

# The Application of Nanoparticles Targeting Cancer-Associated Fibroblasts

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**Abstract:** Cancer-associated fibroblasts (CAF) are the most abundant stromal cells in the tumor microenvironment (TME), especially in solid tumors. It has been confirmed that it can not only interact with tumor cells to promote cancer progression and metastasis, but also affect the infiltration and function of immune cells to induce chemotherapy and immunotherapy resistance. So, targeting CAF has been considered an important method in cancer treatment. The rapid development of nanotechnology provides a good perspective to improve the efficiency of targeting CAF. At present, more and more researches have focused on the application of nanoparticles (NPs) in targeting CAF. These studies explored the effects of different types of NPs on CAF and the multifunctional nanomedicines that can eliminate CAF are able to enhance the EPR effect which facilitate the anti-tumor effect of themselves. There also exist amounts of studies focusing on using NPs to inhibit the activation and function of CAF to improve the therapeutic efficacy. The application of NPs targeting CAF needs to be based on an understanding of CAF biology. Therefore, in this review, we first summarized the latest progress of CAF biology, then discussed the types of CAF-targeting NPs and the main strategies in the current. The aim is to elucidate the application of NPs in targeting CAF and provide new insights for engineering nanomedicine to enhance immune response in cancer treatment.

**Keywords:** nanoparticles, cancer therapy, cancer-associated fibroblasts, drug delivery, cancer, nanomedicine

## Background

In recent years, several barriers to effective tumor eradication, including the formation of distant metastasis, angiogenesis, drug resistance, and immunosuppression, have been identified. Consequently, there has been an increasing interest in the classification of different cells within the tumor parenchyma; these endeavors have led to the emergence of the tumor microenvironment (TME) concept.<sup>1-6</sup> Characterization of the TME has drawn attention to the deleterious role of cancer-associated fibroblasts (CAFs) in this context.<sup>7</sup> Under normal conditions, fibroblasts maintain a quiescent state and mainly contribute to the secretion of collagen, which supports the formation and integrity of the extracellular matrix (ECM), the fundamental tissue scaffold.<sup>8,9</sup> In the context of wound healing, fibroblasts become activated to facilitate tissue repair before undergoing apoptosis.<sup>10</sup> Tumors are often likened to “cancerous wounds”<sup>11</sup> that fail to heal and continuously stimulate fibroblasts through the secretion of cytokines such as TGF- $\beta$ ,<sup>12</sup> preventing them from returning to their resting state and promoting tumor growth. Recent investigations, however, have uncovered the presence of cancer-restraining CAF (rCAF) subtypes, which have tumor suppressor properties.<sup>13</sup> CAFs play significant roles in tumor proliferation, metastasis, chemotherapy resistance, cancer metabolism, immunotherapy resistance, and immune evasion,<sup>14</sup> which will be comprehensively discussed in this review. Consequently, CAF-targeting therapies have emerged; however, despite promising preclinical findings, the desired levels of efficacy have yet to be achieved and the clinical implementation of these therapies remains limited.<sup>15</sup>

The past few decades have hailed rapid advancements in nanotechnology, which have facilitated the development and delivery of targeted drugs for cancer therapy.<sup>16,17</sup> However, results from clinical trials of anti-CAF drugs, such as vismodegib, navitoclax, and sibtrotuzumab, have highlighted their uncontrollable toxicity and unstable action.<sup>7,18</sup> Drug encapsulation in nanoparticles (NPs) promises to overcome these challenges. NPs can optimize drug properties, reduce degradation rates in the body, improve solubility, enhance targeting capabilities, and improve overall drug efficacy.<sup>19</sup> Various types of NPs are available, including those constructed from lipids, metals, polymers, biological macromolecules, and cell membranes; each type has distinct properties and characteristics, which can be matched to a specific application. Moreover, coupling CAF-specific peptides or antibodies to the surface of NPs enables efficient targeting and drug release within these cells.<sup>20,21</sup>

This review provides an overview of the characteristics of CAFs and their interactions with immune cells in the TME. Furthermore, we present a comprehensive summary of nanomaterial selection and construction strategies, which are being used in the development of NPs for CAF-targeted immunotherapy.

