

# Nano-Drug Delivery Systems Targeting CAFs: A Promising Treatment for Pancreatic Cancer

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**Abstract:** Currently, pancreatic cancer (PC) is one of the most lethal malignant tumors. PC is typically diagnosed at a late stage, exhibits a poor response to conventional treatment, and has a bleak prognosis. Unfortunately, PC's survival rate has not significantly improved since the 1960s. Cancer-associated fibroblasts (CAFs) are a key component of the pancreatic tumor microenvironment (TME). They play a vital role in maintaining the extracellular matrix and facilitating the intricate communication between cancer cells and infiltrated immune cells. Exploring therapeutic approaches targeting CAFs may reverse the current landscape of PC therapy. In recent years, nano-drug delivery systems have evolved rapidly and have been able to accurately target and precisely release drugs with little or no toxicity to the whole body. In this review, we will comprehensively discuss the origin, heterogeneity, potential targets, and recent advances in the nano-drug delivery system of CAFs in PC. We will also propose a novel integrated treatment regimen that utilizes a nano-drug delivery system to target CAFs in PC, combined with radiotherapy and immunotherapy. Additionally, we will address the challenges that this regimen currently faces.

**Keywords:** nanoparticle, nano-drug delivery system, cancer-associated fibroblasts, pancreatic cancer, tumor microenvironment

## Introduction

Pancreatic cancer (PC) is a highly aggressive disease that has been causing a gradual increase in morbidity and mortality. In recent decades, only about 4% of patients have been able to survive 5 years after diagnosis. The survival rate of PC has stagnated due to its resistance to current conventional therapies, including surgery, chemotherapy, radiotherapy, and immunotherapy. Additionally, the fact that most patients are detected at an advanced stage contributes to the challenge.<sup>1</sup> The resistance of PC to current therapeutic modalities is primarily attributed to the structural characteristics of PC itself, as it is surrounded by a rich tumor microenvironment (TME). The rich TME not only produces various pro-tumor growth and metastatic active factors, but also serves as a physical barrier preventing drug penetration from reaching the tumor.<sup>2</sup> Cancer-associated fibroblasts (CAFs) play a crucial role in the composition of the TME, which is a major component in the composition and regulation of the tumor's physical barrier. CAFs can be differentiated from normal fibroblasts and PC astrocytes and they can be classified into numerous subtypes.<sup>3</sup> Different subtypes of CAFs secrete a variety of regulatory factors that play crucial roles in PC proliferation, invasion, and metastasis. However, recent studies have shown that excessive depletion of CAFs accelerates tumor progression.<sup>4</sup> CAFs, with their pro-tumorigenic and anti-tumorigenic functional heterogeneity, are promising targets for the treatment of PC. Currently, there are no effective therapeutic modality available to improve the prognosis of PC. Novel regimens targeting the CAFs that comprise the TME may revolutionize the current landscape of PC treatment. The ability to remodel the TME to reverse the physical barrier that protects tumor cells and creates a rapid pathway for drug penetration is crucial. Currently, there is no effective treatment to improve the prognosis of PC. However, there has been limited progress in efficiently targeting CAFs and appropriately converting tumor-promoting CAFs into anti-tumor CAFs. The use of nano-drug delivery systems has made significant advancements in recent years. These systems not only enable precise targeting of the affected area but also

allow for precise temporal, spatial, and dosimetric release of drugs through suitable stimulus-responsive systems.<sup>5</sup> Nanosystems can also target specific organelles and signaling pathways after entering cells to interfere with energy metabolism and signaling in tumor cells. This interference affects the function of tumor cells and has the potential to target CAFs in the treatment of PC.<sup>6</sup> Furthermore, these nanosystems are virtually non-toxic to normal tissues, making them highly promising for targeted therapy. Dual targeting of CAFs and their internal organelles by nanosystems may help alleviate the issue of accelerated tumor progression caused by excessive depletion of CAFs. This review summarizes recent research advances on CAFs in PC and nano-drug delivery system. It explores potential strategies for nanosystem-targeted CAFs in PC as well.

## CAFs in PC

PC is highly lethal and mesenchymal-rich, with a significant proportion of CAFs in the TME. CAFs are a class of cell types that are critical for the generation and development of PC, and they play important roles in the formation, proliferation, and invasion of PC.<sup>1</sup> In TME, CAFs secrete growth factors, inflammatory ligands, and extracellular matrix (ECM) to promote cancer cell proliferation, resistance to treatment, and immune evasion.<sup>7</sup> However, recent studies have revealed that, under certain circumstances, CAFs may also inhibit tumor progression.<sup>4</sup> It has been shown that CAFs are heterogeneous in origin and species.<sup>8</sup> Therefore, it is essential to have a comprehensive understanding of the complex role that CAFs plays in the cancer progression.

## Landscape of CAFs

### Origin of CAFs

A significant reason for the phenotypic difference between CAFs and quiescent fibroblasts in normal tissues is their distinct origins. Fibroblasts in normal and damaged fibrotic tissues originate from mesodermal cells during embryonic development. Although fibroblasts can come from peripheral tissues, bone marrow stem cells, endothelial cells, and bone marrow-derived fibroblasts,<sup>9–12</sup> the origins of CAFs are still a subject of controversy. Furthermore, there is limited information regarding the specific cell types that give rise to different subtypes of CAFs. It has been demonstrated in pancreatic ductal adenocarcinoma (PDAC) that *in vitro*, pancreatic stellate cells (PSCs) can differentiate into inflammatory fibroblasts (iCAF) and myofibroblasts (myCAF).<sup>13</sup> Antigen-presenting CAF (apCAF) isoforms<sup>14</sup> and mesothelial cells were recently identified in PDAC through scRNA-seq and immunohistochemistry analysis.<sup>8</sup> This observation suggests that apCAF may originate from mesothelial cells.<sup>15</sup> Thus, in PDAC, CAFs are primarily derived from PSCs and potentially from mesothelial cells. The different origins create heterogeneity in CAF subpopulations, and these studies provide a foundation for our understanding of the heterogeneity of CAFs in TME and promote further research on the origin and function of CAF subtypes.

### Subtypes of CAFs

The subtypes of CAFs correlate with specific types of cancer. The most consistently observed subtypes across different cancer types are likely the myCAFs and non-myCAFs.<sup>16–18</sup> In addition, the presence of myCAFs and iCAFs has also been confirmed in PC.<sup>19</sup>

With the help of a 3D co-culture system, study found that a small subset of PDAC fibroblasts expresses fibroblast activation protein (FAP) and high levels of alpha smooth muscle actin ( $\alpha$ -SMA), which were defined as myCAF. Additionally, a CAF subtype with low  $\alpha$ -SMA, a high interleukin-6 (IL-6) inflammatory profile, and a loss of myofibroblast characteristics was also defined as iCAF.<sup>13</sup> Elyada et al identified a subtype of CAF that expresses the major histocompatibility complex (MHC) class II family of genes and named it apCAF. MyCAF requires direct contact with cancer cells in the PDAC to be activated and is in the periglandular region. In addition, experimental studies have shown that PDAC depleted of myCAFs exhibits inhibition of angiogenesis and enhancement of tumor hypoxia.<sup>4</sup> Furthermore, myCAFs have been found to be associated with metastasis and immunosuppression in PDAC.<sup>20</sup> iCAF, which is located far away from the tumor cells and myCAF, is able to be activated by paracrine factors secreted by the tumor cells. iCAF can secrete various immunosuppressive factors, such as IL-6 and prostaglandin E2 (PGE2), to inhibit the activity of immune cells. It also releases CXCL12 in an NF- $\kappa$ B-mediated manner, leading to immunosuppression and

promoting tumor growth in PDAC. iCAF is closely associated with inflammation related to the TME.<sup>21–23</sup> ApCAF can express professional antigen-presenting cells (APCs) and other co-stimulatory factors, but their levels are significantly reduced, which suggests that the role of apCAF in PDAC is different from that of professional APCs. apCAF is regulated by IFN- $\gamma$  signaling in vivo and exhibits an antioxidant response, making it inextricably linked to the immunity of the pancreatic TME.<sup>14</sup> The TGF- $\beta$ /SMAD2/3 pathway can induce myCAF activation in PDAC and it is believed that myCAF can be converted to iCAF.<sup>24</sup> However, there is a lack of direct experimental evidence to confirm whether these two cell states can be interconverted in vivo.

## The Role of CAFs in the Progression of PC

### The Interaction Between CAFs and PC

CAFs participate in a complex signaling network that encompasses tumor cells, immune infiltrates, and other cellular constituents within the intricate TME. Within this context, cancer cells are recognized as the instigators of malignancy due to their ability to reprogram normal fibroblasts and quiescent PSCs into CAFs through diverse mechanisms.<sup>25</sup> Biffi et al, utilizing organoids and murine models, demonstrated that gradients of tumor-secreted ligands, primarily TGF- $\beta$  and IL-1, diffuse outward from epithelial lesions, influencing intracellular signaling in fibroblasts and driving the differentiation of distinct CAF subtypes at an epigenetic level.<sup>24</sup> This underscores the potential of TGF- $\beta$  as a crucial target for pancreatic cancer therapy through CAFs. Several therapies targeting ligands or receptors involved in TGF- $\beta$  signaling, such as Trabedersen (targeting TGF- $\beta$ 2 with antisense oligonucleotides), LY3022859 (anti-T $\beta$ RII mAb), LY2157299 (T $\beta$ RI inhibitor), and TEW-7197 (TGF- $\beta$  receptor ALK4/ALK5 inhibitor), have progressed to clinical trials.<sup>26</sup>

The reciprocal communication between cancer cells and CAFs is intricate and intimate. Upon activation by transformed cancer cells, CAFs engage in reciprocal signaling, promoting cancer cell proliferation, metabolism, stemness, and drug resistance.<sup>27</sup> Through systematic proteomic studies of secretory mediators, Shi and colleagues identified leukemia inhibitory factor (LIF) as a critical paracrine factor released by activated PSCs, driving tumor progression and chemoresistance in cancer cells.<sup>28</sup> Clinically, trials targeting LIF as a therapeutic candidate are undergoing Phase I trials.<sup>26</sup> CAFs enhance PDAC cell proliferation and survival by secreting CXCL12 and stimulating the corresponding receptor CXCR4 in cancer cells. Various signaling pathways within cancer cells are activated by the CXCL12-CXCR4 axis, including the MAPK, PI3K, Wnt and Shh pathways, collectively contributing to pancreatic cancer progression.<sup>29</sup> This highlights the potential of the CXCL12-CXCR4 axis as a therapeutic target for pancreatic cancer therapy through CAF modulation. Drugs targeting this axis, such as Olaptosed (anti-CXCL12 oligonucleotide), BL-8040 (CXCR4 peptide antagonist), and Plerixafor/AMD3100 (CXCR4 inhibitor), are currently undergoing clinical trials.<sup>26</sup>

