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Functional diversity of cancer-associated fibroblasts in modulating drug resistance

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Abstract

The effectiveness of current chemotherapies for cancer is gradually progressing; however achieving a complete cure through chemotherapy is still difficult and has been the main goal in treatment of advanced cancer. Drug resistance is an issue in cancer therapy, therefore increasing numbers of investigations into drug resistance have focused on the characteristics of the cancer cells themselves. The interaction between the tumor microenvironment (TME) and cancer cells is also intimately involved in the development of drug resistance. Cancer-associated fibroblasts (CAFs) are a predominant component of the TME and affect tumor progression by secreting soluble factors. This review summarizes the most up-to-date knowledge of CAFs and drug resistance in cancer, with a focus on factors secreted from CAFs including proteins, cytokines, extracellular vesicles, and metabolites. A perspective on the potential role of anti-CAF therapies in overcoming CAF-induced drug resistance is also discussed.

KEYWORDS

cancer stemness, cancer-associated fibroblast, chemoresistance, immunotherapy, metabolic modulation

1 | INTRODUCTION

Over the past few decades, advances in this field have demonstrated that the complexity of cancer is not only dependent on the intrinsic characteristics of tumor cells but also mainly determined by cross-talk between altered cancer cells and various components of the tumor microenvironment (TME). This complexity has become a considerable obstacle in discovering the specific mechanism(s) underlying treatment failure. Many various cell types are present within the TME, including fibroblasts and endothelial, adipose, mesenchymal, and proinflammatory immune cells.[1] Among these cell types, fibroblasts have emerged as a pivotal effector of cancer metabolism and transformation due to their abundance in the tumor stromal tissue and their diverse biological functions. Fibroblasts usually remain in a quiescent state and are flexibly activated and deactivated in response to changes due to tissue damage and wound healing; this results in the generation of myofibroblasts characterized by the expression of α-smooth muscle actin (α-SMA), a fibroblast marker.[2-4] These activated fibroblasts interact closely with tumor cells through multiple mechanisms and produce different results[5], therefore they are defined as cancer-associated fibroblasts (CAFs) rather than normal fibroblasts (NFs). Among the tumor-promoting functions of CAFs, their reinforcement of chemoresistance is a crucial component due to the importance of establishing an effective anticancer

strategy. In recent studies, strategies targeting CAFs in addition to the cancer cells themselves have become a new area of interest. In this review, we summarize some of the current perspectives on how CAFs support the development of drug resistance.

2 | ORIGIN OF CAFS

Research into cancer progression through various mechanisms is widely performed, and the characteristics of different cancer types can be determined by studying their distinct somatic origins and the genetic background of individual patients. Cancer cells are located in different TMEs and present different morphologies and genetic profiles. These conclusions have contributed vastly to the development of successful cancer treatments by illustrating the importance of the assorted steps of cancer progression. Similar to cancer cells, the elucidation of mechanisms of tumor stromal heterogeneity has also had a substantial effect on anticancer therapies. CAFs, as one of the dominant cell types in the tumor stroma, and their role in anticancer treatments have been reported in numerous studies to date. At the CAF origin is an individual factor that reveals its heterogeneity. CAFs originate from diverse cell types, and well known progenitors for CAFs include NFs,[6] epithelial cells,[7] endothelial cells,[8] adipocytes,[9] stellate cells,[10] bone marrow-derived cells,[11,12] and mesenchymal stem cells (MSCs).[13] More specifically, as demonstrated in a xenograft model of breast cancer,[6] fibroblasts can be stimulated by endogenous transforming growth factor-beta 1 (TGF-β1) and stromal cell-derived factor-1 (SDF-1) in an autocrine manner and thereby differentiate into myofibroblasts. In multiple types of cancer, epithelial-mesenchymal transition (EMT) is the mechanism by which epithelial cells are transformed into CAFs.[7] For example, using γGTCreR26R mice to track kidney fibroblasts, it was shown that kidney fibroblasts are derived from 2 sources: 1 small group that migrates from the bone marrow, and a larger group of fibroblast-specific protein 1 (FSP1)-positive fibroblasts that arise through local EMT.[14] Similarly, by irreversibly tagging endothelial cells by crossing Tie2-Cre mice with R26Rosa-lox-Stop-lox-LacZ mice, it was shown that, under TGF-β1 stimulation, FSP1 expression was increased followed by decreased CD31/PECAM expression. This corresponded to the conversion of endothelial cells to CAFs via endothelial-mesenchymal transition (EndMT) in pancreatic cancer and melanoma.[8] In another study, the mouse breast cancer cell line 4T1 was transplanted into fat pads formed by preadipocytes, and showed that adipocytes stimulated by cancer cell-derived WNT3a transformed into adipocyte-derived fibroblasts, which exhibited increased secretion of fibronectin and collagen and promoted tumor invasion.[9] Moreover, the well known pancreatic stellate cells, which are the resident fibroblasts in the pancreas, can be isolated from rat pancreas and cultured *in vitro*. Upon interaction with a tumor, these cells lost vitamin A expression and subsequently presented secretory phenotypes via activation of the mitogen-activated protein kinase (MAPK) pathway, which promotes tumor survival.[10] Bone marrow-derived cells are also considered a major source of CAFs. For instance, in a mouse model of inflammation-dependent gastric dysplasia, bone marrow-derived MSCs were actively recruited to the dysplastic stomach and comprised at least 20%-25% of α-SMA$^+$ CAFs.[12] Similarly, using a mouse model of pancreatic insulinoma, α-SMA$^+$ mesenchymal cells labeled with green fluorescent protein from a male donor were transplanted into a female recipient. It was reported that approximately 25% of the myofibroblasts in these pancreatic tumors were donor-derived.[11] Last, but not least, the use of CXCR6 knockout mice revealed that MSCs activated by the CXCL16/CXCR6 signaling cascade had increased secretion of SDF-1, also known as CXCL12, which binds to CXCR4 on tumor cells to induce EMT, ultimately promoting metastasis.[13] In addition to this evidence, the theory that CAFs are mainly derived from local fibroblasts rather than other precursors has also been demonstrated by a recent study using mice expressing a green fluorescent reporter protein (EGFP) under the control of the type I collagen (Col-I) promoter (COL-EGFP), in which the origin of the fibroblastic stroma was tracked.[15]