## TME and Cancer Therapy

Numerous studies have established the significance of the TME in tumor progression, which can be as important as the genetic and epigenetic alteration of tumor cells.<sup>22,23</sup> The TME provides an external view of the changes occurring within tumor cells and evolves dynamically throughout tumor development. During the initial stages of cancer development, innate immune cells such as natural killer (NK) cells, macrophages, and dendritic cells (DCs) infiltrate the tumor and mount a rapid immune response.<sup>24,25</sup> In addition, antigen-presenting cells, prime and stimulate lymphocytes, which are essential for tumor eradication. Tumor cells employ various mechanisms to evade or impair anti-tumor T cell activity; in addition, they recruit various pro-tumor immune cells, including myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), CAFs, macrophages, and neutrophils. The subsequent increase in the ratio of pro-tumor to anti-tumor immune cells contributes to tumor progression. Given the limitations of conventional tumor treatment approaches, attention has shifted toward immunotherapy as a potent means of anti-tumor eradication. Over the past few decades, diverse forms of cancer immunotherapy have been explored.<sup>26</sup> Current immunotherapy strategies encompass: 1) oncolytic viral therapy, which uses transgenic viruses to infect tumors and establish a pro-inflammatory TME to enhance anti-tumor immune responses;<sup>27</sup> 2) tumor vaccines, which use tumor-associated antigens to prime tumor-specific T cells;<sup>28</sup> 3) cytokine therapy, which modulates cytokine patterns to activate the immune system;<sup>29</sup> 4) adoptive immune cell transfer, which involves the reinfusion of autologous, existing or genetically-engineered (eg, CAR-T cells) anti-tumor immune cells into the patient;<sup>30</sup> and 5) immune checkpoint inhibitor (ICI) therapy.<sup>31</sup> Immunotherapy has emerged as an effective and well-tolerated treatment approach in clinical practice. However, the development of immunotherapy resistance poses a significant challenge. To address this issue, attention has been refocused on specific immune and stromal cells within the TME. Consequently, targeted drugs have been developed to remodel the TME and mitigate immunotherapy resistance.<sup>26</sup> Meanwhile, advancements in nanotechnology have optimized immunotherapy via the use of nanoscale drug carriers. This innovative approach enhances the efficacy of targeted drugs and provides a promising direction for overcoming immunotherapy resistance.<sup>32</sup>