### Functional Heterogeneity of CAFs

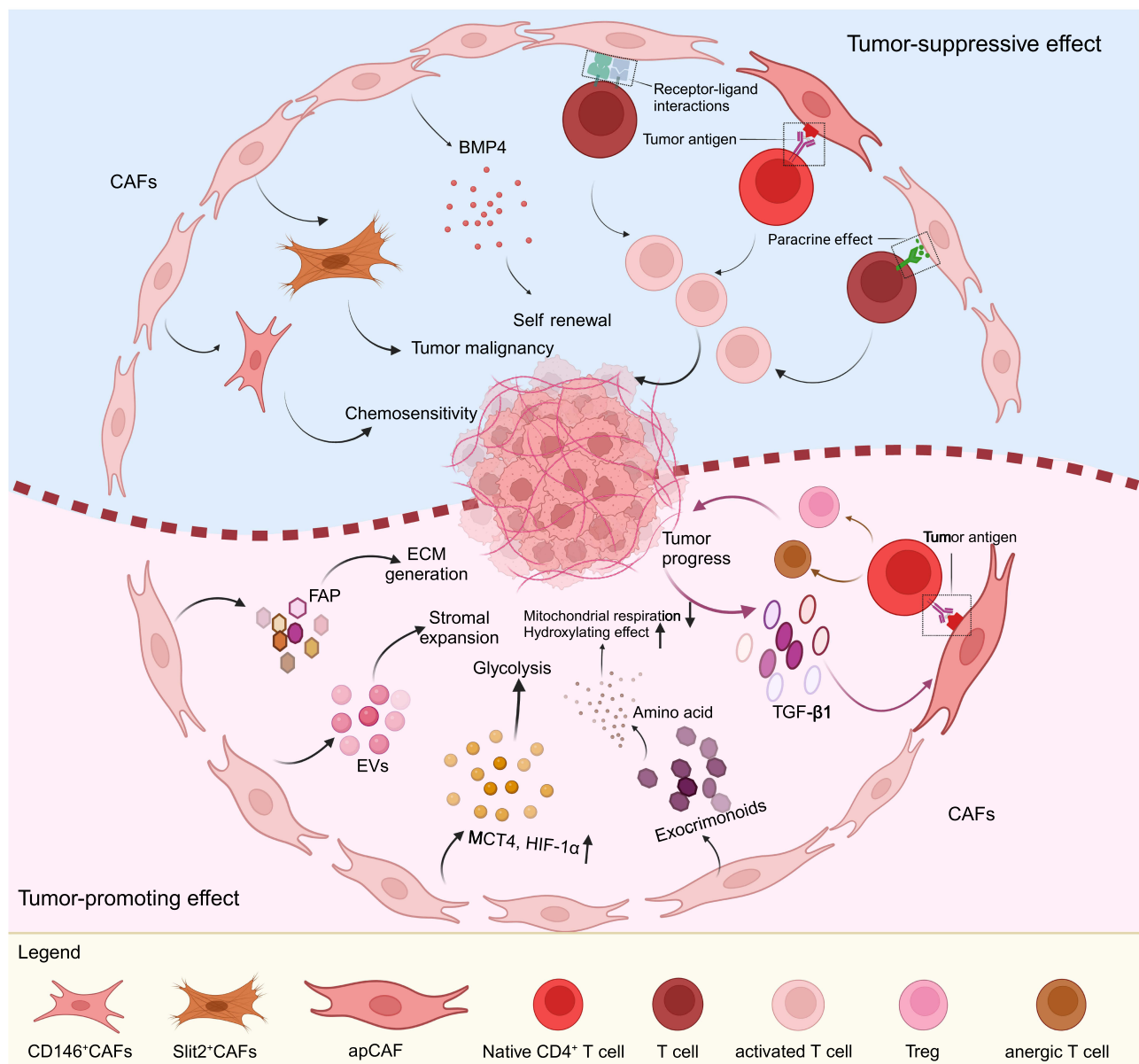
CAFs present a multifaceted array of functions within the realm of tumor biology, profoundly shaped by their distinct phenotypic traits. Broadly, these functions bifurcate into two principal categories: the facilitation and the restraint of tumor progression.<sup>4</sup> Different types of CAFs have distinct pro-tumorigenic and anti-tumorigenic roles (Table 1).

CAFs significantly contribute to the onset and progression of cancers. They enhance tumorigenesis, proliferation, invasion, and metastasis by secreting a myriad of cytokines, chemokines, growth factors, and enzymes that degrade the stromal matrix. These fibroblasts also modulate the TME, establishing direct interactions with cancer cells. Furthermore, they influence the immune response to tumors by releasing inflammatory cytokines, fostering angiogenesis, and providing crucial support for tumor metastasis and invasion. For example, CAFs can secrete a variety of growth factors, cytokines, and soluble factors, such as TGF- $\beta$ , IL-6, HGF, heat shock factor 1 (HSP1), various angiogenic factors, stromal cell-derived factor 1 (SDF-1, also known as CXC motif chemokine ligand 12, CXCL12), and members of the matrix metalloproteinase (MMP) family (including MMP-1, -2, and -3). These factors can promote the growth, proliferation, and metastasis of neighboring cancer cells.<sup>34–38</sup> Expression of associated transcription factors and iconic proteins in CAFs can also promote tumor growth, invasion, and metastasis. For example, overexpression of the transcription factor ETV1 increased tumor volume by promoting stromal expansion, altering stromal morphology, enhancing invasive capacity, and up-regulating regulators of EMT and MMP, including SLUG, SNAIL, TWIST, Vimentin (VIM), zinc finger E-box binding homeobox 1 (ZEB1), ZEB2, and MMP9.<sup>39</sup> Another key player in tumor

**Table 1** Functional Heterogeneity of CAFs in PC: Tumor Promoting and Tumor Suppressive Effects

CAF type	Characteristics (location, gene expression)	Tumor-promoting effects	Tumor-suppressing effects	References
myCAF	Location: Periglandular region of the body Gene expression: $\alpha$ -SMA, VIM, CTGF, collagen type I alpha 1 chain (COL1A1), collagen type V alpha 1 chain (COL5A1), collagen type VI alpha 1 chain (COL6A1), FAP, etc.	Promotes tumor cell proliferation and invasion; promotes tumor neovascularization; matrix deposition; reduces T cell infiltration and maintains tumor stem cell properties.	Generation of derived collagen I alters the chemokine profile of pancreatic cancer cells, thereby preventing the recruitment of myeloid-derived suppressor cells and enabling antitumor B cell and T cell immunity; Prevent tumor dissemination.	[13,30–32]
iCAF	Location: Away from tumor cells and myCAFs Gene expression: IL-6, IL-1, IL-11, LIF, etc.	Specifically expresses hyaluronan synthase 1 (HAS1) and HAS2; inhibits immunity and ECM deposition; promotes the tumor inflammatory microenvironment; secretion of cytokines and chemokines; Inflammatory infiltration; through Immunosuppressive effects promoting tumor escape; reduction of oxidative stress.	iCAFs may inhibit tumor growth in some cases by activating immune responses.	[13,14]
apCAF	Location: – Gene expression: MHC class II family and PDGFR $\alpha$ , SAA3, HLA-DRA (MHCII genes), CD74, SAA3, SLPI, etc.	Activates CD4 <sup>+</sup> T cells and promotes their differentiation into Tregs, directly contributing to tumor immunosuppression and promoting tumor growth.	Inhibition of tumor cell proliferation through secretion of antiproliferative factors such as secreted frizzled related protein 1 (SFRP1) and TGF- $\beta$ .	[14,33]

promotion is FAP, which has been shown to play a crucial role in extracellular matrix (ECM) formation, angiogenesis, cell motility, immunosuppression, and final clinical outcome.<sup>40</sup> Chiwaki et al demonstrated the role of FAP in cancer cell invasion and EMT.<sup>41</sup> A study by McAndrews et al found that specific depletion of FAP<sup>+</sup> CAFs could significantly inhibit PDAC tumor progression and significantly increase overall survival in mice (Figure 1).<sup>42</sup> CAFs also act as promoters of tumor growth and invasion by influencing tumor metabolism. Increased expression of monocarboxylate transporters 4 (MCT4) and hypoxia-inducible factor 1 alpha (HIF-1 $\alpha$ ) in CAFs, compared with healthy fibroblasts, increases glycolytic activity, resulting in the secretion of excess lactic acid. This excess lactic acid can stimulate cancer cells and promote tumor progression.<sup>43</sup> CAFs-derived exosomes carry amino acids, TCA cycle intermediates, and lipids. Once ingested by cancer cells, these exosomes will inhibit mitochondrial respiration while promoting cancer cell proliferation through increased glycolysis and glutamine-dependent reductive carboxylation.<sup>44</sup> CAFs can also exchange lipid-derived metabolites with tumor cells. For example, PSC-derived CAFs secrete large amounts of lysophosphatidylcholine (LPC) compared to their healthy counterparts. The extracellular enzyme autocrine motility factor, secreted by cancer cells and CAFs, subsequently converts LPC into the wound-healing mediator which is called lysophosphatidic acid (LPA). LPA promotes the proliferation, migration, and activation of AKT in PC cells.<sup>45</sup> CAFs can significantly reduce the killing ability of various immune cells against tumor cells, thereby promoting the proliferation and progression of tumor cells. In the case of PC, which is typically characterized by a high degree of connective tissue proliferation, reduced vascularity, and extreme hypoxia, these factors have led to the limited effectiveness of immunotherapy for treating this type of cancer.<sup>46</sup> In addition, even if immune cells enter the TME, their activity is inhibited by immunosuppressive factors and the hypoxic environment.<sup>47</sup>



**Figure 1** Effects of CAFs on pancreatic cancer cells can be categorized into tumor-promoting and tumor-suppressing effects. Tumor suppressive effects mainly include increasing chemosensitivity, reducing malignancy, inhibiting self-renewal of cancer cells, and promoting anti-tumor immunity mainly by T cells. Tumor-promoting effects mainly involve the production of a series of substances by CAFs, such as FAP, EVs, exocrine bodies, TGF- $\beta$ , and others. These substances can promote the functions of ECM generation, stromal expansion and suppression of tumor immunity. Created with BioRender.com.

Contrasting with their tumor-promoting activities, select CAF contingents are known to impede tumor growth and progression.<sup>4,48,49</sup> This is accomplished through the activation of specific signaling pathways, such as the hedgehog (Hh) pathway, which has the capacity to alter the rigidity of the tumor microenvironment and decelerate tumor progression. Emerging research, including pivotal studies by Özdemir et al, underscores the tumor-suppressive capabilities of certain CAF subsets, particularly myCAFs in PC.<sup>4</sup> Experimental depletion of myCAFs has been observed to intensify tumor infiltration and diminish survival rates, indicating a potential defensive role in both the early and advanced stages of PDAC. Additionally, a correlation has been identified between lower  $\alpha$ -SMA scores in patients and an increased prevalence of poorly differentiated cancers, suggesting a role for myCAFs in sustaining immune surveillance.

