancer, CAF-secreted IL-6 induces EMT programming and modulate metastasis-related genes through the JAK2/STAT3 signaling pathway in vitro and in vivo. In our study, IL-6 induces overexpression of EMT-related genes and proteins, including vimentin, N-cadherin, MMP2, MMP9 and VEGF. CAF-secreted hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) induce annexin A2 (ANXA2) expression and phosphorylation through the c-Met pathway, resulting in EMT and EGFR tyrosine kinase inhibitors (EGFR-TKIs) resistance in NSCLC. Also, CAF-secreted complement 3a (C3a), a prominent tumor-promoting factor in TME, activates PI3K/Akt signaling. In breast cancer, the secretion of TGF-β, HGF and PDGF by cancer cells ultimately results in EMT remodeling, invasion and metastasis.

Recent studies reported that miRNAs secreted from CAFs are involved in metastasis, such as the exosomal miR-210. It promotes EMT through targeting UPF1 to activate the PTEN/PI3K/AKT pathway in NSCLC. Besides the above signaling pathways, CAFs secrete high mobility group box 1 (HMGB1) to promote EMT through NF-κB signaling. HMGB participates in multiple cellular processes such as invasion and angiogenesis.

CAFs facilitate local invasion and metastasis of the cancer cells by biomechanically remodeling the ECM. Thus, it may be said that cancer cells invade the matrix by following the footpath of the CAFs. CAFs synthesize structural proteins like collagen type I and IV, heparan sulfate proteoglycans, tenasin-C, secrete connective tissue growth factors and produce digestive factors such as MMPs and plasminogen activators. In particular, MMPs are ECM-degrading proteases, participate in tumorigenesis and activate the inflammatory cytokines. They are categorized into different functional subtypes and have multiple functions in the tumor stroma, including tissue invasion and intravasation, angiogenesis, regulation of inflammation and preparation of the metastatic niche. In collagen invasion assay, podoplanin (PDPN)-expressing CAFs invade the collagen matrix, and then cancer cells invade within the footpaths created by CAFs. PDPN-positive CAFs can be commonly found in clinical samples of lung adenocarcinoma and are also related to poor survival.

### 4.2 Invasion and metastasis

CAFs are a vital component in the TME and can act as a bridge between the TME and cancer cells. CAFs facilitate cancer cell crosstalk within the TME. CAFs stimulate invasion and metastasis through two main aspects, which include EMT and ECM remodeling (Figure 3B). CAFs induce EMT by secreting soluble factors. In lung cancer, CAF-secreted IL-6 induces EMT programming and modulate metastasis-related genes through the JAK2/STAT3 signaling pathway in vitro and in vivo. In our study, IL-6 induces overexpression of EMT-related genes and proteins, including vimentin, N-cadherin, MMP2, MMP9 and VEGF. CAF-secreted hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) induce annexin A2 (ANXA2) expression and phosphorylation through the c-Met pathway, resulting in EMT and EGFR tyrosine kinase inhibitors (EGFR-TKIs) resistance in NSCLC. Also, CAF-secreted complement 3a (C3a), a prominent tumor-promoting factor in TME, activates PI3K/Akt signaling. In breast cancer, the secretion of TGF-β, HGF and PDGF by cancer cells ultimately results in EMT remodeling, invasion and metastasis.

Recent studies reported that miRNAs secreted from CAFs are involved in metastasis, such as the exosomal miR-210. It promotes EMT through targeting UPF1 to activate the PTEN/PI3K/AKT pathway in NSCLC. Besides the above signaling pathways, CAFs secrete high mobility group box 1 (HMGB1) to promote EMT through NF-κB signaling. HMGB participates in multiple cellular processes such as invasion and angiogenesis.

CAFs facilitate local invasion and metastasis of the cancer cells by biomechanically remodeling the ECM. Thus, it may be said that cancer cells invade the matrix by following the footpath of the CAFs. CAFs synthesize structural proteins like collagen type I and IV, heparan sulfate proteoglycans, tenasin-C, secrete connective tissue growth factors and produce digestive factors such as MMPs and plasminogen activators. In particular, MMPs are ECM-degrading proteases, participate in tumorigenesis and activate the inflammatory cytokines. They are categorized into different functional subtypes and have multiple functions in the tumor stroma, including tissue invasion and intravasation, angiogenesis, regulation of inflammation and preparation of the metastatic niche. In collagen invasion assay, podoplanin (PDPN)-expressing CAFs invade the collagen matrix, and then cancer cells invade within the footpaths created by CAFs. PDPN-positive CAFs can be commonly found in clinical samples of lung adenocarcinoma and are also related to poor survival.

### 4.4 Immune escaping

To achieve immune evasion, CAFs are involved in shaping the immunosuppressive TME (Figure 3D). However, the mechanism and crosstalk between the CAFs and immune cells are still to be fully elucidated. Using The Cancer Genome Atlas Lung Squamous Cell Carcinoma database, several genes are highly expressed in cases with PDLPN-expressing CAFs, including interleukin (IL)-1A, IL-1B, IL-6, IL-10, CCL2, colony-stimulating factor 1 (CSF1), FGF2, galectin 1, PDGFA, PDGFB and TGF-β1. Among them, TGF-β1 is a well-known cytokine that participated in M2 macrophage polarization and immunosuppression. In addition, PDLPN-expressing CAFs are associated with a high number of CD204+ TAMs, and a low ratio of CD8+ T cells and FOX3+ T cells in immunohistochemical staining of lung adenocarcinoma specimens, suggesting that PDLPN-expressing CAFs help cancer cells escape host immunosurveillance. CAFs secrete monocytic- and neutrophil-attracting chemokines and cytokines such as CCL2, CCL7, CXCL1, CXCL5, CXCL8, MIF, IL6 and VEGF in a 3D-transwell system. MIF, IL6 and VEGF have been suggested to promote MDSC differentiation. Significantly, CAF-secreted CCL2 induces CCR2+CD14+ monocyte migration in chemotaxis assay and thus promotes monocyte differentiation into monocytic MDSCs. CAF-induced MDSCs inhibit the IFNγ production of CD8+ T cells, thus suppressing the proliferation of CD8+ T cells. At the same time, they express NADPH oxidase-2 (NOX2), which generates ROS to promote immunosuppression in lung cancer cells.