The origin of CAFs has been a controversial topic over the past decades, however, following the development of techniques such as lineage tracking, better accuracy and more convincing evidence can be expected in the future.

3 | MARKERS AND HETEROGENEITY OF CAFS

As most CAFs are considered to be activated fibroblasts, this specific population is identified by its expression of related markers. α-SMA is a well known marker of activated fibroblasts and in pancreatic cancer was reported to reinforce the contractility of connective tissue.[16] Fibroblast activation protein (FAP) functioned as a serine protease in a mouse melanoma model,[17] and platelet-derived growth factor receptor is a pharmacological target.[18] Conversely, FSP is a reliable marker of quiescent, nonproliferating (Ki67$^-$) fibroblasts,[19] and numerous other markers have been identified to classify CAFs under certain conditions. Nonetheless, although CAF-specific gene profiles have already been identified in some studies (compared with the NF gene profile),[20] none of those markers was specific for CAFs, confirming their heterogeneity. When analyzing CAFs, multiple factors should be considering rather than focusing on a single marker.[21] Therefore, in recent years, the concept of dividing CAFs into subtypes has begun to emerge. Distinct types of CAFs displaying either a matrix-secreting (ie, myofibroblast) phenotype or an inflammatory phenotype have been consistently reported and are known as "myCAFs" and "iCAFs," respectively. In pancreatic cancer, TGF-β induced the upregulation of α-SMA resulting in CAFs with a myCAF contractile phenotype.[22] iCAFs have low levels of α-SMA expression and secrete additional inflammatory cytokines, an additional important characteristic of iCAFs is their high levels of secreted IL-6, which in turn activate the Janus kinase/signal transduction and activator of transcription (JAK-STAT) signaling pathway and in a tumor mouse model favored KPC mouse tumor organoid survival. Moreover, this activation could be reversed by knocking out IL-6 in CAFs.[22] These

types of CAFs were generated by TGF-β-mediated suppression of IL-1 receptor expression, which subsequently induced IL-6 expression.[23] In addition to the subtypes mentioned above, another study identified an NF-κB-driven subset of CAFs that expressed GPR77 and CD10, and which in breast cancer promoted cancer cell stemness and chemoresistance.[24]

Single-cell RNA-sequencing techniques have become increasingly ubiquitous over the past decade and have made significant contributions to understanding the heterogeneity of CAFs. For instance, a cross-species single-cell analysis of human and mouse pancreatic ductal adenocarcinoma (PDAC) tumors detected CAFs expressing major histocompatibility complex (MHC) class II and CD74 in pancreatic cancer and that were termed "antigen-presenting CAFs (apCAFs)." This unique population activated $CD4^+$ T cells in mice in an antigen-specific manner.[25] In addition, another study utilizing a novel RNA-in situ hybridization analysis of single cells provided evidence that CAFs stimulated different pathways to drive cancer cells into proliferative types and invasive EMT types. In detail, CAF-conditioned medium enhanced metastatic and proliferative capabilities; single-cell RNA-sequencing data revealed secretory profiles of CAFs in PDAC, indicating the mechanism involved in activating the MAPK and STAT signaling pathways.[26] In addition, a more recent reports demonstrated that CAF-derived prostaglandin E2 (PGE2) can expand a novel type $Sca\text{-}1^+$ "reserve-like stem cell" population. These cells had strong regenerative and tumorigenic features, which were propagated by the Hippo pathway effector Yap.[27]