The paradoxical roles of CAFs in tumor biology sketch a complex therapeutic landscape, pointing to the feasibility of a “stromal switch” strategy to transform tumor-promoting CAFs into tumor-suppressing entities. Nonetheless, the



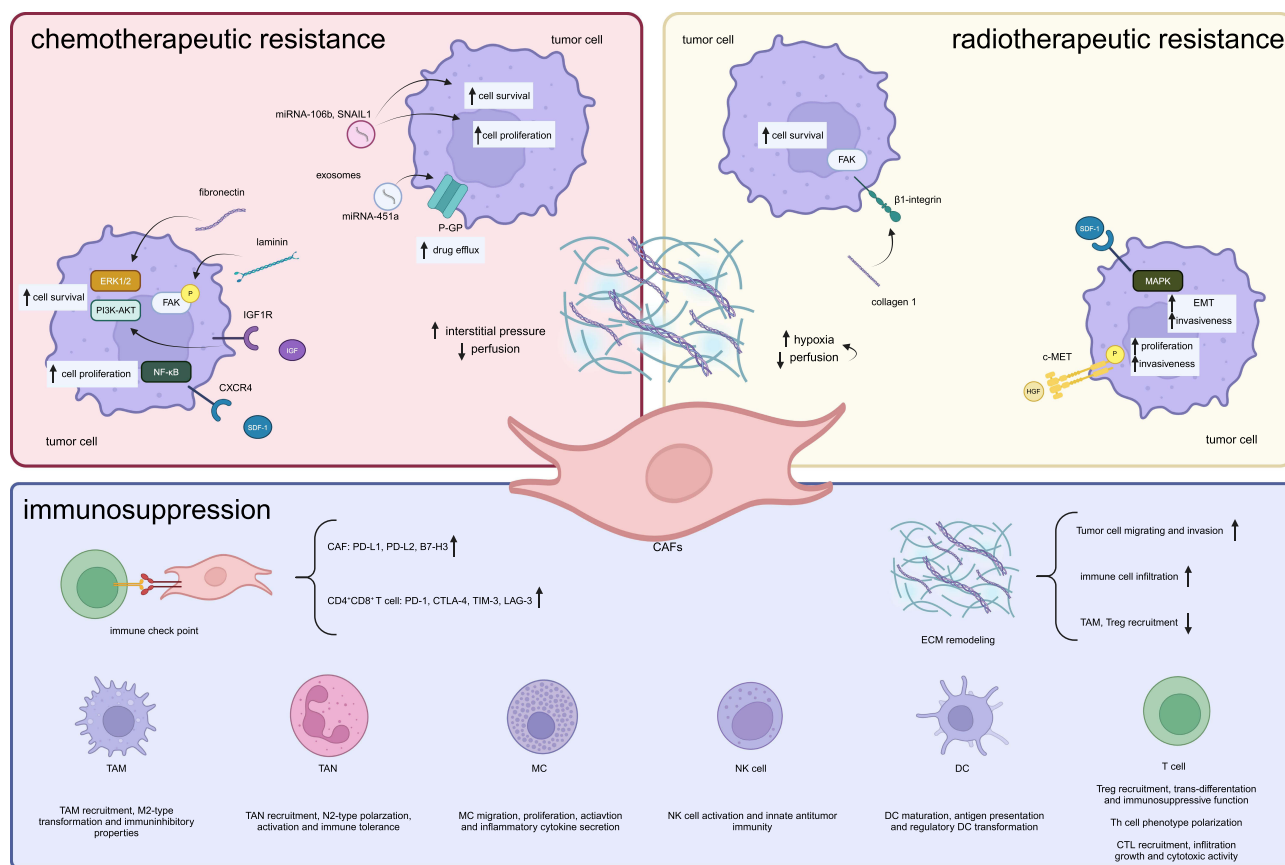
diversity and intricacy of CAF phenotypes demand extensive research to fully decipher their specific functions across different tumor types and to formulate precise therapeutic approaches. The pronounced heterogeneity of CAFs highlights their critical status as therapeutic targets, and a deeper comprehension of this heterogeneity is crucial for enhancing treatment modalities and prognoses for cancer patients, particularly those battling PC.

## Relationship Between CAFs and PC Treatment

Recently, CAFs, as a major component of TME, have been identified as another key factor in promoting tumor progression.<sup>3</sup> Although more specific roles and detailed mechanisms of CAFs in cancer pathogenesis and progression still need to be further explored, many studies have found that CAFs are important markers of PC nowadays. CAFs not only correlate with prognosis and resistance to chemotherapy, radiotherapy and immunosuppression, but also serve as key targets that have the potential to improve the current status of PC treatment. The specific mechanisms of action of CAFs in chemotherapeutic resistance, radiotherapeutic resistance, and immunosuppression are shown in Figure 2.

## CAF Markers for PC Prognosis

Specific markers or certain types of CAFs play a more significant role in the diagnosis and prognosis determination of PDAC. Histologic studies have shown that a higher expression of  $\alpha$ -SMA or a high percentage of stroma predicts a poor clinical outcome in patients with PDAC.<sup>50</sup> Related studies have already discovered that LIF can activate CAFs.<sup>20</sup> Furthermore, alterations in circulating LIF levels have been closely linked to the tumor's response to therapy.<sup>28</sup> Therefore, LIF would be an attractive therapeutic target and circulating marker. Sun et al found a negative correlation



**Figure 2** Mechanisms of therapy resistance in pancreatic cancer orchestrated by CAFs. The diagram situated in the upper left quadrant elucidates the precise molecular pathways through which CAFs instigate resistance to chemotherapy. Similarly, the diagram positioned in the upper right quadrant delineates the specific molecular mechanisms underpinning CAF-induced radiotherapeutic resistance. The diagram located in the lower section of the illustration delineates the intricate molecular interactions between CAFs, diverse immune cells, immune-related molecules, and the immunological microenvironment, elucidating the underlying causes of immunosuppression. Created with BioRender.com.

between IL-33 and CXCL3 levels and the survival of PDAC patients. It can be inferred that IL-33 and CXCL3 can serve as prognostic markers for PDAC.<sup>20</sup> Meanwhile, leucine-rich repeat-containing protein 15 (LRRC15) myCAF surrounds tumor islets and it is absent in normal tissues of PDAC. An immunotherapy clinical trial involving more than 600 patients found that elevated levels of LRRC15 myCAF signaling were associated with poor outcomes after anti-PD-L1 therapy. This suggests that LRRC15 myCAF may serve as a prognostic marker for immune checkpoint blockade therapy in PDAC.<sup>8</sup> Mizutani et al found that PSCs can be activated to become CAFs in PDAC and Meflin expression was detected in CAFs in the tumor stroma of both human PDAC and a PDAC mouse model. Moreover, Meflin CAFs could be used as a marker of a good prognosis in PDAC patients.<sup>51</sup> In a meta-analysis of 29 patients from 4000 studies, the combination of gibberellins and CAFs was found to result in a decrease in overall survival (OS) and disease-free survival (DFS) in patients with solid tumors. This suggests that gibberellins could serve as a prognostic marker with therapeutic potential.<sup>52</sup> In addition to the aforementioned studies, the staging of PDAC disease can be assessed by integrating multiple markers to actively monitor the status and its interconversion of CAFs. This approach is preferable to relying on a single marker as a prognostic criterion, as it provides more robust and dependable evidence for treatment.

## CAFs and Chemotherapeutic Resistance

The modifications in the extracellular environment induced by CAFs constitute a significant contributory element to the emergence of resistance to chemotherapy. Multiple mechanisms have been identified and verified, with the principal mechanism involving the creation of physical barriers that impede the infiltration of chemotherapy agents. The extracellular matrix barrier constructed by CAFs poses a physical obstacle to the transportation of drugs, thereby limiting drug delivery. The excessive expression of polysaccharides, particularly hyaluronic acid, by CAFs activated in PDAC,<sup>53</sup> leads to the development of densely stromal compartments characterized by elevated interstitial pressure. This heightened interstitial pressure, predominantly mediated by hyaluronic acid, is linked to vascular collapse and inadequate perfusion, resulting in alterations to the physiological functions within tumors and consequentially influencing drug delivery. Furthermore, the overexpression of hyaluronic acid is correlated with unfavorable prognoses in PDAC patients.<sup>54</sup>

In addition to external environmental alterations precipitating resistance to chemotherapy, intrinsic mechanisms of chemoresistance inherent to tumor cells can also be directly or indirectly modified by CAFs, thereby facilitating the survival of cancer cells under the influence of chemotherapy. Intrinsic mechanisms such as anti-apoptosis and enhanced survival capabilities in cancer cells are orchestrated through various signaling pathways, including RAS-RAF-MEK-ERK and PI3K-AKT. This interplay between cancer cells and CAFs is facilitated by the expression of diverse molecules (such as laminin, fibronectin, IGF, SDF-1 $\alpha$ , and basement membrane proteoglycans), triggering multiple downstream signaling pathways that bolster the survival of cancer cells.<sup>55–60</sup>

Numerous experiments have illustrated that chemotherapy can induce stromal reactions to promote tumor progression. These acquired resistance mechanisms, mediated through different pathways, furnish a theoretical underpinning for the failure of chemotherapy following prolonged clinical treatment. In vitro experiments have delineated molecular alterations and functional modifications associated with PDAC CAFs, following gemcitabine treatment and corroborated that CAFs subjected to chemotherapy assume a more supportive role in tumor promotion compared to their untreated counterparts.<sup>61</sup> Moreover, gemcitabine chemotherapy can prompt CAFs to release exosomes containing various tumor-promoting molecules.<sup>62</sup> Research has revealed that the generation and secretion of exosomal miRNA-106b and SNAIL1 can augment the viability and proliferation of recipient tumor cells.<sup>61,63</sup> Furthermore, miRNA-451a is abundantly present in the exosomal profile of PDAC CAFs.<sup>64</sup> This miRNA can modulate the drug transport protein P-glycoprotein,<sup>65</sup> potentially fostering chemotherapeutic resistance by exploiting this transport protein.

## CAFs and Radiotherapeutic Resistance

Another significant characteristic of CAFs is their ability to induce radio-resistance, which reduces the efficacy of radiotherapy in tumor treatment and ultimately promotes tumor growth.

Both in vitro and in vivo studies have shown that CAFs can hinder the response of cancer cells to radiation therapy through direct or paracrine interactions. For example, radiation therapy leads to increased secretion of HGF, CXCL12, and elevated c-Met phosphorylation of the HGF receptor in CAFs. This promotes proliferation, metastasis, and stimulates

EMT-associated drug resistance in PC cells.<sup>66,67</sup> In addition, radiotherapy induces CAFs to produce TGF- $\beta$ . This not only promotes the migration and potential metastatic escape of cancer cells but also enhances radio-resistance, ultimately leading to poorer survival outcomes in patients.<sup>68,69</sup> The proliferation of connective tissues in TME can also be mediated through integrin  $\beta$ 1 in cancer cells, as well as downstream FAK and MAPK-AKT signaling pathways, which are involved in radio-resistance.<sup>70</sup> The hypoxic tumor microenvironment generated by the pro-fibroproliferative response will further exacerbate radio-resistance in cancer cells.<sup>71</sup>

## CAFs and Immunosuppression

CAF assumes a central role in modulating the anti-tumor efficacy of immune cells within the tumor immune micro-environment (TIME).<sup>72,73</sup> Furthermore, they facilitate the expression of immune checkpoint molecules and ECM remodeling, thereby indirectly influencing the recruitment and function of immune cells.<sup>72</sup> Through the secretion of cytokines, chemokines, and other effector molecules, CAFs foster the initiation and progression of cancer while promoting ECM degradation and remodeling.<sup>74,75</sup> Numerous investigations have substantiated that the interplay between CAFs and immune cells, alongside other immune constituents, can govern the TIME, consequently impeding anti-tumor immune responses.<sup>76,77</sup>

### CAFs Inhibit Innate Immunity

The innate immune system of the human body is a sophisticated entity comprising macrophages, neutrophils, natural killer (NK) cells, antigen-presenting cells, mast cells, and their respective secreted molecules. CAFs, functioning as cells exerting immunosuppressive effects in tumors, can interact with various cellular molecules in the innate immune system, diminishing their anti-tumor effects and thereby promoting tumor progression.