To promote an immunosuppressive environment, CAFs diminish the antitumorigenic activity of natural killer cells (NK cells). To escape the attack from the immune cell, CAFs also modulate the immune checkpoints such as the programmed cell death protein 1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1). PD-1/PD-L1 pathway suppresses the antitumor immune activity of T cells. CAF secreted cytokines such as IL-8, osteoprotegerin (OPG) and CXCL2 can increase the expression of PD-L1 in lung adenocarcinoma cells. More interestingly, CAFs express inhibitory ligands, including PD-L1 and PD-L2, which inhibit the

### 4.3 Angiogenesis

Angiogenesis plays an important role in tumor growth and metastasis. The process requires several regulatory molecules such as VEGF receptors (VEGFR), bFGF, type I collagen and fibronectin. CAFs express these regulatory molecules to initiate angiogenesis (Figure 3C). There is also evidence that in NSCLC, when nonsmall-cell lung cancer tumor cells are cocultured with fibroblasts, gene expressions related to tumor angiogenesis, ECM degradation, cell growth and survival are enhanced in the tumor cells.

Other studies have shown that myofibroblast transformation can be induced by cisplatin and 5-fluorouracil treatment through CCAAT/enhancer-binding protein delta (CEBPΔD), thereby promoting proliferation, migration in vascular endothelial cells and angiogenesis in NSCLC. CEBPΔD elevates SDF4 (a C-X-C chemokine) expression in CAFs in response to cisplatin and 5-fluorouracil treatment in HFL1 cells. SDF4 is secreted and directly interacts with CXCR4 to induce vascular endothelial growth factor D (VEGF-D) expression for angiogenesis via ERK1/2 and p38 pathways in endothelial cells.
CD8^+ T cells in NSCLC. Apart from that, CD39^+ T cells highly colocalized with FAP^+ CAFs in NSCLC. These T cells and CAFs may cooperate in mediating immune escape: Activated T cells upregulate the expression of MHC, coinhibitory ligands PD-L1 and PD-L2, and CD73 on CAFs, increase production of IL-6 and initiate production of IL-27. On the other hand, CAFs enhance the level of coinhibitory receptors PD-1, Tim3, LAG-3 and CD39 on T cells, resulting in their transformation into tumor infiltrating T cells, and leading T-cell apoptosis in NSCLC.252 The above studies suggested that CAFs play a critical role in immune checkpoint biology. However, the interplay between CAFs and cancer cells remains elusive and insufficiently delineated.

5 THE ROLE OF CAFs IN CHEMORESISTANCE

The two primary mechanisms used by CAFs to help cancer cells evade therapy have been demonstrated, including the physical barrier method and interplay of CAFs and lung cancer (Figure 3E).

5.1 The physical barrier formed by CAFs

ECM can become rigid and acts as a barrier to protect the tumor cell from chemotherapy.253 ECM stiffness is characterized by an aggregation of ECM proteins with hyaluronic acid (HA) at the core. CAFs enhance the expression of ECM proteins such as collagen, HA and fibronectin. Collagen and fibronectin provide resistance to tensile stress in the periphery of tumor cells.254-256 On the one hand, CAF-expressed integrins α11β1 is a collagen-binding receptor and increases ECM stiffness in NSCLC.191 A semisolid Matrigel-embedded cell culture system provides a clear picture of how ECM stiffness induces chemoresistance. Lung cancer cells line A549 cells within the semisolid Matrigel matrix are arrested in the G0/G1 cell cycle, with decreased cell proliferation and invasion.257

5.2 The ligand-receptor pathways between CAFs and cancer cells

CAF-secreted stromal cell-derived factor 1 (SDF-1) enhances the chemoresistance of lung cancer cells to cisplatin by suppressing CXCR4 expression, suggesting CAFs facilitate drug resistance via the CXCR4-mediated signaling pathway.175 Notably, the increased SDF-1 was caused by a downregulation of miR-1 which is a tumor-suppressor microRNA and is required for transforming NFs to CAFs.258 On the other hand, CAFs express C-C motif chemokine ligand 5 (CCL5) and inhibit the cisplatin-induced apoptosis in NSCLC cells. CCL5 enhances the expression level of long noncoding RNA (IncRNA) HOX transcript antisense RNA (HOTAIR), which inhibits tumor cell apoptosis259,260 via the caspase-3/BCL-2 signaling pathway.199

Activating EGFR mutations are common in lung cancers and can be treated by EGFR-TKIs such as erlotinib and gefitinib. Unfortunately, most patients develop drug resistance to EGFR-TKI. One possible explanation is that CAFs can induce EGFR-TKI resistance. Choe et al demonstrated that coculture with CAFs induces erlotinib resistance in lung cancer cells via 7-transmembrane protein smoothened (SMO) mediated Hh signaling. Besides, CAF-secreted IL-6 induces drug resistance by promoting EMT and acquiring stemness of lung cancer cells. Using a cancer tissue-originated spheroid experiment, CAF-secreted IL-6 and TGF-β contribute to tumor progression, the acquisition of stemness and drug resistance.57

6 CONCLUSION AND FUTURE PERSPECTIVES

The research about CAFs remains going on. Recently, tumor organoid studies have become popular since it is described as “cancer surro-gates” that mimic the tumor’s biological characteristic.261 An organoid derived from the patients’ tumor tissue seed within the Matrigel culture system of 3D cell culture technique in vitro.126,262-268 The Matrigel combinational culture system can simulate the ECM environment, yet it still has some limitations. The Matrigel was different from the composition of the ECM. Thus, it may not exert the entire functions and properties of fibroblasts. Moreover, the system does not contain all cell populations, such as immune cells. Thus, it is hard to investigate the crosstalk of CAFs with immune cells. Different culture systems will be developed for the deep investigation of CAFs.

Due to the heterogeneity of CAFs from the molecular aspect, several scientific and technical concerns about CAFs remain to be addressed. First, the relationship and function between CAFs at the metastatic and primary sites remains unresolved. It is unclear if primary CAFs may migrate to the metastatic site or NFs at the metastatic site are transformed to CAFs by similar cytokines produced at the primary site. Second, the classification and subtypes of CAFs are not well-defined clearly in different cancer types. Third, it is well documented that CAFs promote tumorigenesis by remodeling the cancer cells, while how CAFs communicate with other microenvironmental components has not been clearly elucidated. Further investigations will be performed to unravel the cell-cell chat between CAFs and endothelial, myeloid, T, B cells based on ligand-receptor pathways. Fourth, it is urgent to develop novel research platforms for the investigation of CAFs. Apart from single-cell RNA sequencing for the expression profiling analysis of each CAF, it also needs to develop the DNA sequencing technique in single-cell resolution to investigate the copy number changes and mutation spectrum in CAFs.