Despite the vast numbers of reports demonstrating the tumor-promoting roles of CAFs, contrary ideas on their tumor-suppressing roles have arisen in the past few years. A study using a xenograft model in NSG mice exhibited 2 types of fibroblasts, namely, CD146-negative and CD146-positive fibroblasts; the former sustained chemoresistance in tumors, whereas the latter enhanced drug sensitivity to tamoxifen.[28] Another study went a step further and identified a subtype of CAFs termed cancer-restraining CAFs. In these cells, Meflin expression combined with low expression of α-SMA in the early stages of pancreatic cancer played an essential role in weakening tumor aggressiveness. Patients with high Meflin-expressing cells had a better prognosis, and in a syngeneic transplantation model knocking out Meflin in CAFs resulted in poor differentiation.[29] Moreover, in a mouse model of PDAC with shh deletion, either direct elimination of $\alpha\text{-SMA}^+$ CAFs or suppression of Hedgehog signaling (which sustains stromal fibroblasts) led to an undifferentiated and more aggressive phenotype of cancer with more severe angiogenesis. This finding indicated that the Hedgehog-driven stroma suppressed tumor growth in part by restraining tumor angiogenesis.[30] Furthermore, depending on the α-SMA expression level, CAFs could be divided into 2 types: C1 (low α-SMA) and C2 (high α-SMA). C1 CAFs were more supportive of cell proliferation but suppressed the self-renewal capacity of oral stem-like cancer cells via bone morphogenetic protein 4 (BMP4).[31] Finally, experiments with transgenic mice with the ability to deplete $\alpha\text{-SMA}^+$ myofibroblasts in pancreatic cancer indicated that CAF depletion led to enhanced hypoxia, elevated EMT, more pronounced cancer stem cell (CSCs) properties and increased animal mortality. Immune suppression was also observed in CAF-depleted tumors with elevated numbers of $CD4^+Foxp3^+$ regulatory T cells (Tregs). Despite the limitation that this was an animal-based study, these data provided evidence for a cancer suppressive role of CAFs.[16]

The complexity of the heterogeneity of CAFs has been observed in various studies (Figure 1). Although this heterogeneity appeared to be the main obstacle to understanding the functions of CAFs, novel sequencing techniques, immunohistochemical staining with multiple antibodies, and other quantitative methods have allowed the expansion of knowledge on this topic. The reason why the tumor stroma, including CAFs, has been extensively studied is their important role in resistance to anticancer therapies. Thus, revealing the mechanism underlying this resistance will bring us a step closer to translating these findings from the bench to the clinic.

4 | DRUG RESISTANCE MEDIATED BY CAF-SECRETED FACTORS

During treatment with anticancer drugs, cancer cell activities are impaired through multiple pathways, which simultaneously alter the TME. These anticancer therapies induce CAFs to secrete numerous cytokines that activate signaling cascades to prevent the elimination of the cancer cells and possibly cause recurrence.

The canonical Wnt signaling pathway is known for its cancer-promoting function. In prostate cancer, CAFs produce wingless-type mouse mammary tumor virus integration site family member 16B (WNT16B), a member of the Wnt family, to decrease the cytotoxicity of chemotherapy and enhance tumor progression.[32] In one report, WNT16B was shown to be regulated by NF-κB through a post-DNA damage mechanism, and could be induced by both tumor necrosis factor α (TNF-α) and radiotherapy. This mechanism subsequently triggered the canonical Wnt pathway as a paracrine signal, eventually resulting in drug resistance. A complementary study from the same group reported that secreted frizzled-related protein 2 (SFRP2), another soluble factor secreted from CAFs after genotoxic treatments, supported β-catenin-mediated activities induced by WNT16B. This process was interpreted as SFRP2 coordinating the recognition of frizzled (FZD) 3/4/6 and low-density lipoprotein receptor-related protein 6 (LRP6) on the cancer cell surface by WNT16B, which prevented cell death and increased the proliferation, migration, and invasion of cancer cells.[33] In pancreatic cancer, CAFs protected cancer cells from gemcitabine-induced apoptosis in an NF-κB-dependent manner. this apoptosis was regulated by IL-1β and IL-1 receptor-associated kinase 4 (IRAK4) autocrine signaling based on the observation that inhibiting IL-1β or knocking down IRAK4 increased chemosensitivity to gemcitabine and decreased fibrosis.[34] Furthermore, IL-6 was mainly secreted by CAFs cocultured with esophageal squamous cell carcinoma (ESCC) cancer cells and increased CXCR7 expression through the STAT3/NF-κB pathway, thus enhancing the chemoresistance of ESCC to cisplatin treatment and highlighting