Infiltrating macrophages within tumors, known as tumor-associated macrophages (TAMs), are divided into two distinct subgroups activated by different polarizing cytokines, termed M1 (induced by lipopolysaccharide alone or in conjunction with Th1 cytokines) and M2 (Th2 cytokines).<sup>78</sup> As a critical component of the TIME, TAMs play a crucial role in its regulation, particularly in tumor immune suppression.<sup>79,80</sup> TAMs are the most prominent immune cells near areas of CAF aggregation, indicating close interaction between these two cell types.<sup>81</sup> Increasing evidence suggests that tumor-associated neutrophils (TANs), as an important component of the TIME, also exhibit phenotypic heterogeneity and functional diversity.<sup>82,83</sup> Notably, CAFs may regulate neutrophil polarization. As recently reported in a hepatocellular carcinoma study, CAFs-derived cardiostrophin-like cytokine factor 1 (CLCF1) induces polarization of N2 phenotype neutrophils by upregulating CXCL6 and TGF- $\beta$  expression in tumor cells, thereby promoting tumor progression.<sup>84</sup> Furthermore, CAFs may be involved in all stages of malignant progression of TANs, ultimately inhibiting anti-tumor immune responses in the TME. By secreting SDF-1 $\alpha$ , CAFs can recruit peripheral neutrophils to the tumor.<sup>85</sup> In recent decades, increasing attention has been focused on the role of mast cells (MC) in cancer, rather than their role in allergic diseases.<sup>86,87</sup> Excessive presence of CAFs and MCs in the tumor stroma is closely associated with cancer invasiveness, and their interaction directly leads to tumor progression.<sup>88</sup> Natural killer (NK) cells are members of the innate immune system with natural responses to tumor cells.<sup>89</sup> Increasing evidence suggests that CAFs inhibit NK cells through various processes, including direct or indirect activation of NK cell receptors, cytotoxic activity, and cytokine production.<sup>90,91</sup> In recent years, several studies have shown that CAFs can drive immune escape of tumor cells by blocking dendritic cell (DC) maturation, antigen presentation, and related adaptive immune responses.<sup>92</sup>

### CAFs Inhibit Acquired Immunity

The adaptive immune system primarily comprises T lymphocytes, B lymphocytes, their respective activated types, and a myriad of cytokines, exhibiting extensive interactions with the innate immune system. It constitutes a pivotal component in tumor immunity, serving as the vanguard in tumor immune responses. CAFs employ various pathways to diminish the functionality of the acquired immune system, thereby effecting immune suppression.

T lymphocytes play a pivotal role in regulating acquired immune responses, encompassing diverse subgroups such as Treg cells, helper T (Th) cells, and cytotoxic T lymphocytes (CTLs).<sup>93</sup> Extensive research has elucidated the role of CAFs in modulating T cell activity and function. Treg cells characterized by high Foxp3 expression are known to play



a crucial role in curtailing anti-tumor immune responses.<sup>94</sup> Kinoshita et al employed immunohistochemical staining to confirm the proximity of Treg cells to CAFs. Furthermore, clinical data indicate an association between the infiltration of Foxp3<sup>+</sup> Tregs and CAFs in the tumor stroma and adverse prognosis.<sup>95</sup> These findings suggest potential crosstalk between CAFs and Treg cells. Some reports suggest significant effects of CAF-related activity on Th cell polarization, although their specific role remains unclear. For instance, when DNA vaccines target CAF-activating proteins, polarization of the Th2 subset is concurrently inhibited, suggesting that activated CAFs may promote the aforementioned differentiation.<sup>96</sup> Extensive research has reported interactions between CAFs and CD8<sup>+</sup> T cells, detailing CAFs' inhibitory effects on infiltration, proliferation, and anti-tumor immunity of CD8<sup>+</sup> T cells.<sup>97</sup>

### Interactions Between CAFs and Other Immune Components

In addition to directly influencing the innate and acquired immune systems, CAFs can achieve immune suppression by interacting with other immune components. Specific mechanisms involve upregulating the expression of cell surface immune checkpoint molecules on CAFs, inducing immune tolerance, and reshaping the ECM to promote immune suppression.

The heightened expression of immune checkpoint molecules on the surface of T cells and tumor cells has been identified as the primary cause of T cell dysfunction in the TME.<sup>98</sup> CAF itself can express various immune checkpoint molecule ligands on its cell surface, including PD-L1, PD-L2, B7-H3/H4, galectins, and IDO enzymes.<sup>99</sup> In addition to upregulating molecules on their own surface, CAFs also produce various types of cytokines and exosomes to upregulate checkpoint molecules on other cells, such as tumor cells and immune cells in the TME, indirectly inhibiting T cell function and anti-tumor immune responses. For example, it has been reported that CAFs in pancreatic cancer upregulate the expression of certain immune checkpoint molecules, including PD-1, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), T cell immunoglobulin, mucin domain-containing-3 (TIM-3), and lymphocyte activation gene-3 (LAG-3), on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, thereby inhibiting the proliferation of T cells and their specific recognition of tumor cells.<sup>100</sup>

Changes in ECM in the TME are a common phenomenon in tumor tissues, often associated with cancer progression.<sup>101</sup> Several studies have demonstrated the crucial role of CAFs in reshaping the ECM.<sup>102</sup> CAFs can promote the degradation of normal ECM structure by secreting various matrix proteins (such as fibronectin and type I collagen) and producing various MMPs, while increasing matrix stiffness.<sup>103</sup> The modified ECM, in turn, promotes CAF activation and tumor function. Increasing evidence suggests that CAF-induced ECM modifications are associated with cancer cell migration and invasion.<sup>104</sup> Moreover, this modified matrix is involved in inducing immune suppression in the TME. The ECM protein network reshaped by CAFs can act as a physical barrier to immune cells (especially T lymphocytes), thereby inhibiting their recruitment to cancer sites and reducing their opportunities to participate in TME immune responses.<sup>105</sup> Additionally, CAF-modified ECM can also regulate the activity of other immune cell populations. Aberrant carcinogenic collagen matrices are involved in the recruitment and function of TAMs.<sup>106</sup>

### Potential Targets of CAFs in PC

The abundant stromal components in pancreatic cancer tissues can not only promote pancreatic cancer invasion and metastasis but also limit drug infiltration and reduce the killing effect of drugs on cancer cells. CAFs are associated with PC development, metastasis, immunosuppression, and drug resistance in many aspects, so targeting CAFs is an increasingly important direction for the treatment of PC. With the advancement of nanotechnology, various nanocarriers with different sizes, structures, and surface properties are now available, allowing systemic or localized drug delivery. Besides, nanosystems have the advantage of directly targeting CAF-related targets, which has great potential in the treatment of pancreatic cancer. The potential targets of CAFs and their advantages and disadvantages are shown in [Table 2](#).

#### FAP

FAP is a fibroblast-activated protein produced by CAFs and an oncogenic factor. FAP is highly expressed in CAFs. It has long been customary to consider FAP as a better target for CAFs.<sup>117</sup> Yu et al achieved this goal by encapsulating paclitaxel-albumin nanoparticles (HSA-PTX) in CAP-modified heat-sensitive liposomes (CAP-TSL) and then adding the photothermal agent IR-TSL to form HSA-PTX@CAP-ITSL, resulting in the development of novel dual-responsive

**Table 2** Potential Targets and Advantages of CAF Treatment

Potential target	Advantage	References
FAP	HSA-PTX@CAP-ITSL kills tumor cells through the thermal effect and enhances the release of HSA-PTX and facilitates deep penetration into tumor tissues.	[107,108]
$\alpha$ -SMA	MMF-LA@DSPE-PEG significantly reduced the density of CAFs and exhibited higher anti-tumor activity.	[4,109]
IL-6 and its receptor	Chemotherapy can be improved by blocking IL-6 signaling which is to increase MAPK signaling activation and STAT3.	[110]
Sonic Hedgehog signaling	SHH pathway inhibition with the Smoothened antagonist LDE225 impairs tumor growth and reduces myCAF numbers and increases iCAF numbers, which correlates with a decrease in cytotoxic T cells and an expansion in regulatory T cells, consistent with increased immune suppression.	[111]
IL-1/LIF/JAK/STAT3 pathway	Tumor released IL-1 $\alpha$ and IL-1 $\beta$ and ASC are key regulators of TSLP secretion by CAFs and their targeting should ultimately dampen Th2 inflammation and improve overall survival in pancreatic cancer.	[112]
TGF- $\beta$	CAFs secrete large amounts of TGF- $\beta$ , which amplifies the stromal reaction and induces an autocrine signaling loop that maintains the differentiation of fibroblasts into myofibroblasts, which means that TGF- $\beta$ may be listed as a research target for PDAC treatment.	[113]
CXCL-associated axis	FAP <sup>+</sup> CAFs are the only tumoral source of chemokine (C-X-C motif) ligand 12 (CXCL12), and administering AMD3100, an inhibitor of chemokine (C-X-C motif) receptor 4, a CXCL12 receptor, also revealed the antitumor effects of an immunotherapeutic antibody and greatly diminished cancer cells.	[114]
Hyaluronic acid	Metastatic pancreatic ductal adenocarcinoma is characterized by excessive hyaluronan (HA) accumulation in the tumor microenvironment, elevating interstitial pressure and impairing perfusion. Preclinical studies demonstrated pegvorhyaluronidase alfa (PEGPH20) degrades HA, thereby increasing drug delivery.	[115]
EGFR	EGFR-activated myCAFs promote PDAC metastasis in mice, unmasking functional significance in myCAF heterogeneity. Finally, analyses of other cancer datasets suggest that these processes might operate in other malignancies. These data provide functional relevance to myCAF heterogeneity and identify a candidate target for preventing tumor invasion in PDAC.	[116]

nanoparticles. HSA-PTX@CAP-ITSL first accumulates at the tumor site and releases HSA-PTX through the cleavable CAPs that are responsive to FAP- $\alpha$ . Upon irradiation with near-infrared (NIR) laser light, the nanoparticle not only kills tumor cells through the thermal effect but also enhances the release of HSA-PTX and facilitates deep penetration into tumor tissues. The anti-tumor effects were verified by *in vivo* and *in vitro* experiments in this study.<sup>118</sup> Several studies have combined targeted therapy for CAFs with photodynamic therapy (PDT). Since FAP occupies an important position in CAFs-targeted therapy, an anti-FAP antibody and its humanized version, sibtrotuzumab, have been used in clinical studies for various experiments. However, after conducting preliminary studies, researchers discovered that the expression of FAP in normal tissues cannot be ignored.<sup>108</sup> Additionally, treatment with sibtrotuzumab resulted in severe side effects, highlighting the need for a more targeted approach to specifically target CAFs. Equally disappointing, however, is the variety of FAP-targeting agents, including bispecific antibodies, antibody-drug couplings, vaccines, and chimeric antigen receptor (CAR) T-cells, all of which are designed to eliminate FAP fibroblasts. But anti-FAP therapy leads to muscle loss, osteotoxicity, cachexia, and even death, which may be related to the tumor suppressor function of CAFs. Excessive depletion of CAFs leads to loss of tumor suppressor function, which leads to malignant outcomes.<sup>107</sup>

### $\alpha$ -SMA

$\alpha$ -SMA is a classical CAF membrane surface marker, MMF precursor (MMF-LA) by chemically derivatizing MMF with linoleic acid (LA) was constructed to develop MMF-LA@DSPE-PEG nanoparticles by encapsulating MMF-LA using DSPE-PEG2000. It was observed by immunofluorescence that MMF-LA@DSPE-PEG could aggregate in the  $\alpha$ -SMA-positive region. The results showed that MMF-LA@DSPE-PEG significantly reduced the density of CAFs and exhibited higher anti-tumor activity.<sup>109</sup>

Unfortunately, ablation of  $\alpha$ -SMA CAFs resulted in reduced survival in a PDAC animal model. This outcome was closely associated with an increase in Treg cells and a suppression of immune surveillance.<sup>4</sup>

### IL-6 and Its Receptor

iCAFs are characterized by low  $\alpha$ -SMA and high IL-6. IL-6 has the ability to inhibit NK cells and promote PDAC metastasis. In order to promote the development of PDAC by increasing mitogen-activated protein kinase (MAPK) signaling activation and to reduce the therapeutic response by inhibiting anticancer immunotherapy for PDAC in multiple ways, targeting IL-6 is a wise choice. Relevant clinical trial studies have demonstrated that IL-6 activates STAT3 and leads to chemo-resistance of PDAC in mouse models. Furthermore, the efficacy of chemotherapy in PDAC has been shown to improve after blocking IL-6 signaling.<sup>110</sup> As the most important marker of iCAF, IL-6 plays a very important role, and the inhibition of IL-6 or its receptor is significant in arresting tumor progression.