CAFs provide a tumor-friendly microenvironment for cancer cells and reshape their biological behaviors by cytokine secretion, ECM modification and EMT reprogramming. In this review, we summarized the molecular mechanisms and clinical significance of CAFs in NSCLC. Hopefully, the future work will shed light on developing novel therapeutic approaches by accurately targeting CAFs based on the recognized molecular mechanisms.
AUTHOR CONTRIBUTIONS
Ka Fai To conceived the project, provided direction and guidance on the whole project. Kit Yee Wong and Alvin Ho-Kwan Cheung drafted the manuscript. Bonan Chen and Wai Nok Chan analyzed the data and interpreted the results. Ka Fai To, Wei Kang, Kwok Wai Lo and Jun Yu reviewed the manuscript and made significant revisions. The final manuscript has been approved by all authors. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

ACKNOWLEDGMENTS
The figures in the manuscript were partly generated from BioRender (https://biorender.com/). We acknowledge the TCGA Research Network (http://cancergenome.nih.gov/) for providing the datasets and analysis. We also acknowledge the technical support from Core Utilities of Cancer Genomics and Pathobiology of the Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

ORCID
Alvin Ho-Kwan Cheung https://orcid.org/0000-0002-3913-8117
Wei Kang https://orcid.org/0000-0002-4651-677X