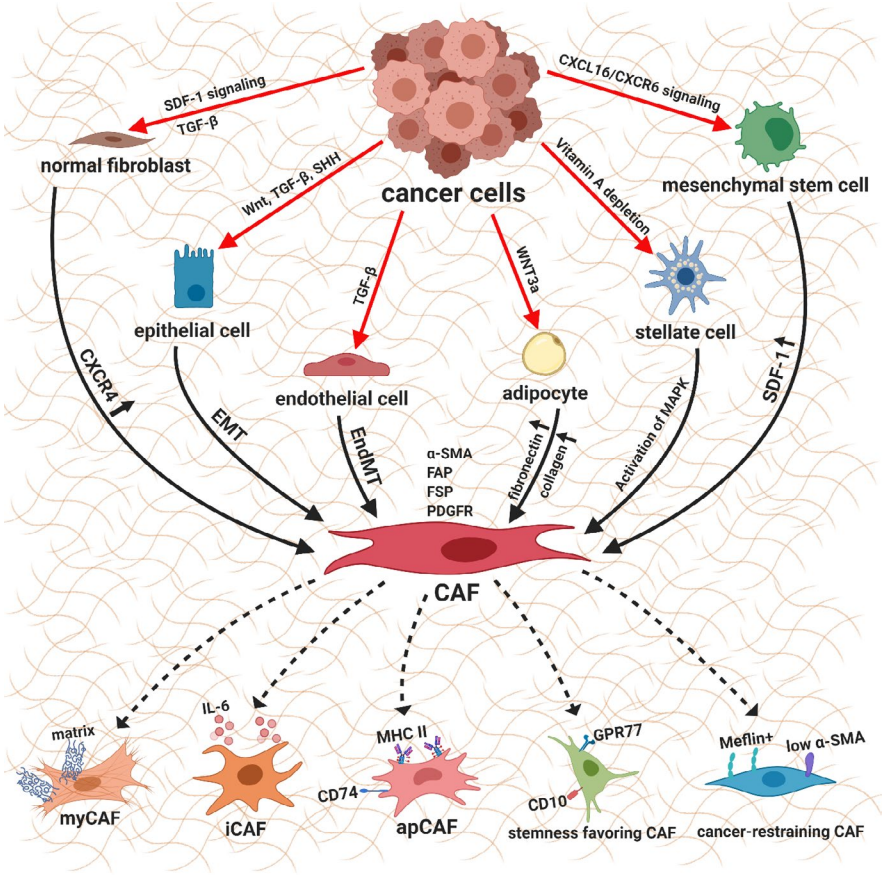

FIGURE 1 Heterogeneity of cancer-associated fibroblasts (CAFs). CAFs (black arrows) originate from a variety of cell types upon exposure to several different stimuli (red arrows). Normal resident fibroblasts stimulated with transforming growth factor-beta (TGF-β) transform into CAFs through upregulation of CXCR4, whereas the application of TGF-β to endothelial cells will cause EndMT and convert endothelial cells into CAFs. Through Wnt and SHH signaling, epithelial cells transform into CAFs via EMT, and WNT3a triggers a signal cascade that remodels adipocytes into CAFs. Similar types of differentiation occur in stellate cells and mesenchymal stem cells, which are activated by vitamin A depletion and CXC16/CXCR6 signaling, respectively. Different subtypes (broken lines) of CAFs have also been identified, including myofibroblast-like CAFs, inflammatory CAFs, antigen-presenting CAFs, stemness-supporting CAFs, and cancer-restraining CAFs

the importance of the IL-6/CXCR7 axis in ESCC.[35] The important role of IL-6 secreted by CAFs in chemoresistance was also supported by recent secretome and transcriptome analyses in CAFs from gastric cancer that showed that IL-6 was a CAF-specific secretory protein that protected gastric cancer cells through the JAK-STAT3 pathway, resulting in antiapoptotic activities and supporting tumor survival in a paracrine manner. Moreover, additional work demonstrated that IL-6 production from CAFs could be caused by coculture with gastric cancer cells exposed to chemotherapeutic agents.[36] Additionally, according to a more recent study, IL-8 expression was induced in CAFs, and CAFs cultured in conditioned medium from gastric cancer cells exhibited increased levels of PI3K, phosphorylated AKT (p-AKT), phosphorylated Iκb (p-Iκb), phosphorylated p65 (p-p65) and ABCB1, all of which were accompanied by NF-κB activation to enhance cisplatin resistance in cancer cells.[37] Previous studies have stated that both IL-6 and IL-8 were required for chemoresistance. A multidrug-resistant human breast cancer cell line, MCF-7/R, was established and analyzed using a cytokine antibody array, and levels of IL-6, IL-8, and 13 other proteins were significantly increased. However, MCF-7/R cells with knockdown of IL-6 or IL-8 showed increased drug sensitivity.[38] In addition, the significance of extracellular vesicles (EVs) in CAFs was demonstrated in gastric cancer. Comprehensive proteomic analysis of CAF-EVs identified that annexin A6 played a pivotal role in the drug resistance of gastric cancer cells via activation of the β1 integrin-focal adhesion kinase (FAK)-YAP axis.[39]

Oncogene-targeted methods are also considered vital approaches in antitumor therapy. *BRAF* inhibitors are widely used for melanoma treatment, and CAFs have an important role in hindering their activity. A proteomic analysis of a coculture system revealed that stromal cell-secreted hepatocyte growth factor (HGF) activated the MAPK and PI3K/Akt signaling pathways in tumor cells via the MET receptor, which ultimately induced resistance to *BRAF* inhibitors.[40] Similarly, the effects of another *BRAF* inhibitor, PLX4720, were weakened by the high levels of matrix produced by CAFs via increased integrin β1/FAK/Src signaling, indicating another type of factor secreted by CAFs that could sabotage oncogene-targeted drugs.[41] Based on these findings, chemotherapy can eliminate cancer cells but potentially induce the secretion of stroma-derived factors to produce beneficial environments that promote drug resistance and increase tumor survival. Therefore, when developing curative treatments for cancer, changes in the tumor stroma should be strongly considered.