### Sonic Hedgehog (SHH) Signaling

A study found that the SHH signaling pathway is specifically activated in myCAFs. The antagonist LDE225 effectively inhibited SHH signaling, leading to a reduction in the number of myCAF and an increase in the number of iCAF in mice. This led to the accumulation of fibroblasts and immune infiltration in the PDAC microenvironment.<sup>111</sup> In another experiment, a combination of gemcitabine and the SHH inhibitor IPI-926 was used in a mouse model of PC. The results showed that the concentration of gemcitabine in the tumors increased and the treatment stabilized the disease process.<sup>119</sup>

### Other Relevant Targets

The IL-1/LIF/JAK/STAT3 pathway, TGF- $\beta$  and integrins, and the CXCL-associated axis are among the other targets. Biffi et al demonstrated that the IL-1/LIF/JAK/STAT 3 pathway promotes tumor progression and activates iCAF in the PDAC, thereby promoting tumor growth. Inhibiting this pathway can slow down tumor progression. Current experiments using Anakinra to inhibit IL-1R have shown prolonged overall survival in pancreatic cancer. This study also found that TGF- $\beta$  promotes the transformation from fibroblasts to myCAF. It listed TGF- $\beta$  as a research target for PDAC treatment as well.<sup>112</sup> Additionally, FAP<sup>+</sup>CAF secretes CXCL12, which leads to immunosuppression in PDAC. Inhibition of CXCR4, a receptor for CXCL12, using the inhibitor AMD3100 resulted in the accumulation of T cells in cancerous tissues, along with PD-L1 blockade, leading to a reduction in cancer cells in KPC mice.<sup>114</sup> Several studies have confirmed the effectiveness of these two inhibitory PDAC pathways.<sup>20,120</sup> CAFs also have many potential targets, such as hyaluronidase, ECM and so on. The multiple targets of CAFs offer various alternative pathways for treating pancreatic cancer by targeting CAFs. With the continuous advancement of biotechnology and chemical synthesis technology, an increasing number of suitable targets have been discovered and characterized. The discovery of these markers provides more options for nanomedicine research that targets CAFs. The study of specifically targeting certain types of CAFs, rather than all CAFs, is likely to address the drawback of over-depletion of CAFs that worsens the deterioration of PC. It can also preserve the tumor suppressor function of CAFs to some extent. This approach may provide a new direction for the treatment of PDAC and suggest a new perspective for future experimental studies.

## Nanomaterials for Targeting CAFs

### Efficient Drug Delivery Systems

As one of the most lethal and devastating human cancers, conventional treatments for PC are extremely ineffective. Therefore, nano-drug delivery systems are ideal for transporting anticancer drugs deep into cancerous tissues as well as reducing both damage and side effects against their healthy cells. Many nanomaterials have been used as delivery carriers, including inorganic particles such as silica particles, metallic nanoparticles (eg, gold, silver, and titanium), carbon nanostructures (eg, nanotubes, nanodiamonds, and graphene), organic materials such as liposomes, polymeric carriers (eg, micelles, hydrogels, polymersomes, dendrimers, and nanofibers), and hybridized nanomaterials.<sup>120</sup> Various nanomaterials are suitable for carrying different drugs because of their distinct properties. The specific categorization is shown in [Table 3](#).

Various nanomedicine delivery systems possess distinct advantages and drawbacks. As listed in [Table 3](#), within the realm of inorganic nanoparticles, exemplified by TGNS, their application as imaging agents or for molecular targeting

**Table 3** Classification of Nanotherapeutic Drugs and Their Effects

Kinds	Materials	Size	Type of Drug	Target Point	Highly Effective	References
Inorganic nanoparticles	Therapeutic Diagnostic Gold Nanoshells (TGNS)	810 nm	–	Neutrophil gelatinase-associated lipid transport protein (NGAL)	Molecularly targeted imaging and photothermal therapy can be achieved at concentrations more than two orders of magnitude lower than conventional gold nanoparticles.	[121]
	Loaded Fe@Au/ Gemcitabine Chitosan Nanoparticles (CGFe@Au Nps)	20–40 nm	Gemcitabine (gemcitabine)	PC Cells	When the system undergoes thermal therapy, 67% of the drug is released within 15 minutes, allowing for effective T2-weighted MRI imaging and excellent CT imaging contrast, sustained drug release and higher toxicity to PanCI cells.	[122]
	Iron-doped zinc oxide nanoparticles (Fe:ZnO NPs)	136.6 nm	Zinc ions Zn <sup>2+</sup>	Passive targeting	Cytocompatibility and internalization rate were higher than that of pristine ZnO NPs, which showed selective toxicity to tumor cells, affecting the cytoskeleton and cellular membranes, redox homeostasis, and genotoxic effects.	[123]
	Phosphates	10–80 nm	Drugs such as mitoxantrone, verapamil, doxorubicin, therapeutic agents such as proteins and enzymes	Folic acid, hyaluronic acid, etc.	High efficiency in combination with photodynamic therapy and magneto-thermal therapy induces an imbalance in Ca <sup>2+</sup> homeostasis.	[124]
Orous structure	Mesoporous silica nanoparticles	50–200 nm	Endogenous stimulus-sensitive drugs such as gemcitabine (gemcitabine) pirfenidone (PFD), etc.	Tumor cells or tumor vasculature or extracellular matrix (ECM)	Fine control of the release, maintaining the optimal drug concentration for a long period of time, avoiding the potential degradation of the drug, and simultaneous loading of multiple drugs to address multi-drug resistant tumors.	[125,126]
	Metal-organic framework (loaded with NV in ZIF-8)	102–160 nm	NV	PC Cells	Achieved about 73% cancer inhibition using NV-ZIFMCF in a 4T1 mouse breast cancer model. Achieves tumor-specific recognition (cell-specific membrane coating (CC) for targeted delivery), reduces off-target delivery and immune-related side effects, improves TME sensitivity to NV, prolongs retention of NV-ZIF within the tumor, and triggers tumor-specific immunity.	[127]

Organic nanoparticles	Polymer	103.4 nm	Oxaliplatin, irinotecan and 5-fluorouracil	Passive targeting	Nanoparticles containing multiple drugs have a synergistic effect, optimized biocompatibility, and reduced particle agglomeration, resulting in prolonged circulation time and improved half-life of the drug in the in vivo circulation.	[128]
	Dendritic nanomicelles	<20 nm	Gemcitabine	PC Cells	High drug loading capacity, small size, stable formulation, low toxicity, and acid-promoted drug release are promising novel drugs that can enhance cellular uptake in an effective and sustained manner. With a high drug loading capacity of 33%, gemcitabine nanoformulations show more potent anticancer activity in vitro and in vivo compared to free gemcitabine.	[129]
	Liposomes	146–188 nm	Berbamine, sodium cromoglycate, gemcitabine and other	S13P proteins	The drug encapsulation efficiency was 28%, which inhibited the viability, proliferation, migration and invasion of pancreatic cancer cells while promoting apoptosis.	[130,131]
Hybridized nanoparticles	Magnetic liposomes (MLs)	44–73 nm	Superparamagnetic iron oxide nanoparticles encapsulated in a phospholipid bilayer	Passive targeting	Prolonged circulation time, comparable tumor deposition to liposomes of the same composition, good MRI T2-contrast agent, tumor retention up to 1 week.	[132]
	Lipid bilayer-coated mesoporous silica nanoparticles	<83 nm	Oxaliplatin (OX) and indolimod (IND, an IDO inhibitor)	Passive targeting	Dual-delivery vehicle to achieve potent anti-PDAC disruption, with IND prodrugs acting synergistically with OX at PDAC tumor sites to induce immunogenic cell death (ICD).	[133]
	PLGA-based magnetic polymer nanoparticles USPIO@CKAAKN-PEG-PLGA nanoparticles	(104.5 ± 4.1) nm	PC cells	CKAAKN peptide	The modification of CKAAKN peptide increased USPIO binding capacity, enhanced the relative fluorescence intensity of BxPC-3 cells, and significantly enhanced tumor specificity.	[134]



imaging and photothermal therapy can achieve efficacy at concentrations over two orders of magnitude lower than conventional gold nanoparticles. Nonetheless, they are linked to numerous adverse biological outcomes including inhibiting bacterial iron uptake, renal injury, and mediating inflammatory activity.<sup>121</sup> Another illustration of inorganic nanoparticles is phosphate, which when employed as a drug delivery medium, can effectively integrate photodynamic therapy and magnetic hyperthermia, thereby inducing  $\text{Ca}^{2+}$  homeostasis imbalance to augment tumor cytotoxicity. However, the substantial size of these particles and the intricate synthesis process pose significant impediments to their prospective clinical utility.<sup>124</sup> Among porous structure materials, Mesoporous silica nanoparticles emerge as a quintessential example. They provide meticulous control over drug release, sustained maintenance of optimal drug concentrations, prevention of potential drug degradation, and the capacity to accommodate multiple drugs to surmount tumor multidrug resistance. Nevertheless, this type of nano-carrier may harbor inherent toxicity and may not be completely eliminated from the body via hepatic and renal routes.<sup>125</sup> Dendritic nanomicelles, a pivotal class of diminutive organic nanoparticles, showcase notable characteristics including high drug loading, diminutive size, stable formulations, low toxicity, and acid-triggered drug release. These attributes render them auspicious candidates for novel drugs that can efficaciously and continuously enhance cellular uptake. Preparations concomitantly composed of dendritic nanomicelles and gemcitabine nanoagents exhibit a substantial drug loading capacity of 33%. Comparative to free gemcitabine, they evince enhanced anticancer efficacy both *in vitro* and *in vivo*. Nevertheless, these materials manifest cytotoxicity towards normal cells, thereby impeding their clinical application.<sup>129</sup> Lastly, within the domain of Hybridized nanoparticles, MLs are prominently discussed materials. Their utilization as carriers significantly prolongs circulation time and achieves substantial tumor deposition comparable to liposomes of analogous composition. They function as superb MRI T2 contrast agents, persisting in tumors for up to one week. However, the clinical application of this substance entails certain limitations. Relative to traditional hyperthermia, the technology for controlled release utilizing low-frequency magnetic fields remains nascent. Consequently, further elucidation of its release mechanism and optimization of magnetic field conditions are imperative.<sup>132,135</sup>