REFERENCES
1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-249.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71:7-33.
3. Inamura K. Lung cancer: understanding its molecular pathology and the 2015 WHO classification. Front Oncol. 2017;7:193.
4. Stünzi H, Head K, Nielsen S. Tumours of the lung. Bull World Health Organ. 1974;50:9-19.
5. Tsoulos N, Papadopoulou E, Metaxa-Mariatou V, et al. Tumor molecular profiling of NSCLC patients using next generation sequencing. Oncol Rep. 2017;38:3419-3429.
6. Rotow J, Bivona TG. Understanding and targeting resistance mechanisms in NSCLC. Nat Rev Cancer. 2017;17:637-658.
7. Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic signaling pathways in the cancer genome atlas. Cell. 2018;173:321-327.e10.
8. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21:309-322.
9. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-674.
10. Hui L, Chen Y. Tumor microenvironment: sanctuary of the devil. Cancer Lett. 2015;368:7-13.
11. Paget S. The distribution of secondary growths in cancer of the breast. Lancet. 1889;133:571-573.
12. Chen F, Zhuang X, Lin L, et al. New horizons in tumor microenvironment biology: challenges and opportunities. BMC Med. 2015;13:1-14.
13. Wang M, Zhao J, Zhang L, et al. Role of tumor microenvironment in tumorigenesis. J Cancer. 2017;8:761-773.
14. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6:392-401.
15. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002;3:349-363.
16. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16:582-598.
17. Öhlund D, Elyada E, Tuveson D. Fibroblast heterogeneity in the cancer wound. J Exp Med. 2014;211:1503-1523.
18. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. Cancer Cell. 2016;30:836-848.
19. Maacha S, Bhat AA, Jimenez L, et al. Extracellular vesicles-mediated intercellular communication: roles in the tumor microenvironment and anti-cancer drug resistance. Mol Cancer. 2019;18:1-16.
20. Luga V, Zhang L, Viloria-Petit AM, et al. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell. 2012;151:1542-1556.
21. Webber JP, Spary LK, Sanders AJ, et al. Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes. Oncogene. 2015;34:290-302.
22. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. Oncogene. 2017;36:1770-1778.
23. Giusti I, Di Francesco M, D’Ascenzo S, et al. Ovarian cancer-derived extracellular vesicles affect normal human fibroblast behavior. Cancer Biol Ther. 2018;19:722-734.
24. Giusti I, Di Francesco M, Poppa G, Esposito L, D’Ascenzo S, Dolo V. Tumor-derived extracellular vesicles activate normal human fibroblasts to a cancer-associated fibroblast-like phenotype, sustaining a pro-tumorigenic microenvironment. Front Oncol. 2022;12:839880.
25. Fang T, Lv H, Lv G, et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nat Commun. 2018;9:1-13.
26. Dvorak HF. Tumors: wounds that do not heal. New Engl J Med. 1986;315:1650-1659.
27. Sahai E, Asatsurov I, Cukierman E, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20:174-186.
28. Ping Q, Yan R, Cheng X, et al. Cancer-associated fibroblasts: overview, progress, challenges, and directions. Cancer Gene Ther. 2021;28:1-16.
29. Wang Z, Yang Q, Tan Y, et al. Cancer-associated fibroblasts suppress cancer development: the other side of the coin. Front Cell Dev Biol. 2021;9:146.
30. Louault K, Li R-R, DeClerck YA. Cancer-associated fibroblasts: understanding their heterogeneity. Cancers. 2020;12:3108.
31. Gnieiec KA, Butler LM, Worthley DL, Woods SL. Cancer-associated fibroblasts—heroes or villains? Br J Cancer. 2019;121:293-302.
32. Gabbiani G, Ryan G, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971;27:549-550.
33. Rodemann HP, Müller GA. Characterization of human renal fibroblasts in health and disease: II. In vitro growth, differentiation, and collagen synthesis of fibroblasts from kidneys with interstitial fibrosis. Am J Kidney Dis. 1991;17:684-686.
34. Simian M, Hirai Y, Navre M, Werb Z, Lochter A, Bissell MJ. The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. Development. 2001;128:3117-3131.
35. Fukumura D, Xavier R, Sugiura T, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell. 1998;94:715-725.
36. Rockey DC, Weymouth N, Shi Z. Smooth muscle α Actin (Acta2) and myofibroblast function during hepatic wound healing. PLoS One. 2013;8:e77166.
37. Wiseman BS, Werb Z. Stromal effects on mammary gland development and breast cancer. Science. 2002;296:1046-1049.
38. Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granuloma tissue and scar. *Am J Pathol.* 1995;146:56-66.
39. Eyden B, Banerjee SS, Shenjere P, Fisher C. The myofibroblast and its tumours. *J Clin Pathol.* 2009;62:236-249.
40. Bierie B, Moses HL. TGFβ: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer.* 2006;6:506-520.
41. Saito A, Horie M, Nagase T. TGFβ signaling in lung health and disease. *Int J Mol Sci.* 2018;19:2460.
42. Liu S, Chen S, Zeng J. TGFβ signaling: a complex role in tumorigenesis. *Mol Med Rep.* 2018;17:699-704.
43. Pickup M,Novitskiy S, Moses HL. The roles of TGFβ in the tumour microenvironment. *Nat Rev Cancer.* 2013;13:788-799.
44. Navab R, Strumpf D, Bandarchi B, et al. Prognostic gene-expression signature of carcinoma-associated fibroblasts in non-small cell lung cancer. *Proc Natl Acad Sci.* 2011;108:7160-7165.
45. Heldin C-H, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev.* 1999;79:1283-1316.
46. Elbenbaas B, Weinberg RA. Heterotypic signaling between epithelial tumor cells and fibroblasts in carcinoma formation. *Exp Cell Res.* 2001;264:169-184.
47. Bronzert D, Pantazis P, Antoniades H, et al. Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines. *Proc Natl Acad Sci.* 1987;84:5763-5767.
48. Dong J, Grunstein J, Tejada M, et al. VEGF-null cells require PDGFRα signaling-mediated stromal fibroblast recruitment for tumorigenesis. *EMBO J.* 2004;23:2800-2810.
49. Cadamuro M, Nardo G, Indraccolo S, et al. Platelet-derived growth factor-D and Rho GTPases regulate recruitment of cancer-associated fibroblasts in cholangiocarcinoma. *Hepatology.* 2013;58:1042-1053.
50. Si H, Lv X, Guo A, Jiang H, Li J. Suppressive effect of leflunomide on rat hepatic stellate cell proliferation involves on PDGF-BB-elicited activation of three mitogen-activated protein kinas. *Cytokine.* 2008;42:24-31.
51. Shao Z-M, Nguyen M, Barsky SH. Human breast carcinoma desmoplasia is PDGF initiated. *OncoGene.* 2000;19:4337-4345.
52. Auguste P, Lemiere S, Larrieu-Lahargue F, Bikfalvi A. Molecular mechanisms of tumor vascularization. *Crit Rev Oncol Hematol.* 2005;54:53-61.
53. Sinz IZ, Zeisberg M, Hemberlein B, et al. Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. *Kidney Int.* 2000;57:1521-1538.
54. Burgess WH, Maclag T. The heparin-binding (fibroblast) growth factor family of proteins. *Annu Rev Biochem.* 1989;58:575-602.
55. Marrual A, Ojeda L, Paz-Ares I, Molina-Pinelo S, Ferrer I. Proteomic-based approaches for the study of cytokines in lung cancer. *Dis Markers.* 2016;2016:1-12.
56. Albregen J, Bourget I, Pons C, et al. ILF mediates proinvasive activation of stromal fibroblasts in cancer. *Cell Rep.* 2014;7:1664-1678.
57. Shinan Y, Fujikawa A, Kimura T, et al. IL-6 secreted from cancer-associated fibroblasts mediates chemoresistance in NSCLC by increasing epithelial-mesenchymal transition signaling. *J Thorac Oncol.* 2016;11:1482-1492.
58. O’Donoghue R, Knight DA, Richards CD, et al. Genetic partitioning of interleukin-6 signalling in mice dissociates Stat3 from Smad3-mediated lung fibrosis. *EMBO Mol Med.* 2012;4:939-951.
59. Le Q-T, Chen E, Salim A, et al. An evaluation of tumor oxygenation and gene expression in patients with early stage non-small cell lung cancers. *Clin Cancer Res.* 2006;12:1507-1514.
60. Huang Y, Lin D, Taniguchi CM. Hypoxia inducible factor (HIF) in the tumor microenvironment: friend or foe? *Sci China Life Sci.* 2017;60:1114-1124.
61. Kim J-w, Evans C, Weidemann A, et al. Loss of fibroblast HIF-1α accelerates tumorigenesis. *Cancer Res.* 2012;72:3187-3195.
62. Doedens AL, Stockmann C, Rubinstein MP, et al. Macrophage expression of hypoxia-inducible factor-1α suppresses T-cell function and promotes tumor progression. *Cancer Res.* 2010;70:7465-7475.
63. Liu Y, Song X, Wang X, et al. Effect of chronic intermittent hypoxia on biological behavior and hypoxia-associated gene expression in lung cancer cells. *J Cell Biochem.* 2010;111:554-563.
64. Zhang Y, Bian Y, Wang Y, et al. HIF-1α is necessary for activation and tumour-promotion effect of cancer-associated fibroblasts in lung cancer. *J Cell Mol Med.* 2021;25:5457-5469.
65. Komatsu M, Kurokawa H, Waguri S, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol.* 2010;12:213-223.
66. Kang Ji, Kim DH, Sung KW, et al. PDGF-induced cancer-associated fibroblast activation via the Nrf2-ATF6 pathway promotes lung tumorigenesis. *Cancer.* 2021;13:864.
67. Hiebert P, Wietecha MS, Cangkrama M, et al. Nrf2-mediated fibroblast reprogramming drives cellular senescence by targeting the matrisome. *Dev Cell.* 2018;46:145-161.e10.
68. Rusek AM, Abba M, Eljaszewicz A, Moniuszko M, Niklinski J, Allgayer H. MicroRNA modulators of epigenetic regulation, the tumor microenvironment and the immune system in lung cancer. *Mol Cancer.* 2015;14:1-10.
69. Du H, Che G. Genetic alterations and epigenetic alterations of cancer-associated fibroblasts. *Oncol Lett.* 2017;13:3-12.
70. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004;116:281-297.
71. Reddy KB. MicroRNA (miRNA) in cancer. *Cancer Cell Int.* 2015;15:1-6.
72. Lin P, Yu S, Yang P. MicroRNA in lung cancer. *Br J Cancer.* 2010;103:1144-1148.
73. Cummins J, Velculescu V. Implications of micro-RNA profiling for cancer diagnosis. *Oncogene.* 2006;25:6220-6227.
74. Lee S, Hong JH, Kim JS, et al. Cancer-associated fibroblasts activated by miR-196a promote the migration and invasion of lung cancer cells. *Cancer Lett.* 2021;508:92-103.
75. Fan J, Xu G, Chang Z, Zhi L, Yao J. miR-210 transferred by lung cancer cell-derived exosomes may act as proangiogenic factor in cancer-associated fibroblasts by modulating JAK2/STAT3 pathway. *Clin Sci.* 2020;134:807-925.
76. Kunita A, Morita S, Irisa TU, et al. MicroRNA-21 in cancer-associated fibroblasts supports lung adenocarcinoma progression. *Sci Rep.* 2018;8:1-14.
77. Pottier N, Maurin T, Chevalier B, et al. Identification of keratinocyte growth factor as a target of miRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. *PLoS One.* 2009;4:e6718.
78. Huleihel L, Ben-Yehudah A, Milosevic J, et al. Let-7d miRNA affects mesenchymal phenotypic properties of lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol.* 2014;306:L534-L542.
79. Albregen J, Bertero T, Grasset E, et al. Epigenetic switch drives the conversion of fibroblasts into proinvasive cancer-associated fibroblasts. *Nat Commun.* 2015;6:1-15.
80. Ren Y, H-h J, Xu Y-q, et al. Paracrine and epigenetic control of CAF-induced metastasis: the role of HOTAIR stimulated by TGF-ss1 secretion. *Mol Cancer.* 2018;17:1-14.
81. Yang X, Li Y, Zou L, Zhu Z. Role of exosomes in crosstalk between cancer-associated fibroblasts and cancer cells. *Front Oncol.* 2019;9:356.
82. Yang F, Ning Z, Ma L, et al. Exosomal miRNAs and miRNA dysregulation in cancer-associated fibroblasts. *Mol Cancer.* 2017;16:1-10.
83. Ding L, Ren J, Zhang D, et al. A novel stromal lncRNA signature of carcinoma-associated fibroblasts in non-small cell lung carcinoma via LncRNA-CAF/interleukin-33. *Carcinogenesis.* 2018;39:397-406.
84. Webber J, Steadman R, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. *Cancer Res.* 2010;70:9621-9630.
85. Skog J, Würdinger T, Van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol. 2008;10:1470-1476.

86. Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie. 2007;89:205-212.

87. Zhang J, Fu B, Li M, Mi S. Secretome of activated fibroblasts induced by exosomes for the discovery of biomarkers in non-small cell lung cancer. Small. 2021;17:2004750.

88. Chen P-Y, Wei W-F, Wu H-Z, Fan L-S, Wang W. Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. J Hematol Oncol. 2019;12:1-15.

89. Arina A, Ide C, Hyjek EM, et al. Tumor-associated fibroblasts predominantly come from local and not circulating precursors. Proc Natl Acad Sci. 2016;113:7551-7556.

90. Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest. 2007;117:50-59.

91. Barcellos-de-Souza P, Comito G, Pons-Segura C, et al. Mesenchymal stem cells are recruited and activated into carcinoma-associated fibroblasts by prostate cancer microenvironment-derived TGF-β1,6-fucosyltransferase, FUT8. Am J Pathol. 2012;172:5198-5208.

92. Iwano M, Kugler MC, Loomis CA, et al. Hedgehog signaling in neonatal and adult lung. Am J Respir Cell Mol Biol. 2013;48:703-710.

93. Cigna N, Moshtai EF, Brayer S, et al. The hedgehog system machinery controls transforming growth factor-β-dependent myofibroblastic differentiation in humans: involvement in idiopathic pulmonary fibrosis. Am J Pathol. 2012;181:2126-2137.

94. Barolasi AL, Milla CM, Lira JC, et al. Role of sonic hedgehog in idio-pathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2012;309:L197-L206.

95. Kahounová Z, Kurfürstová D, Bouchal J, et al. The fibroblast surface markers FAP, anti-fibroblast, and FSP are expressed by cells of epithelial origin and may be altered during epithelial-to-mesenchymal transition. J Physiol Pharmacol. 2013;64:293-306.

96. Brechbuhl HM, Barrett AS, Kopin E, et al. Fibroblast subtypes define a functionally distinct stromal cell population in breast cancer. Physiol Rev. 2011;91:755-766.

97. Ihara H, Ikeda Y, Toma S, et al. Crystal structure of mammalian α,1,6-fucosyltransferase, FUT8. Glycobiology. 2007;17:455-466.

98. Sasaki H, Toda T, Furukawa T, et al. α,1,6-Fucosyltransferase (FUT8) inhibits hemoglobin production during differentiation of murine and K562 human erythroleukemia cells. J Biol Chem. 2013;288:16839-16847.

99. Sun W, Tang H, Gao L, et al. Mechanisms of pulmonary fibrosis induced by core fucosylation in pericytes. Int J Biochem Cell Biol. 2017;88:44-54.

100. Walter K, Omura N, Hong S-M, et al. Overexpression of smooth-ened activates the sonic hedgehog signaling pathway in pancreatic cancer-associated fibroblasts. Clin Cancer Res. 2010;16:1781-1789.

101. Wei R, Lv M, Li F, et al. Human CAFs promote lymphangioegenesis in ovarian cancer via the Hh-VEGF-C signaling axis. Oncotarget. 2017;8:67315-67328.

102. Popiceli CV, Lewis PM, McMahon AP. Sonic hedgehog regulates branching morphogenesis in the mammary gland. Curr Biol. 1998;8:1083-1086.

103. Liu L, Kugler MC, Loomis CA, et al. Hedgehog signaling in neonatal and adult lung. Am J Respir Cell Mol Biol. 2013;48:703-710.

104. Ihara H, Ikeda Y, Toma S, et al. Crystal structure of mammalian α,1,6-fucosyltransferase, FUT8. Glycobiology. 2007;17:455-466.

105. Chen P-Y, Wei W-F, Wu H-Z, Fan L-S, Wang W. Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. J Hematol Oncol. 2019;12:1-15.

106. Arina A, Ide C, Hyjek EM, et al. Tumor-associated fibroblasts predominantly come from local and not circulating precursors. Proc Natl Acad Sci. 2016;113:7551-7556.
126. Öhlund D, Handly-Santana A, Biffi G, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med. 2017;214:579-596.

127. Ligorio M, Sil S, Malagon-Lopez J, et al. Stromal microenvironment shapes the intratumoral architecture of pancreatic cancer. Cell. 2019;178:160-175.e27.

128. Hao J, Zeltz C, Pintiile M, et al. Characterization of distinct populations of carcinoma-associated fibroblasts from non-small cell lung carcinoma reveals a role for STRBIA2 in cancer cell invasion. Neoplasia. 2019;21:482-493.

129. Lambrechts D, Wauters E, Boeckx B, et al. Prognostic significance of combined EGFR and RET inhibition with osimertinib and BLU-667 for cancer-associated fibroblasts in metastatic lymph nodes predicts poor prognosis in pathological N2 stage III lung adenocarcinoma. Ann Surg Oncol. 2012;19:3953-3962.

130. Su S, Chen J, Yao H, et al. Cancer-associated fibroblasts inhibit small cell lung cancer growth. Oncotarget. 2015;6:9531-9541.