5 | CAFS MEDIATE DRUG RESISTANCE BY PROMOTING CANCER STEMNESS

In addition to increasing cancer cell proliferation or facilitating tumor survival, another essential pathway that promotes chemoresistance is the maintenance of CSCs. Over recent years, the concept of stemness as a dynamic trait rather than a fixed trait has increased

rapidly.[42] Studies have shown that the stem cell fraction maintains both a slow-cycling state and a proliferative state through regulation by some key molecules. For example, in LGR5$^+$ colorectal CSCs, upregulation of F-box/WD repeat-containing protein 7 (FBXW7) resulted in altered c-*Myc* expression and consequent conversion of cells into slow-cycling and drug-resistant CSCs; FBXW7 was identified as an important molecule responsible for this conversion.[43] Furthermore, among the members of the Cip/Kip family of cyclin-dependent kinase (CDK) inhibitors (p21, p27, p57), p57 was required for maintaining quiescence in hematopoietic stem cells (HSCs) based on experiments with p57-deficient mice; this provided another candidate for regulating the states of stem cells.[44]

Another feature of CSCs is their multiple defense mechanisms against chemotherapy.[45-47] This ability is due not only to intrinsic mechanisms in CSCs but also to the tumor stroma that provides vital support to maintain CSCs. In response to chemotherapy, colorectal cancer-initiating cells exhibited chemotherapeutic resistance, which was enhanced by interleukin-17A (IL-17A) secreted from CAFs. This finding suggested that chemotherapy induced remodeling of the TME to support the cellular hierarchy of the tumor through secreted factors from CAFs.[48] TGF-β signaling in fibroblasts could specifically enhance the tumor-initiating potential of colorectal cancer cells. Moreover, the use of TGF-β signaling inhibitors to block crosstalk between cancer cells and the microenvironment attenuated tumor progression.[49] Impaired drug delivery is another possible mechanism of chemoresistance, and the efficacy of gemcitabine delivery was increased after the depletion of tumor stromal tissue through inhibition of Sonic hedgehog (SHH) signaling.[50] Given the independent mechanisms described above, researchers further identified a role for noncanonical SHH signaling in TGF-β2 signaling, with hypoxia-inducible factor (HIF-1α) functioning cooperatively to allow CAFs to activate the transcription factor GLI2 in a paracrine manner. This led to the upregulation of some well known stem cell markers, such as NANOG and SOX2, to reinforce chemoresistance in colorectal CSCs by decreasing the apoptosis rate. Interestingly, enhanced cancer cell stemness is usually considered to be due to activation of Wnt/β-catenin signaling, however, in this scenario, the authors declared the importance of TGF-β2/GLI2 signaling in promoting cancer stemness, while molecules such as IL-6 and WNT5A failed to do so. The study also revealed a positive correlation between the poor prognosis of patients with colorectal cancer and TGF-β2/GLI2/HIF1α expression, which extended the clinical importance of strategies targeting this signaling pathway.[51] A similar mechanism was also reported in breast cancer, in which CAFs secreted soluble factors such as ACTIVIN A, IGF-1, and LIF, all of which enhanced CSC proliferation and self-renewal.[52] In addition to the routes mentioned above, exosomal pathways and noncoding RNA from CAFs also deserve attention for supporting CSCs.[53,54] As discussed previously, Wnt signaling is crucial for establishing chemoresistance during cancer progression, and exosomal Wnt from CAFs stimulated differentiated colorectal cancer cells to restore their CSC features, consequently endowing them with a drug-resistant phenotype.[55] Moreover, one research group discovered that, in breast cancer, CAF-derived microvesicles transferred miR-221 to cancer cells and activated an ER^{low}/Notch^{high} feed-forward loop responsible for the generation of CD133^{high} CSCs. They further observed therapy-resistant metastasis using patient-derived xenograft (PDX) models.[56] In addition to miRNAs, the long noncoding RNA H19 from CAFs promoted cancer stemness and chemoresistance in colorectal cancer via activation of the β-catenin pathway and by acting as a competing endogenous RNA sponge for miR-141, which reduces the ability of miR-141 to inhibit the stemness of CRC cells.[57] Finally, the specific mechanism by which CAFs established niches for CSCs has been described in recent studies. Loss of H3K27me3 in gastric cancer led to substantially increased secretion of multiple stem cell niche factors from CAFs, including WNT5a, thereby enhancing tumorigenesis and facilitating chemoresistance.[58] In a subsequent study, a specific subtype of CAFs that established survival niches for CSCs was defined. This type of CAF was distinctly enriched in biopsies of chemoresistant tissues and was characterized by positive expression of CD10 and GPR77 and the secretion of large amounts of IL-6 and IL-8.[24]

As a crucial cell type contributing to cancer cell survival and maintenance, CSCs play an important role in chemoresistance. Therefore efforts should focus on the crosstalk between the tumor stroma and CSCs to ensure that the strategies used to treat cancer are integrated into this phenomenon.