Among the common nanomaterials used for PC treatment and detection, liposomes are the first nanoparticles approved as carriers for cancer therapy. The lipid bilayer of liposomes is very similar to that of mammalian cell membranes in terms of both composition and structure. This property contributes to better targeting the PC cells and being uptaken by them.<sup>136</sup> Liposome nanoparticles carrying gemcitabine have been confirmed to be more effective than the drug alone in the treatment of PC in mice.<sup>134</sup> Meanwhile, Liposome-encapsulated paclitaxel for the treatment of PC is under study.<sup>137</sup> Kim et al found that coupling liposomes with polymeric compounds, such as polyethylene glycol, enhanced the cytotoxicity of liposomes and improved their delivery to target sites.<sup>138</sup> A new type of liposomal nanoparticles loaded with 1, 3-disubstituted hydrofuran-2yl-5FU (MFU), which is called Zhubech liposomal nanoparticles, is currently under investigation. They have enhanced anticancer effects under normal physiological conditions. However, methods to enhance their stability need to be explored in the future due to drawbacks such as poor retention and a short half-life.<sup>139</sup> Hybridized nanoparticles that combine liposomes with magnetic nanomaterials have been shown to exhibit improved therapeutic efficacy. These nanoparticles not only serve as a traceable alternative to MRI, but also increase TME permeability and drug perfusion, and enhance the deposition of liposomal drug carriers at the organ and tissue levels.<sup>78</sup> Recent studies have found that cationic polymer nanoparticles (cNPs) can be excreted in the form of extracellular vehicles (EVs), encapsulated within cNP@EVs. This suggests that cNP@EVs are an effective form of nanoparticles for intercellular transport and have the potential to be used as an efficient biomimetic drug delivery system.<sup>140</sup>

## Response Mode of Nano-Drug Delivery System

The high efficiency of a nano-drug delivery system is demonstrated not only by its ability to efficiently target and deliver drugs to the tumor site, but also by the design of an appropriate stimulus response system that can achieve the goal of time, space, and dose control of the nano-drug delivery system, resulting in a more efficient therapeutic effect. The nanosystem response can be categorized into endogenous stimulus response and exogenous stimulus response.<sup>5</sup>

Endogenous stimulation of nanosystem responses includes pH response and enzyme stimulation response. The pH response of nanosystems is achieved through the acidic nature of TME in PC. This property allows the nanosystems to release the drug controllably at specific pH conditions, which not only protects the drug from the harsh conditions in the

gastric lumen but also improves its absorption in the intestine.<sup>140</sup> The stimulation of nanosystems by enzymes refers to the ability of nanoparticles to be recognized and broken down by specific enzymes. For example, pancreatic elastase IIIA and its variants, as well as amyloidogenic proteases, are highly expressed in the pancreas. By utilizing a sensitive nanoparticle drug-carrying system, when these nanoparticles enter pancreatic cancer tissues, proteolytic enzymes hydrolyze the nanoparticles and release the drug. This process increases the concentration of the drug at the tumor site, thereby enhancing the therapeutic effect.<sup>141,142</sup>

Exogenous stimuli activate the nanosystem response through temperature, magnetic, ultrasound, light, and electrical responses. Temperature-responsive nanosystems maintain their payload under body temperature (approximately 37°C) and deliver drugs rapidly within a locally heated tumor (40–42°C) to counteract the rapid blood passage time and tumor washout. Thermoresponsive systems typically consist of liposomes, polymeric micelles, or nanoparticles. Among these, thermosensitive liposomes (TSLs) are probably the most advanced thermoresponsive nanosystems, currently being investigated for their potential use in treating breast cancer, liver metastases from colorectal cancer, and further experimental studies are needed for their application in PC.<sup>143</sup> Modification of magnetic materials on the surface of nanoparticles can be directed to the target region in vivo through the application of an external magnetic field, enabling localized and controlled drug release. For example, Indocyanine Green (ICG) carrying iron oxide and NIR dye can specifically target PC cells expressing the early disease marker NGAL. When coupled with NGAL, TGNS can enable molecularly targeted imaging and targeted photothermal therapy through in vitro NIR photothermal therapy.<sup>144</sup> Other external stimuli, such as ultrasound, light, and electricity, can also stimulate the response of nanosystems.<sup>5</sup> The choice of these nanosystem response modalities depends on the specific therapeutic needs and goals. Different response modalities can achieve precise treatment for pancreatic cancer with varying characteristics.

## Nano-Drug Delivery System Acting on PC-Associated CAFs

CAFs are densely distributed in pancreatic tumor sites, and their presence can also be detected in some benign tissues and ducts.<sup>145–147</sup> Emerging studies have emphasized the role of CAFs in PC, revealing their mechanism of action in the development of drug resistance. Specifically, CAFs contribute to the development of drug resistance and the hindering effect in drug delivery by upregulating the expression of  $\alpha$ -SMA, increasing the secretion of vascular endothelial growth factor (VEGF), and releasing pro-angiogenic molecules.<sup>148</sup> Considering these findings, therapeutic strategies that target CAFs have been considered to have potential therapeutic benefits, especially in the treatment of pancreatic cancer. Targeting CAFs via nano-drug delivery systems may offer a potential solution to overcome drug resistance and improve the effectiveness of pancreatic cancer treatment. The implementation of this strategy is expected to yield more positive outcomes for patients with pancreatic cancer.

Proliferation and activation of CAFs are prerequisites for driving tumor progression and drug resistance. Therefore, intervening in this process may become an important strategy to achieve tumor suppression. A novel approach was developed to establish biodegradable polymer nanoparticles targeted towards CAFs. These nanoparticles were loaded with  $\alpha$ -Mangostin and coated with the CREKA peptide.  $\alpha$ -Mangostin is known to regulate TME by interfering with the TGF- $\beta$ /SMAD signaling pathway and blocking the activation of CAFs. On the other hand, the CREKA peptide exhibits a specific affinity for fibronectin, which is overexpressed on the membrane surface of CAFs. Thus, the peptide coating of nanoparticles enhances the uptake of nanoparticles by CAFs. Additionally, the combined application of  $\alpha$ -Mangostin strengthens the inhibitory effect on CAFs, thereby remodeling the TME by compromising the matrix barrier.<sup>149</sup>

The formation of CAFs is associated with two types of cells: fibroblasts, which are important components of CAFs, and pancreatic stellate cells, which serve as precursor cells to tumor-associated fibroblasts. Fibroblast formation is influenced by TGF- $\beta$ 1, which increases levels of reactive oxygen species (ROS) and  $\alpha$ -SMA, and is inhibited by antioxidants.<sup>150</sup> Ariely et al found that cerium oxide nanoparticles were able to regulate fibroblast formation and reduce  $\alpha$ -SMA levels, thereby inhibiting tumor cell infiltration. These results suggest that cerium oxide nanoparticles may be an effective and safe therapeutic strategy.<sup>151</sup> Mardhian et al developed a superparamagnetic iron oxide nanomaterial by modifying relaxin-2. This nanomaterial inhibits the differentiation of pancreatic stellate cells by suppressing SMAD2 signaling. It reveals that relaxin-2 nanoparticles could facilitate targeted drug delivery and inhibit collagen deposition.<sup>152</sup> In addition, other studies have designed an injectable peptide hydrogel nanoparticles with achieving self-assembly and

losartan encapsulation. These nanoparticles were successfully retained for several days in a mouse tumor in situ model and inhibited the levels of collagen and CAFs.<sup>153</sup> A study reported a co-delivery and pH-sensitive nanoparticle system consisting of p-GEM and paclitaxel nanoparticles. This system could not only deliver drugs but also target deeper layers of the matrix. It was confirmed that these nanoparticles were able to specifically reduce  $\alpha$ -SMA levels in tumor tissues while destroying cancer cells without affecting the extracellular matrix.<sup>154</sup> Thus, these nanoparticles exhibit higher efficiency in drug delivery and specific targeting characteristics.

Despite evidence that CAFs act as a barrier to antitumor therapy, recent studies have shown that specific subsets of CAFs can have antitumor effects under certain circumstances. It was found that quiescent fibroblasts may differentiate into cancer-inhibiting (F1 subtype) and cancer-promoting (F2 subtype) CAFs, depending on the stage of tumorigenesis. If CAFs are directly eliminated, it may disrupt their homeostasis in the body and lead to tumor progression.<sup>7</sup> Consequently, placing CAFs in an inactivated state or converting them to oncogenic phenotypes may be a safe and effective treatment. Several studies have attempted to reprogram CAFs at the transcriptional level. For example, Kim et al determined in a study that the small molecule Scriptaid, an HDAC 1/3/8 inhibitor, reduced the TGF- $\beta$ -induced differentiation of CAFs. This also resulted in the reduction in ECM secretion, cell invasiveness and stiffness in preclinical animal models.<sup>155</sup> Albregues et al demonstrated the preclinical efficacy of restoring CAFs to a wild-type phenotype by targeting the pro-inflammatory cytokine LIF. This was achieved using a DNA methyltransferase (DNMT) inhibitor and a JAK inhibitor.<sup>156</sup> Another study identified the methyltransferase nicotinamide N-methyltransferase (NNMT) as a central regulator of CAF activation in the TME. Treatment with NNMT inhibitors alone reduced the tumor burden in a mouse model. In addition, kinases involved in the complex signaling network of CAFs may also act as potential targets for inhibiting the function of CAFs.<sup>157</sup> A study found that the multikinase receptor inhibitor Nintedanib downregulated the induction of collagen and  $\alpha$ -SMA in TGF- $\beta$ 1-stimulated fibroblasts.<sup>158</sup> Experiments investigated the effects of inhibiting the JAK2/STAT3 and MEK/ERK1/2 pathways using ruxolitinib and trametinib to reverse the activation of a subset of pro-tumor CAFs. They observed an increase in tumor tissue response to etoposide after inhibiting JAK2/STAT3 and MEK/ERK1/2 via using ruxolitinib and trametinib. In the meantime, there was an increase in overall survival in hormone-treated mice.<sup>159</sup> The study by Ford et al focused on the inhibition of NOX4, a ROS-generating enzyme that is a downstream target of TGF- $\beta$ 1 and regulates the CAF phenotype. siRNA knockdown or pharmacological inhibition GKT137831 (Setanaxib) of NOX4 “normalized” CAFs to a quiescent phenotype and promoted intratumoral infiltration of CD8<sup>+</sup> T cells, thereby overcoming the immunosuppressive effect. These findings suggest that inhibiting NOX4 can effectively overcome CAF-mediated immunotherapy resistance and potentially improve outcomes in various types of cancers.<sup>160</sup> Miao et al discovered that nanoparticles loaded with a plasmid encoding secreted tumor necrosis factor (TNF)-related apoptosis-inducing ligand (sTRAIL) triggered apoptosis in tumor cells located near CAFs in a mouse xenograft model. Interestingly, it restored the remaining CAFs to a quiescent state, remodeled the TME, and further inhibited tumor growth, thus facilitating a second wave of nanotherapeutics.<sup>161</sup> A TME-responsive nano-system based on PEGylated polyethylenimine-coated gold nanoparticles was developed. This system was used to simultaneously deliver all-trans retinoic acid (ATRA), which induces quiescence in PSCs, and siRNA targeting heat shock protein 47 (HSP47), a collagen-specific molecular chaperone, for remodeling of PSCs. This nanosystem concurrently induces quiescence in PSC and inhibits ECM proliferation, which facilitated drug delivery to PC and significantly enhancing the antitumor efficacy of chemotherapeutic agents.<sup>162</sup>

In summary, various strategies have been utilized in preclinical and clinical research to target the tumor-promoting effects of CAFs. These approaches include efficient depletion of TME and inhibition of signaling pathways, with the aim of making significant advancements in the therapy of CAFs tumors. However, the heterogeneity of the CAFs population in the TME poses a significant challenge in terms of the necessity to “eliminate the tumor-promoting population without completely abolishing their anti-tumor properties”. As nano-drug delivery systems and methods for reprogramming CAFs continue to be advanced, we expect greater success in related therapeutic areas in the coming years.