131. Yu Y, Schuck K, Friess H, Konig B. Targeting aggressive fibroblasts to enhance the treatment of pancreatic cancer. Expert Opin Ther Targets. 2021;25:5-13.

132. Engelmann JA, Zejnilolu A, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316:1039-1043.

133. Piotrowska Z, Isokazi H, Lennerz JK, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. Cancer Discov. 2018;8:1529-1539.

134. Sequist LV, Waltman BA, Dias-Santagata D, et al. Histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011;3:75ra26.

135. Cortez E, Roswall P, Pietras K. Functional subsets of mesenchymal cell types in the tumor microenvironment. Semin Cancer Biol. 2014;25:3-9.

136. Nazareth MR, Broderick L, Simpson-Abelson MR, Kelleher RJ, Yokota SJ, Bankert RB. Characterization of human lung tumor-associated fibroblasts and their ability to modulate the activation of carcinoma-associated fibroblasts in three-dimensional in vitro co-culture. J Immunol. 2007;178:5552-5562.

137. Shu H, Li H. Prognostic effect of stromal myofibroblasts in lung adenocarcinoma. Neoplasma. 2012;59:658-661.

138. Kihara TK, Khaenekenari MR, Hellevik T, et al. Cancer associated fibroblasts in stage I-IIIa NSCLC: prognostic impact and their correlations with tumor molecular markers. PLoS One. 2015;10:e0134965.

139. Kihara TK, Rakae M, Hellevik T, et al. Tissue analyses reveal a potential immune-adjunct function of FAP-1 positive fibroblasts in non-small cell lung cancer. PLoS One. 2018;13:e0192157.

140. Hegab AE, Ozaki M, Kameyama N, et al. Effect of FGFR expression of blocking on lung adenocarcinoma and its cancer-associated fibroblasts. J Pathol. 2019;249:193-205.

141. Zeltz C, Pasko E, Cox TR, Navab R, Tsao M-S. LOXL1 is regulated by integrin α11 and promotes non-small cell lung cancer tumorigenicity. Cancer. 2019;11:705.

142. Zhou Z, Zhou Q, Wu X, et al. VCAM-1 secreted from cancer-associated fibroblasts enhances the growth and invasion of lung cancer cells through AKT and MAPK signaling. Cancer Lett. 2020;473:62-73.

143. Kawase A, Ishii G, Nagai K, et al. Podoplanin expression by cancer associated fibroblasts predicts poor prognosis of lung adenocarcinoma. Int J Cancer. 2008;123:1053-1059.

144. Zhou Z, Zhou Q, Wu X, et al. Transforming growth factor-β1 and α-smooth muscle Actin in stromal fibroblasts are associated with a poor prognosis in patients with clinical stage I–IIIA nonsmall cell lung cancer after curative resection. Tumor Biol. 2014;35:6707-6713.

145. Mahale J, Smagurauskaite G, Brown K, Miik Y, Saito R, Okada Y, Sasano H. Prognostic significance of combining immunohistochemical markers for
cancer-associated fibroblasts in lung adenocarcinoma tissue. 

165. Lee HW, Park YM, Lee SJ, et al. Alpha-smooth muscle actin (ACTA2) is required for metastatic potential of human lung adenocarcinoma. 

166. Zhang HY, Gharaee-Kermani M, Zhang K, Karmiol S, Phan SH. Lung fibroblast alpha-smooth muscle actin expression and contractile phenotype in bleomycin-induced pulmonary fibrosis. 

168. Wang L, Cao L, Wang H, et al. Cancer-associated fibroblasts reveal distinctive ultrastructure and function. 

169. Scott AM, Wiseman G, Welt S, et al. A phase I dose-escalation study 

170. Vicent S, Sayles LC, Vaka D, et al. Cross-species functional analysis of cancer-associated fibroblasts identifies a critical role for Clec11a and Il-6 in non-small cell lung cancer in vivo. 

171. Li H, Zhang Q, Wu Q, et al. Interleukin-22 secreted by cancer-associated fibroblasts regulates the proliferation and metastasis of lung cancer cells via the PI3K-Akt-mTOR signaling pathway. 

172. Wald O, Izhar U, Amir G, et al. Interaction between neoplastic cells and cancer-associated fibroblasts activates protein-positive cancer. 

173. Wei J-R, Dong J, Li L. Cancer-associated fibroblasts-derived gamma-glutamyltransferase 5 promotes tumor growth and drug resistance in lung adenocarcinoma. 

174. Horie M, Saito A, Noguchi S, et al. Relationship between podoplanin-αβ-signaling and chemotherapy-induced cisplatin resistance in lung adenocarcinoma. 

175. Li J, Guan J, Long X, Wang Y, Xiang X. mir-1-mediated paracrine role in non-small cell lung cancer tumor proliferation. 

176. Wei J-R, Dong J, Li L. Cancer-associated fibroblasts-derived gamma-glutamyltransferase 5 promotes tumor growth and drug resistance in lung adenocarcinoma. 

177. Zhang J, Han L, Yu J, Li H, Li Q. miR-224 aggravates cancer-associated fibroblast-induced progression of non-small cell lung cancer by modulating a positive loop of the SIRT3/AMPK/mTOR/HIF-1α axis. 

178. Arandkar S, Furth N, Elisha Y, et al. Altered p53 functionality in cancer-associated fibroblasts contributes to their cancer-supporting features. 

179. Saito R-A, Micke P, Paulsson J, et al. Forkhead box F1 regulates tumor-promoting properties of cancer-associated fibroblasts in lung cancer. 

180. Abulaiti A, Shintani Y, Funaki S, et al. Interaction between non-small-cell lung cancer cells and fibroblasts via enhancement of TGF-β signaling by IL-6. 

181. You J, Li M, Cao L, et al. Snail1-dependent cancer-associated fibroblasts induce epithelial-mesenchymal transition in lung cancer cells via exosomes. 

182. Yi Y, Zeng S, Wang Z, et al. Cancer-associated fibroblasts promote epithelial-mesenchymal transition and EGFR-TKI resistance of non-small cell lung cancers via HGF/IGF-1/ANXA2 signaling. 

183. Guo J, Hsu H, Tyan S, et al. Serglycin in tumor microenvironment promotes non-small cell lung cancer aggressiveness in a CD44-dependent manner. Oncogene. 2017;36:2457-2471. 

184. Neri S, Miyashita T, Hashimoto H, et al. Fibroblast-led cancer cell invasion is activated by epithelial-mesenchymal transition through platelet-derived growth factor BB secretion of lung adenocarcinoma. Cancer Lett. 2017;395:20-30. 