6 | CAFS INDUCE DRUG RESISTANCE BY MODULATING METABOLISM

Cancer metabolism has been a vital research topic due to the importance of obtaining a better understanding of cancer biology at the molecular level and developing new and effective therapies. Tumors primarily survive through using glutamine and glucose as energy sources, in addition to participating in crosstalk with numerous other cells in the TME. As a main components of the TME, CAFs frequently share or exchange metabolites with cancer cells, and this may trigger a signaling cascade that results in drug resistance. The reason for the connections between tumor cells and CAFs is most likely to be attributed to the need for both cell types to adapt to a low-nutrient environment.[59,60] In a study of non-small-cell lung cancer, stromal cells, particularly CAFs, predominantly expresses glucose uptake genes. Among these genes, glutamine-fructose-6-phosphate transaminase 2 (*GFPT2*), which participates in the process of glycosylation, was the primary focus of these authors. GFPT2 functioned independently of the primary glucose transporter GLUT1 but exerted a significant effect on prognosis.[61] Additionally, increased glutamine synthesis and macropinocytosis of extracellular fluid by CAFs were reported to initiate *Ras* signaling in prostate cancer. CAFs supplied cancer cells with glutamine and triggered neuroendocrine differentiation as a response to androgen deprivation therapy (ADT). Relatively higher glutamine levels have been detected in patients with prostate cancer after undergoing ADT. Consistent with these findings, inhibiting macropinocytosis and glutamine transport resulted in

tumor suppression in an orthotopic xenograft model.[62] To adapt to glucose deprivation, cancer cells tended to switch their metabolic energy production to aerobic glycolysis, also known as the Warburg effect. Surprisingly, cancer cells underwent this process and guided stromal cells in the surrounding TME to also use aerobic glycolysis, resulting in multidrug resistance.[63] Specifically, the PI3K/AKT pathway was activated by cancer cells, which led to the induction of the Warburg effect in CAFs through cytoplasmic translocation of the nuclear G-protein-coupled estrogen receptor (GPER) and aberrant activation of the GPER/cAMP/PKA/CREB signaling pathway. CAFs subsequently delivered lactate transporters to cancer cells, forming a coupled energy metabolism process that increased drug resistance. Other metabolic changes in CAFs have also attracted attention in recent studies. For example, pyruvate and lactate from CAFs or cancer cells promoted drug resistance in cancer cells. EGFR- or MET-expressing cancer cells exhibited elevated glycolysis activity and increased production of lactate that induced CAFs to secrete large amounts of HGF through an NF-κB-dependent mechanism. Subsequently, HGF activated MET-dependent signaling and enabled cancer cells to resist tyrosine kinase inhibitors (TKIs).[64] Metabolic reprogramming in cancer cells by CAFs has attracted considerable attention over the past decade. Undoubtedly, it also plays a vital role in resistance to antitumor therapies, therefore further studies in this area are surely worth pursuing.

7 | ROLE OF CAFS IN IMMUNOTHERAPY RESISTANCE

As mentioned previously, compared with NFs, CAFs are highly activated in the TME and exhibit a more complex secretory profile in the majority of tumors. Among all the secretomes identified from CAFs, immunomodulatory secretomes are novel targets in the era of immunotherapy. CAFs exhibited an immunomodulatory secretory profile that was characterized by proteins with multiple roles in regulating the immune response through several pathways.[65,66] In the TME, macrophages are present as 2 distinct types: M1 and M2. M1 macrophages produce large amounts of proinflammatory cytokines and reactive oxygen species and have the capacity to orchestrate a type 1 T helper (Th1)-mediated antitumor immune response, whereas M2 macrophages promote tissue repair and angiogenesis while also producing immunosuppressive factors such as IL-10, IDO and TGF-β.[67] CAFs have been reported to be actively engaged in polarizing macrophages toward the M2 phenotype and in hindering the therapeutic response in individuals with pancreatic cancer.[68] Similarly, chitinase-3-like-1 (Chi3L1), a secreted glycoprotein involved in several diseases (including chronic inflammatory conditions), is expressed at high levels in CAFs and is related to macrophage recruitment and M2 polarization; depleting Chi3L1 impaired tumor growth and increased the infiltration of $CD8^+$ and $CD4^+$ T cells.[69] In addition to their influence on macrophages, CAFs also affected natural killer (NK) cell activities. CAFs are constantly stimulated by TGF-β while also serving as the main source of TGF-β.[70,71] TGF-β was reported to induce miR-183 expression to inhibit DAP12 transcription, which led to a reduction in the expression of NK-activating receptors, ultimately decreasing the cytotoxic activity of NK cells.[72] Likewise, quadruple-mutant mice with 4 of the main colorectal cancer mutations showed that TGF-β suppressed the differentiation and activation of T cells and antagonized Th1 cells. Inhibition of TGF-β boosted the susceptibility cancer cells to anti-PD1/PD-L1 therapies, which indicated the importance of TME-derived TGF-β in immunotherapy.[73] Finally, CAFs were activated by TGF-β from cancer cells carrying distinct gene profiles, thus producing cancer-related extracellular matrix and developing a unique immunosuppressive TME leading to PD-1 blockade failure in multiple cancers.[74]