# Feasibility of Using Nanosystems to Target CAFs for the Treatment of PC and Proposal of New Strategies

## Comparison with Conventional Treatments

The therapy of PC, with high degree of malignancy, poor prognosis and high mortality rate of PC, has shown little improvement in recent decades.<sup>163</sup> Nowadays, the treatment of PC mainly includes surgery, chemotherapy, radiotherapy, targeted therapy and combined treatment options.<sup>164</sup> Most patients with pancreatic cancer are typically treated with chemotherapy. However, due to the presence of vascular infiltration and distant metastasis, only a small percentage (less than 15%) of these patients are eligible for surgical removal.<sup>165</sup> Despite ongoing efforts to improve prognosis, the 5-year survival rate for pancreatic cancer remains extremely low.<sup>166</sup> Therefore, it is urgent to explore new therapeutic approaches. Nanoparticle-targeted therapy for pancreatic cancer has been rapidly developing in recent years and offers unique advantages over traditional treatment options. The following aspects are compared to introduce a novel treatment option using nanosystems that target CAFs to treat PC.

**Targeting and biosafety:** We can achieve highly specific recognition and targeted delivery of drugs to PC tissues or even specific subcellular organelles by modifying the targeting ligands on the surface of the particles.<sup>6,167</sup> The utilization of nanosystems to specifically target CAFs can either damage PC cells by delivering PC-sensitive drugs or remodeling the TME to diminish its protective effects on PC. Nano-drug delivery systems, such as liposomes, have been widely used in the treatment of PC. Nanoparticle-targeted therapy can achieve localized release of drugs and reduce damage to normal tissues, leading to minimizing toxic side effects.<sup>164</sup> In contrast, conventional surgery, chemotherapy, and radiotherapy are poorly targeted and often result in systemic side effects, such as infection, nausea, vomiting, and hair loss. Immunotherapy may cause side effects related to the immune response, such as inflammatory reactions and autoimmune diseases.<sup>168</sup>

**Drug delivery efficiency and control of recurrent metastasis:** Nanoparticles can cross the vessel wall and enter the TME and metastatic foci by altering their shape, size and surface properties. They can also enhance the solubility and stability of the drug, thereby improving the efficiency of drug delivery in tumor tissue. Many nanoparticle delivery systems for PC CAFs have been reported, such as the utilization of nanoparticles for targeted drug delivery.<sup>109</sup> Nanosystems can also be designed to activate nanoparticles by designing response systems that enable precise release of drugs in time, space, and dose.<sup>5</sup> It is due to these features that nanosystems have more efficient and accurate drug delivery, as well as greater control over the recurrence and metastasis of PC, compared to traditional therapeutic methods.

The specific clinical trials of nanoparticle targeted CAFs for the treatment of pancreatic cancer are shown in Table 4. In these experiments, the delivery system targeting CAFs mediated by nanoparticles has exhibited various effective anti-tumor effects. These effects include reducing the percentage of poorly to moderately differentiated tumor phenotypes, enhancing apoptosis as well as decreasing the expression of collagen,  $\alpha$ -SMA, and Glioma-associated oncogene-1 (GLI-1) in tumor tissues. Jonas Schnittert et al devised novel peptide-based nanocomplexes (NCs) for delivering anti-miRNA oligonucleotides to human-derived pancreatic stellate cells (hPSCs) and inhibiting their differentiation into a tumor-promoting CAFs phenotype. This protocol may show significant and long-term anti-tumor effects in stromal-rich PC models.<sup>169</sup>

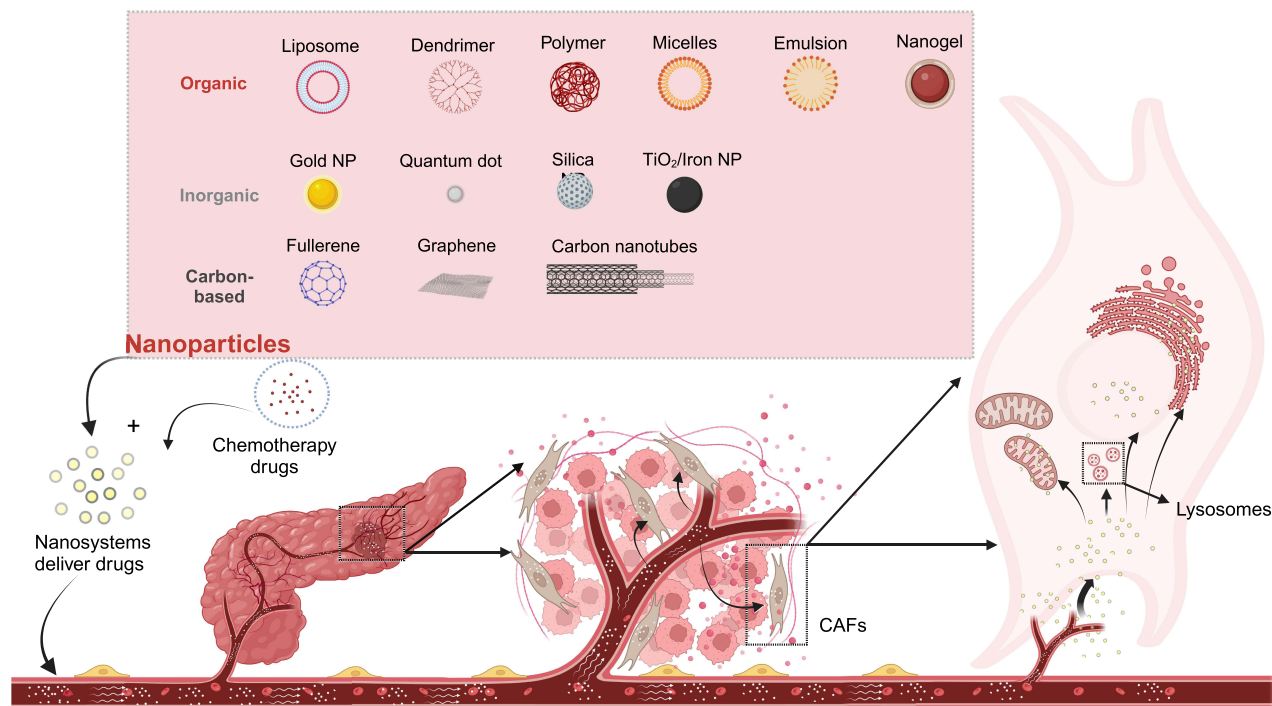
## Nanoparticle Targeting of CAFs and Organelles with a View to Obtaining Enhanced Anti-Tumor Efficacy

Conventional treatments do not significantly contribute to the survival of patients with PC due to drug resistance and toxicity to normal tissues. In order to achieve better results, nanoparticles are dual-modified so that they can accurately deliver the drug to CAFs and target the nanoparticles internalized by the CAFs to specific organelles. As a result, the appropriate concentration of the drug is delivered directly to the specific site of action (Figure 3). Nanoparticles can impact the function of different organelles. For instance, biological selenium nanoparticles (SeNPs) can safeguard against intestinal barrier dysfunction by influencing endoplasmic reticulum stress (ERS) and mitochondrial autophagy-related AMPK signaling pathways.<sup>174</sup> Nano-drug delivery systems targeting mitochondria, nucleus, lysosomes, and Golgi have been investigated.<sup>6</sup> For example, Yu et al constructed a pillar arene-based rotaxane (R1) with tetraphenylene (TPE) and triphenylphosphine (TPP) groups as plugs. The nanoparticles loaded with DOX formed by this construction are able to release significant amounts of DOX after being internalized by HeLa cells and the DOX will enrich in the mitochondria

**Table 4** Clinical Trial Proves Feasibility of Nanoparticles Targeting CAFs in Pancreatic Cancer

Therapeutic regimen (type + drug)	Size	Target	Mode of administration	PC type	Outcome	References
PEG5K-P(HEMASN38)x vismodegib + irinotecan	70 nm	Passive targeting	Intravenous injection	Pancreatic ductal adenocarcinoma	CAFs depletion + chemotherapy	[170]
M-CPA/PTX Cyclophamide + paclitaxel	(45.2 ± 4.5) nm	Passive targeting	Intravenous injection	Pancreatic ductal adenocarcinoma	CAFs depletion + chemotherapy	[171]
RLX-SPOIN relaxin-2	(61.16 ± 3.07) nm	Passive targeting	Intravenous injection	Pancreatic cancer	ECM remodeling + chemotherapy	[172]
DGL/GEM@PP/GA 18β-glycyrrhetic acid + gemcitabine	(151.1 ± 6.3) nm	Passive targeting	Intratumoral injection	Pancreatic cancer	ECM remodeling + chemotherapy	[169]
Polymeric delivery system (cellax) + docetaxel	120 nm	SPARC	Intravenous injection	Pancreatic cancer	CAFs depletion + chemotherapy	[173]
Peptide delivery system (dimeric CPP) + anti-miRNA -199α	< 40 nm	CPP receptor	Processing cells	Pancreatic ductal adenocarcinoma	Inhibited the differentiation of PSC into CAFs	[174]

to kill cancer cells.<sup>6</sup> The strategy of using lipid nanoparticles modified with peptides and biofilm encapsulation to target the endoplasmic reticulum and mitochondria has been employed for targeted therapy in breast, cervical, and lung cancers. This strategy not only holds the promise of overcoming multidrug resistance (MDR) but also of amplifying the



**Figure 3** Drug delivery via nano-drug delivery system acting on pancreatic cancer-associated CAFs. A variety of nanoparticles (in combination with each other or in combination with chemotherapeutic agents) are assembled into nano-drug delivery systems that are capable of aggregating towards the pancreas upon entry into the body. They are able to penetrate the TME with relative ease and target CAFs and the organelles within them. This approach may have the effect of interfering with the promitogenic function of CAFs to treat pancreatic cancer. Created with BioRender.com.



therapeutic effect by sharing drugs and/or signaling molecules to achieve crosstalk between endoplasmic reticulum and mitochondria.<sup>173</sup> Nanoparticle-targeted CAFs in combination with chemotherapy, can enhance anti-tumor efficacy by specifically targeting CAFs or even subcellular organelles in the TME. This approach disrupts the interactions between tumor cells and their interactions, ultimately increasing the sensitivity of tumor cells to chemotherapy.