185. Neri S, Ishii G, Hashimoto H, et al. Podoplanin-expressing cancer-associated fibroblasts lead and enhance the local invasion of cancer cells in lung adenocarcinoma. 

186. Zhang J, Han L, Yu J, Li H, Li Q. miR-224 aggravates cancer-associated fibroblasts in lung cancer. 

187. Li S, Ou Y, Liu S, et al. The fibroblast TIA42 promotes lung cancer cell invasion and metastasis. 

188. Ren Y, Cao L, Wang L, et al. Autophagic secretion of HMGB1 from cancer-associated fibroblasts promotes metastatic potential of non-small cell lung cancer cells via NFkB signaling. Cell Death Dis. 2021; 12:1-13. 

189. Choe C, Shin Y-S, Kim S-H, et al. Tumor-stromal interactions with direct cell contacts enhance motility of non-small cell lung cancer cells through the hedgehog signaling pathway. 

190. Wang L, Cao L, Wang H, et al. Cancer-associated fibroblasts enhance metastatic potential of lung cancer cells through IL-6/STAT3 signaling pathway. 

191. Wang L, Cao L, Wang H, et al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition in lung cancer cells via exosomes. 

192. Guo J, Hsu H, Tyan S, et al. Serglycin in tumor microenvironment promotes non-small cell lung cancer aggressiveness in a CD44-dependent manner. Oncogene. 2017;36:2457-2471. 

193. Neri S, Miyashita T, Hashimoto H, et al. Fibroblast-led cancer cell invasion is activated by epithelial-mesenchymal transition through platelet-derived growth factor BB secretion of lung adenocarcinoma. Cancer Lett. 2017;395:20-30. 

194. Neri S, Ishii G, Hashimoto H, et al. Podoplanin-expressing cancer-associated fibroblasts lead and enhance the local invasion of cancer cells in lung adenocarcinoma. Int J Cancer. 2015;137:784-796. 

195. Choe C, Shin Y-S, Kim S-H, et al. Tumor-stromal interactions with direct cell contacts enhance motility of non-small cell lung cancer cells through the hedgehog signaling pathway. 

196. Wang L, Cao L, Wang H, et al. Cancer-associated fibroblasts enhance metastatic potential of lung cancer cells through IL-6/STAT3 signaling pathway. 

197. Wang L, Cao L, Wang H, et al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition in lung cancer cells via exosomes.
l lung cancer A549 cells by up-regulating the PI3K/Akt and GRP78 signaling on a microfluidic platform. PLoS One. 2015;10:e0129593.

202. Zhang Q, Yang J, Bai J, Ren J. Reverse of non-small cell lung cancer drug resistance induced by cancer-associated fibroblasts via a paracrine pathway. Cancer Sci. 2018;109:944-955.

203. Chen W-J, Ho C-C, Chang Y-L, et al. Cancer-associated fibroblasts regulate the plasticity of lung cancer stemness via paracrine signaling. Nat Commun. 2014;5:1-17.

204. Kinugasa Y, Matsui T, Takakura N. CD44 expressed on cancer-associated fibroblasts is a functional molecule supporting the stemness and drug resistance of malignant cancer cells in the tumor microenvironment. Stem Cells. 2014;32:145-156.

205. Majety M, Pradel LP, Gies M, Ries CH. Fibroblasts influence survival and therapeutic response in a 3D co-culture model. PLoS One. 2015;10:e0127948.

206. Mukaıd N, Sasaki S-i, Baba T. Chemokines in cancer development and progression and their potential as targeting molecules for cancer treatment. Mediators Inflamm. 2014;2014:1-15.

207. Räsänen K, Vaheeri A. Activation of fibroblasts in cancer stroma. Exp Cell Res. 2010;316:2713-2722.

208. Mishra P, Banerjee D, Ben-Baruch A. Chemokines at the crossroads of tumor-fibroblast interactions that promote malignancy. J Leukoc Biol. 2011;89:31-39.

209. Ramachandran S, Verma AK, Dev K, et al. Role of cytokines and chemokines in NSCLC immune navigation and proliferation. Oxid Med Cell Longev. 2021;2021:1-20.

210. Inoue C, Miši Y, Saito R, et al. PD-L1 induction by cancer-associated fibroblast-derived factors in lung adenocarcinoma cells. Cancer. 2019;11:1257.

211. Kinoshi K, Nakagawa K, Hamada J-I, et al. Imatinib mesylate inhibits the proliferation-stimulating effect of human lung cancer-associated stromal fibroblasts on lung cancer cells. Jpn J Cancer Res. 2016;107:334-342.

212. Zhang Z, Ren X, Lu X, et al. GZD856, a novel potent PDGFR inhibitor, suppresses the growth and migration of lung cancer cells in vitro and in vivo. Cancer Lett. 2016;375:172-178.

213. Lim C, Savan R. The role of the IL-22/IL-22R1 axis in cancer. Cytokine Growth Factor Rev. 2014;25:275-271.

214. Hogan B, Grindley J, Bellusci S, Dunn N, Emoto H, Itoh N. Branching morphogenesis of the lung: new models for a classical problem. Cold Spring Harb Symp Quant Biol. 1997;62:249-256.

215. Bermudez O, Hennen E, Koch I, Lindner M, Eckelberg O. Gli1 mediates lung cancer cell proliferation and sonic hedgehog-dependent mesenchymal cell activation. PLoS One. 2013;8:e63226.

216. Staudigl C, Concín N, Grömm C, et al. Prognostic relevance of pretherapeutic gamma-glutamyltransferase in patients with primary metastatic breast cancer. PLoS One. 2015;10:e0125317.

217. Wang Q, Shu X, Dong Y, et al. Tumor and serum gamma-glutamyl transpeptidase, new prognostic and molecular interpretation of an old biomarker in gastric cancer. Oncotarget. 2017;8:36171-36184.

218. He W, Guo G, Cx X, et al. Gamma-glutamyl transpeptidase level is a novel adverse prognostic indicator in human metastatic colorectal cancer. Color Dis. 2013;15:e443-e452.

219. Mezawa Y, Orima A. The roles of tumor-and metastasis-promoting carcinoma-associated fibroblasts in human carcinomas. Cell Tissue Res. 2016;365:675-689.

220. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol. 2006;172:973-981.

221. Gaggioli C, Hooper S, Hidalgo-Carcedo C, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007;9:1392-1400.

222. Sato R, Semba T, Saya H, Arima Y. Concise review: stem cells and epithelial-mesenchymal transition in cancer: biological implications and therapeutic targets. Stem Cells. 2016;34:1997-2007.