In addition, PGE2 and IDO secreted from CAFs in hepatocellular carcinoma also caused NK cell dysfunction, thereby providing tumor cells with a suitable environment for progression.[75] Due to the important role of T cell activity in the antitumor response, the function of CAFs in directly regulating T cell activity is another point that should be discussed. Similar to NK cells, TGF-β released from CAFs may also regulate $CD8^+$ and $CD4^+$ T cells: TGF-β and IL-15 are 2 key regulators of short-lived $CD8^+$ T cells, in which TGF-β limits $CD8^+$ T cell activity by decreasing Bcl-2 expression and promoting apoptosis; by contrast, IL-15 promotes their survival.[76] Another study on triple-negative breast cancer clearly demonstrated a specific subgroup of myofibroblasts. This immunosuppressive subtype of CAFs secreted CXCL12, attracted $CD4^+CD25^+$ T lymphocytes and retained them in the TME through OX40L, PD-L2, and JAM2. Moreover, CAF-S1 increased T-lymphocyte survival and promoted their differentiation into $CD25^{high}FOXP3^{high}$ via B7H3, CD73, and DPP4. They also elevated Treg capacity, which was closely related to the effects of immunotherapies.[77] Furthermore, TGF-β directly altered the cytotoxic functions of $CD8^+$ T cells by inhibiting the expression of cytolytic genes, namely for perforin, granzyme A, granzyme B, Fas ligand, and IFN-γ,[78] and excess secretion of TGF-β from CAFs can greatly inhibit $CD8^+$ T cells. In a murine model of breast cancer, the elimination of CAFs *in vivo* with a DNA vaccine targeting FAP resulted in a shift of the immune TME from Th2 to Th1 polarization. This shift was characterized by increased expression of IL-2 and IL-7, along with an increase in the $CD8^+$ T cell population and a decrease in the recruitment of TAMs, MDSCs, and Tregs.[79]

In a study using PDAC-bearing mice, the immune checkpoint inhibitors anti-cytotoxic T-lymphocyte-associated protein 4 (α-CTLA-4) and α-programmed cell death 1 ligand 1 (α-PD-L1) failed to suppress tumor progression. However, the antitumor effect was rescued for α-CTLA-4 and α-PD-L1 by depleting FAP^+ CAFs, which eventually were discovered to be related to CXCL12 expression from CAFs. Consistent with these findings, CXCL12 inhibition increased the accumulation of T cells after anti-PD-L1 treatment.[80] The immune response plays an important role in supporting the antitumor activities of cells and is recognized as an indispensable contributor to the effectiveness of antitumor