## Nanoparticle-Targeted CAFs Combined with Radiotherapy and Immunotherapy

Conventional chemotherapy has significant side effects and is being used more frequently as an adjuvant therapy. Radiotherapy is a commonly used adjuvant therapy for PC. However, its efficacy is often limited by tumor hypoxia-induced radiation resistance. Additionally, radiotherapy is associated with severe side effects and carries a high risk of recurrence and metastasis.<sup>175,176</sup> Nanomedicines synergize effectively with standard chemotherapeutic treatments. Recently, the combination of liposomal irinotecan (Onivyde<sup>®</sup>) with 5-fluorouracil (5-FU) and calcium folinate (LV) has received approval for systemic combination therapy in PC.<sup>177</sup> Currently, nanoparticles (NPs) are being increasingly used for radiosensitization. Chen Q made poly (lactic-co-glycolic acid) (PLGA)-based core-shell nanoparticles by encapsulating water-soluble catalase (Cat) in the core and loading hydrophobic imiquimod (R837) in the PLGA shell. The PLGA-R837@Cat nanoparticle that could alleviate tumor hypoxia and significantly improve the efficacy of radiotherapy.<sup>178</sup> In addition to metal nanoparticles such as Cu, Bi, and Gd, there has been rapid development of nano-delivery carriers capable of delivering tumor-specific radiosensitizing drugs in recent years. These carriers have the potential to enhance immune checkpoint blockade and improve radiosensitization.<sup>179</sup> Gao et al developed a new mitochondria-targeted nanoplatfrom called hydrogel-based plasmonic nanosensors. This nanoplatfrom can be utilized for the combined radiotherapy of tumors by simultaneously inhibiting dual-energy metabolism. The study demonstrated that the nanosensors have excellent radiosensitization effects, enhancing the effectiveness of radiotherapy.<sup>180</sup> Proton therapy has unique dose deposition properties which can protect normal tissues and improve patient prognosis.<sup>181</sup> An important reason for the high lethality of PC is its acquired immunosuppressive privilege. Therefore, immunotherapy, which can disrupt this immunosuppressive privilege, holds promise as an approach to treating PC. This includes the utilization of immune checkpoint inhibitors, therapeutic vaccines, engineered T-cells and the advancement of prophylactic vaccines.<sup>182</sup> However, there are certain challenges associated with immunotherapy, such as autoimmune responses, cytokine syndromes, and vascular leakage syndrome.<sup>182</sup> The combination of immunotherapy with other treatments can modulate the immune response of tumor cells and produce synergistic therapeutic effects. A significant portion of nanomedicines can be targeted to modulate the adaptive immune system.<sup>183</sup> CAFs can promote the presence of immunosuppressive cells in the TME by secreting pro-inflammatory cytokines and chemokines.<sup>184,185</sup> Nanoparticles can convert immunosuppression in TME to an immunosupportive state by targeting CAFs, which play an important role in immunotherapy for PC. The main principle behind nanosystems in conjunction with immunotherapy is to deliver checkpoint inhibitors that can suppress immune checkpoints. Therapeutic peptide-assembled nanoparticles, which are antagonists of D-peptide programmed cell death ligand 1 (DPPA-1), co-assembled with NLG919, an inhibitor of indoleamine 2, 3-dioxygenase 1 (IDO-1), inhibited both immune checkpoints and tryptophan metabolism. Additionally, they increased cytotoxic T-lymphocyte (CTL) activation and survival, resulting in effective anti-tumor immunity.<sup>186</sup> It has been shown that targeting hyaluronan synthesis by incorporating inhibitors into nanocarriers can result in ECM remodeling and enhance infiltration of  $\gamma\delta$ -T cells.<sup>187</sup> pH-responsive nanomicelles (P/A/B@NM) containing paclitaxel (PTX), the CXCR1 antagonist AMD1, and the PD-4/PD-L1 inhibitor BMS-1 are used to activate the T-cell-mediated anti-tumor immune response. These nanomicelles can remodel the tumor stroma mediated by CAFs and the immunosuppressive microenvironment. As a result, CD8<sup>+</sup> T-cell infiltration in breast cancer is enhanced, leading to the reactivation of anti-tumor immunity in unresponsive triple-negative breast cancer (TNBC) cases.<sup>188</sup> Lipid poly- $\gamma$ -glutamic acid (PGA)/PolyMet-pRLN nanoparticles (LPPR) can rapidly penetrate tumors and weaken the proliferation of CAFs, remodeling the TME, and decreasing the infiltration of immunosuppressive cells to achieve efficient breast cancer immunotherapy.<sup>189</sup>

Tumor immunotherapy and radiotherapy are the best partners based on nanosystems governance. Radiotherapy has certain immunostimulatory effects, and the synergistic effect of the two is beneficial for tumor control.<sup>190</sup> Guan et al developed a novel modality combining radiotherapy and immunotherapy with a unique nanosystem based on the

IPI549@HMP. They used subcutaneous melanoma in mice as a model and achieved a highly effective synergistic effect. This approach not only induced systemic anti-tumor immune memory but also demonstrated minimal toxicity.<sup>191</sup> Chen Q fabricated multifunctional PLGA-R837@Cat nanoparticles that could be used for combination radioimmunotherapy of cancer. Cat is able to reduce the degree of tumor hypoxia and modulate the tumor's immune-suppressing microenvironment to enhance the efficacy of radiotherapy. Additionally, R837 can further stimulate strong immune responses in residual tumors after radiotherapy-induced ICD.<sup>178</sup> There is also an increasing number of nanoparticles that have been designed to be more conducive to combining radiotherapy and immunotherapy to achieve better therapeutic effects. For example, bacterial membrane-encapsulated nanoparticles (BNPs), natural herb *Astragalus* polysaccharide NPs (ANPs), and multifunctional core-shell PLGA nanoparticles have been shown to overcome tumor hypoxia-related radio-resistance.<sup>175</sup>

## Clinical Application and Prospects of Nano-Drug Delivery Systems

In recent years, significant efforts in scientific research and clinical trials have aimed to refine therapeutic approaches to enhance the quality of life and prolong the survival of pancreatic cancer patients. The treatment strategies for PDAC can be broadly classified into three categories based on their specific targets. Firstly, interventions directly addressing CAFs. For instance, certain compounds such as ATRA and vitamin D analogs have demonstrated the ability to modulate various cellular pathways within CAFs, leading to a partial reversal of their activated state to a more quiescent state. Secondly, strategies involve inhibiting soluble proteins in the TME that contribute to CAF-mediated signaling. This includes blocking the production and secretion of tumor-promoting or immunosuppressive factors by CAFs, as well as inhibiting the activation of PSCs and normal fibroblast growth factors. Thirdly, approaches focus on suppressing cell surface receptors through which CAFs communicate with other cell types.<sup>26</sup> In castration-resistant prostate cancer (CRPC), communication between TAMs and the bone microenvironment (BME) plays a significant role in disease development. Ongoing research targeting the overexpression of CAF-related proteins such as  $\alpha$ 5 $\beta$ 1 Integrin and Metalloproteinase Domain 9 (ADAM9) and CAFs Monoamine Oxidase A (MAO-A) has shown promising progress in CRPC therapy. Immunotherapies directed against CAFs within the TME or BME hold crucial potential in the treatment of metastatic CRPC patients.<sup>192</sup>

Several chemotherapy formulations delivered through nanocarrier systems have progressed through various stages of clinical trials, with some reaching Phase III and nearing approval. These formulations encompass a range of nanoparticles, including albumin-bound paclitaxel nanoparticles, pathogenic nanoparticle-based gene delivery systems, micelle nanoparticles, and liposome nanoparticles.<sup>193</sup> For instance, liposomes, polymer nanoparticles, and inorganic nanoparticles exhibit precise targeting of the melanoma tumor microenvironment, offering potential in targeted drug delivery for melanoma therapy while enhancing the efficacy of conventional drugs and reducing toxicity.<sup>194</sup> Regin-G, comprising nanoparticles/gene delivery vectors based on a pathogenic retrovirus, was evaluated by Gordon et al in pancreatic cancer treatment in the Philippines. The study demonstrated tumor stability and growth inhibition in two-thirds of patients without dose-limiting toxicity.<sup>195</sup>

Circular RNAs (circRNAs) transmitted via exosomes have been identified as significant contributors to chemotherapy resistance in cancer by influencing the tumor or immune microenvironment.<sup>196</sup> Additionally, interference-based RNA (iRNA) therapy has emerged as a promising alternative in pancreatic cancer treatment, exhibiting progress in specificity, toxicity, and overcoming resistance to existing drugs. iRNA therapy, an endogenous process involving RNA sequences that complement genes in the body acting through corresponding mRNA, faces challenges in efficient siRNA delivery into target cells due to stability and degradation issues.<sup>197–199</sup> Nanoparticle-based delivery systems, including liposomes, lipid polymers, and dendritic polymer-based nanoparticles, offer potential solutions by encapsulating siRNA, protecting it from degradation, and facilitating targeted delivery to specific cells.<sup>200–202</sup>

Furthermore, nanoparticle-based systems present opportunities for both direct and indirect immune modulation in pancreatic cancer. Direct modulation involves interactions between nanodrug delivery systems and immune cells, influencing their activation and function. These nanoparticles serve as carriers for immune stimulants, enhancing immune responses against pancreatic cancer cells. Indirectly, nanoparticle delivery systems target the tumor microenvironment, delivering therapeutic molecules to reprogram immunosuppressive cells and enhance anti-tumor immune responses.

Leveraging the potential of nanodrug delivery systems, combined with emerging molecular biology approaches and innovative immune modulation strategies, holds promise in fundamentally altering the therapeutic landscape of pancreatic cancer.<sup>203,204</sup>

## Prospects or Questions Raised

Conventional therapies offer limited benefits for PC. However, a potential solution lies in the use of nanosystem-targeted CAFs to deliver drugs in combination with radiotherapy and immunotherapy. This integrated treatment strategy shows promise in breaking the resistance of PC. Modified lipid nanoparticles, which are more biocompatible and like exosomes secreted by CAFs, enter the TME to interfere with the original exosome-related signaling pathways and deliver drugs to target CAFs for TME remodeling. However, due to the functional heterogeneity of CAFs, indiscriminate killing of CAFs may have counterproductive effects. The specific molecular mechanisms underlying the functional heterogeneity of CAFs are not yet clear. Therefore, designing nano-drug delivery systems that target specific tumor-promoting CAFs or release appropriate concentrations of cytotoxic substances into the organelles through nanoparticles internalized by CAFs could be the key to making breakthroughs in nano-targeting technology for CAFs. This would affect the function of CAFs and their specific regulatory mechanisms. However, achieving a balance in the quantity and ratio of various types of CAFs to maximize the anti-tumor effect necessitates a comprehensive understanding of the specific mechanisms underlying CAFs' tumor-promoting or anti-tumor properties. This, in turn, calls for further in-depth research and exploration. Reversing the tumor-promoting TME of PC to an anti-tumor TME by targeting CAFs through a nano-drug delivery system, followed by a combination of radiotherapy and immunotherapy, could potentially offer new survival opportunities for patients with difficult-to-treat PC cases. This combination therapy strategy may become an important direction for future tumor therapy.

## Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

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## Disclosure

The authors report no conflicts of interest in this work.

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