223. Sun B, Zhang D, Zhao N, Zhao X. Epithelial-to-endothelial transition and cancer stem cells: two cornerstones of vasculoigenic mimicry in malignant tumors. Oncotarget. 2017;8:30502-30510.

224. Zha H, Wang X, Zhu Y, et al. Intracellular activation of complement C3 leads to PD-L1 antibody treatment resistance by modulating tumor-associated macrophages. Cancer Immunol Res. 2019;7:193-207.

225. Cho MS, Vasquez HG, Rupaimoole R, et al. Autocrine effects of tumor-derived complement. Cell Rep. 2014;6:1083-1095.

226. Shu C, Zha H, Long H, et al. C3a-C3aR signaling promotes breast cancer lung metastasis via modulating carcinoma associated fibroblasts. J Exp Clin Cancer Res. 2020;39:1-14.

227. He S-J, Cheng J, Feng X, Yu Y, Tian L, Huang Q. The dual role and therapeutic potential of high-mobility group box 1 in cancer. Oncotarget. 2017;8:64534-64550.

228. Neri S, Hashimoto H, Ki H, et al. Cancer cell invasion driven by extracellular matrix remodeling is dependent on the properties of cancer-associated fibroblasts. J Cancer Res Clin Oncol. 2016;142:437-446.

229. Friedl P, Lockier J, Sahai E, Segall JE. Classifying collective cancer cell invasion. Nat Cell Biol. 2012;14:777-783.

230. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. J Cell Biol. 2008;123:2229-2238.

231. Miles FL, Sikes RA. Insidious changes in stromal matrix fuel cancer progression. Mol Cancer Res. 2014;12:297-312.

232. Mueller MM, Fusenig NE. Friends or foes—bipolar effects of the tumour stroma in cancer. Nat Rev Cancer. 2004;4:839-849.

233. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell. 2010;141:52-67.

234. Folkman J. Angiogenesis. Annu Rev Med. 2006;57:1-18.

235. Folkman J. Angiogenesis. In: Jaffe, E.A. eds. Biology of Endothelial Cells. Developments in Cardiovascular Medicine. Vol. 27. Boston, MA: Springer. https://doi.org/10.1007/978-1-4613-2825-4_42.

236. Risau W. Mechanisms of angiogenesis. Nature. 1997;386:671-674.

237. Folkman J. Tumor angiogenesis. Adv Cancer Res. 1985;43:175-203.

238. Leung DW, Cathelaine G, Kuang W-J, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989;246:1306-1309.

239. Maia-Malarz A, Sobol G, Woi H. Proangiogenic factors: vascular-endothelial growth factor (VEGF) and basic fibroblast growth factor—the characteristics and function. Przegląd Lekarski. 2008;65:353-357.

240. Marneros AG, Olsen BR. The role of collagen-derived proteolytic fragments in angiogenesis. Matrix Biol. 2001;20:337-345.

241. Kim S, Bell K, Mousa SA, Varner JA. Regulation of angiogenesis in vivo by ligation of integrin α5β1 with the central cell-binding domain of fibronectin. Am J Pathol. 2000;156:1345-1362.

242. Nicosia RF, Bonanno E, Smith M. Fibronectin promotes the elongation of microvessels during angiogenesis in vitro. J Cell Physiol. 1993;154:654-661.

243. Fromigue O, Louis K, Dayem M, et al. Gene expression profiling of normal human pulmonary fibroblasts following coculture with non-small-cell lung cancer cells reveals alterations related to matrix degradation, angiogenesis, cell growth and survival. Oncogene. 2003;22:8487-8497.

244. Harper J, Sainson RC. Regulation of the anti-tumour immune response by cancer-associated fibroblasts. Semin Cancer Biol. 2014;25:69-77.

245. Rööser T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm. 2015;2015:1-16.

246. Zhang F, Wang H, Wang X, et al. TGF-β induces M2-like macrophage polarization via SNAIL-mediated suppression of a pro-inflammatory phenotype. Oncotarget. 2016;7:52294-52306.
247. Lechner MG, Megiel C, Russell SM, et al. Functional characterization of human Cd33+ and Cd11b+ myeloid-derived suppressor cell sub-sets induced from peripheral blood mononuclear cells co-cultured with a diverse set of human tumor cell lines. J Transl Med. 2013;2:e23337.

248. Simpson KD, Cross JV. MIF: metastasis/MDSC-inducing factor? Onco Targets Ther. 2013;2:e23337.

249. Yang N, Lode K, Berzaghi R, Islam A, Martinez-Zubiaurre I, Hellevik T. Irradiated tumor fibroblasts avoid immune recognition and retain immunosuppressive functions over natural killer cells. Front Immunol. 2021;11:3567.

250. Liang SC, Latchman YE, Buhlmann JE, et al. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol. 2003;33:2706-2716.

251. Lakins MA, Ghorani E, Munir H, Martins CP, Shields JD. Cancer-associated fibroblasts induce antigen-specific deletion of CD8+ T cells to protect tumour cells. Nat Commun. 2018;9:1-9.

252. O'Connor RA, Chauhan V, Mathieson L, et al. T cells drive negative feedback mechanisms in cancer associated fibroblasts, promoting expression of co-inhibitory ligands, CD73 and IL-27 in non-small cell lung cancer. Onco Targets Ther. 2021;10:1940675.

253. Najafi M, Farhood B, Mortezaee K. Extracellular matrix (ECM) stiffness and degradation as cancer drivers. J Cell Biochem. 2019;120:2782-2790.

254. Gkretsi V, Stylianopoulos T. Cell adhesion and matrix stiffness: coordinating cancer cell invasion and metastasis. Front Oncol. 2018;8:145.

255. Liu Y, Lv J, Liang X, et al. Fibrin stiffness mediates dormancy of tumor-repopulating cells via a Cdc42-driven Tet2 epigenetic program. Cancer Res. 2018;78:3926-3937.

256. Nakamura H, Sugano M, Miyashita T, et al. Organoid culture containing cancer cells and stromal cells reveals that podoplanin-positive cancer-associated fibroblasts enhance proliferation of lung cancer cells. Lung Cancer. 2019;134:100-107.

How to cite this article: Wong KY, Cheung AH-K, Chen B, et al. Cancer-associated fibroblasts in nonsmall cell lung cancer: From molecular mechanisms to clinical implications. Int J Cancer. 2022;151(8):1195-1215. doi:10.1002/ijc.34127