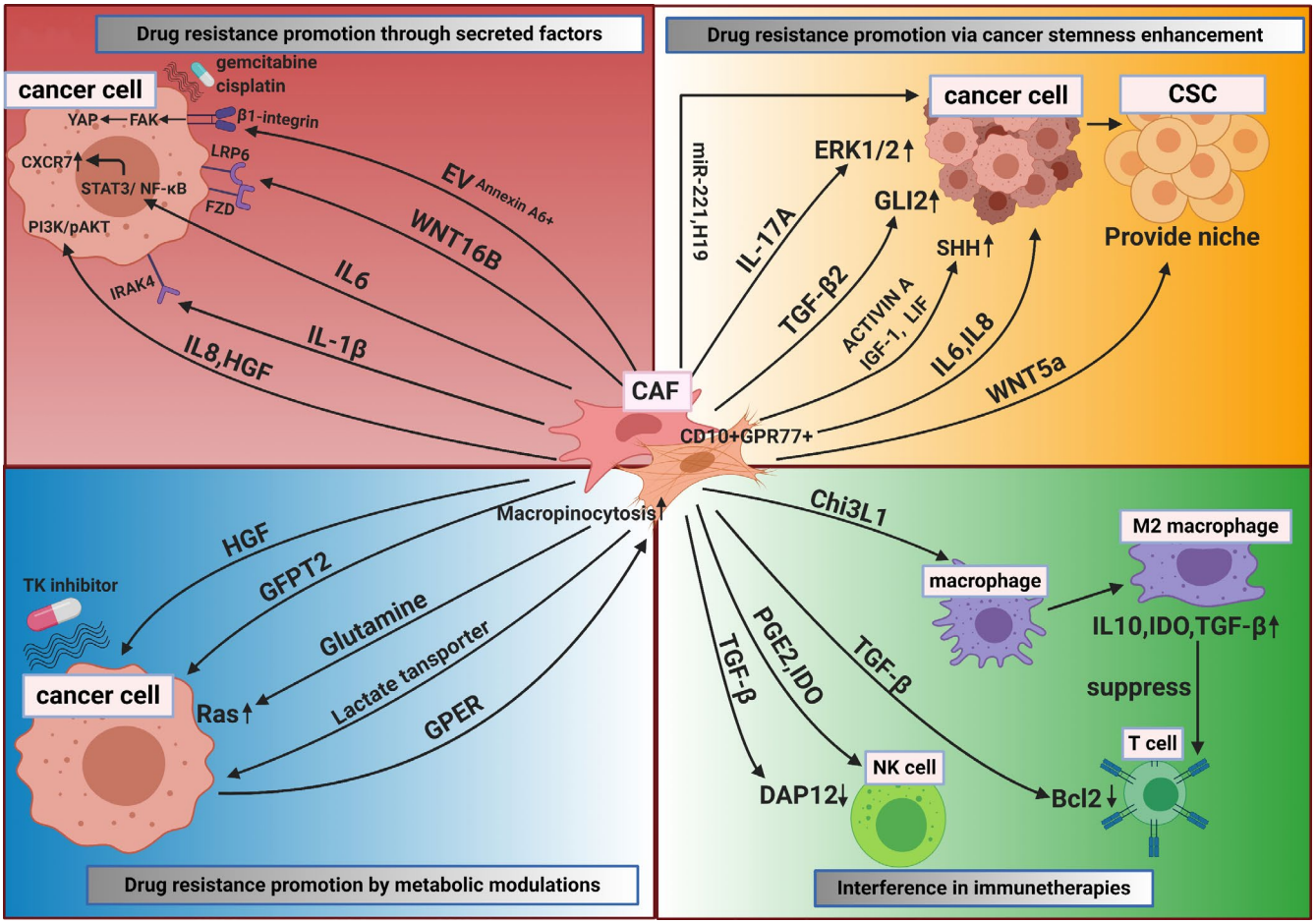

FIGURE 2 Roles of cancer-associated fibroblasts (CAFs) in cancer chemoresistance. CAFs affect anticancer resistance through various mechanisms, including secreted factors (red), the promotion of cancer stemness (orange), metabolic modulations (blue), and interference with immunotherapy (green). Upon stimulation with factors such as annexin A6, WNT16B, IL-6, IL-1β, IL-8, and HGF, chemoresistance is enhanced via downstream pathways, including the STAT3, FAK-YAP, NF-κB and PI3K/p-AKT signaling pathways. Stemness is increased by activation of ERK1/2, upregulation of GLI2, induction of IL-6 and/or IL-8 secretion from $CD10^{+}GPR77^{+}$ CAFs and activation of the SHH pathway. WNT5a also induces CAFs to produce stem cell niche factors. Furthermore, *Ras* signaling is facilitated by glutamine from CAFs through macropinocytosis, GFPT2 and HGF from CAFs reprograming cancer cells to engage in glycolysis, and GPER from cancer cells induced CAFs to produce lactate transporters that support cancer metabolism; all these activities eventually lead to drug resistance. Finally, CAFs polarize macrophages into the M2 phenotype, which suppress T cell activity. At the same time, CAFs secrete TGF-β, PGE2, and IDO to alter the functions of NK cells and T cells

therapies. Nonetheless, CAFs impair the immune response through multiple mechanisms and thus represent an essential target for combination treatment during immunotherapy against cancer.

8 | CONCLUSION

Despite the constant emergence of novel drugs targeting cancer progression, issues relating to drug resistance remain a challenge in the field. Previously, studies mainly focused on drugs targeting the properties of tumor cells, however, in the past few decades, the importance of the TME in chemoresistance has become the main topic, and has been investigated by numerous researchers. CAFs are a pronounced target in the analysis of the role of the TME in chemoresistance due to their unique cell states and tumor-promoting functions mediated by various mechanisms. Currently, CAFs are more frequently recognized as a special cell transition state rather than a distinct cell type, mostly as the identification of a specific marker or even a combination of markers for CAFs has long been a difficult task. Because TME tends to maintain its homeostasis, a fixed marker for CAFs might not be reliable. Due to the constant stimulation of the TME, the contributions of CAFs to drug resistance are complex and diverse (Figure 2). Thus, their crucial role in building an invisible wall that reduces drug sensitivity and prevents drug infiltration might significantly affect anticancer treatment, and this new challenge surely deserves more in-depth study.

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CONFLICT OF INTEREST

The authors have no ethical issues or conflicts of interest to disclose regarding this manuscript.

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