## CAFs Characteristics

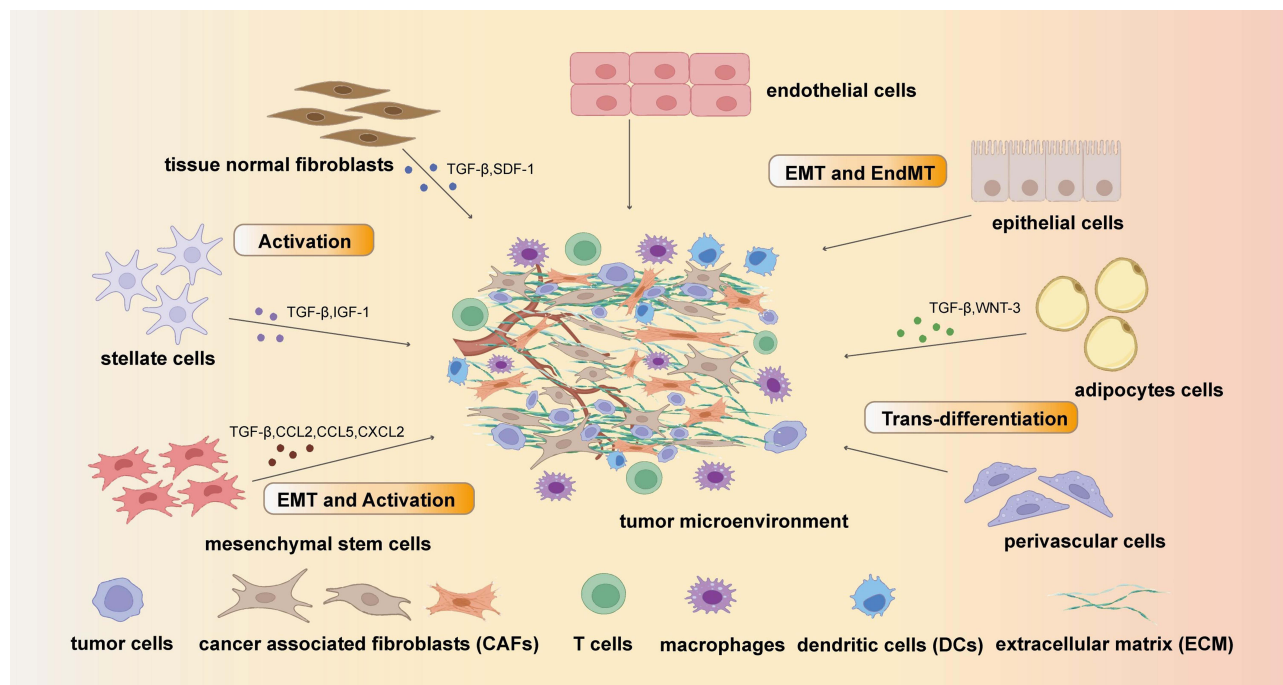
### CAFs Biology

Fibroblasts are the most abundant stromal cells in normal tissues; they play a crucial role in maintaining ECM stability and structural integrity.<sup>8</sup> They typically originate from mesenchymal stem cells (MSCs) and remain in a quiescent state as individual cells primarily located near the basement membrane. Fibroblasts are activated during processes such as wound repair, tissue inflammation, and fibrosis, before subsequently undergoing apoptosis or returning to a resting state upon completion of the healing and remodeling process.<sup>33</sup> During tumor development, a prolonged state of chronic inflammation, often referred to as the “tumor wound”, arises.<sup>11</sup> This sustained state of injury and repair leads to the accumulation of numerous complex cytokines within the TME, triggering persistent fibroblast activation. These hyperactivated fibroblasts, otherwise known as CAFs, are characterized by heightened proliferation, increased migration, and resistance

to apoptosis.<sup>34</sup> In addition, CAFs produce substantial amounts of ECM components and proteases, including collagen, matrix metalloproteinases, and plasmin.<sup>35</sup> This accumulation of ECM building blocks, in turn, increases tumor stiffness, which impedes immune cell infiltration, promotes immunosuppression, and facilitates tumor immune evasion.<sup>36</sup> CAFs also secrete numerous pro-tumor factors such as vascular endothelial growth factor (VEGF), which stimulates angiogenesis and promotes tumor proliferation and metastasis.<sup>37</sup> Moreover, CAFs significantly contribute to the remodeling of cancer metabolism,<sup>38</sup> particularly lipid metabolism<sup>39</sup> and mitochondrial oxidative stress.<sup>40</sup> Additionally, CAFs can induce resistance to chemotherapy or immunotherapy via the release of autocrine exosomes and the creation of self-established physical barriers within the tumor.<sup>41–44</sup> Understanding the functions of CAFs in tumor tissues is crucial for the effective design and implementation of NP-based, CAF-targeting therapeutic strategies.

## CAFs Origin

There is mounting evidence indicating that CAFs do not arise from a single cell source but rather represent a complex and heterogeneous cell population.<sup>45–47</sup> Moreover, CAFs differ between tumors arising in different tissues and displaying tissue-specific characteristics.<sup>7</sup> The majority of CAFs originate from fibroblasts in normal tissues with a particularly high fibroblast content, such as the breast, lung, and colorectal tissues. Additionally, innate stellate cells of the pancreas<sup>48</sup> and liver<sup>49</sup> are common sources of CAFs; upon activation these cells differentiate into CAFs with a muscle-like phenotype, characterized by the expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA).<sup>50</sup> And bone marrow (BM)-derived fibroblasts can be recruited to the TME and transform into CAFs. Moreover, both BM- and tissue-derived MSCs serve as precursor cells for normal fibroblasts and can directly differentiate into CAFs.<sup>51–54</sup> Finally, other cell types, such as epithelial or endothelial cells, adipocytes, and perivascular cells, can undergo metaplasia or trans-differentiation to transform into CAFs.<sup>55–57</sup> The transition processes of CAFs are portrayed in the Figure 1.



**Figure 1** The origin of cancer associated fibroblast (CAF): the main original cell of CAFs are tissue normal fibroblasts, stellate cells, mesenchymal stem cells, endothelial cells, epithelial cells, adipocytes cells and perivascular cells. These cells can transform into CAFs by Epithelial–mesenchymal transition (EMT) pathway and Endothelial-to-mesenchymal transition (EndMT) or be activated by cytokines including TGF- $\beta$ , SDF-1, WNT-3, IGF-3, CCL2, CCL5 and CXCL2.

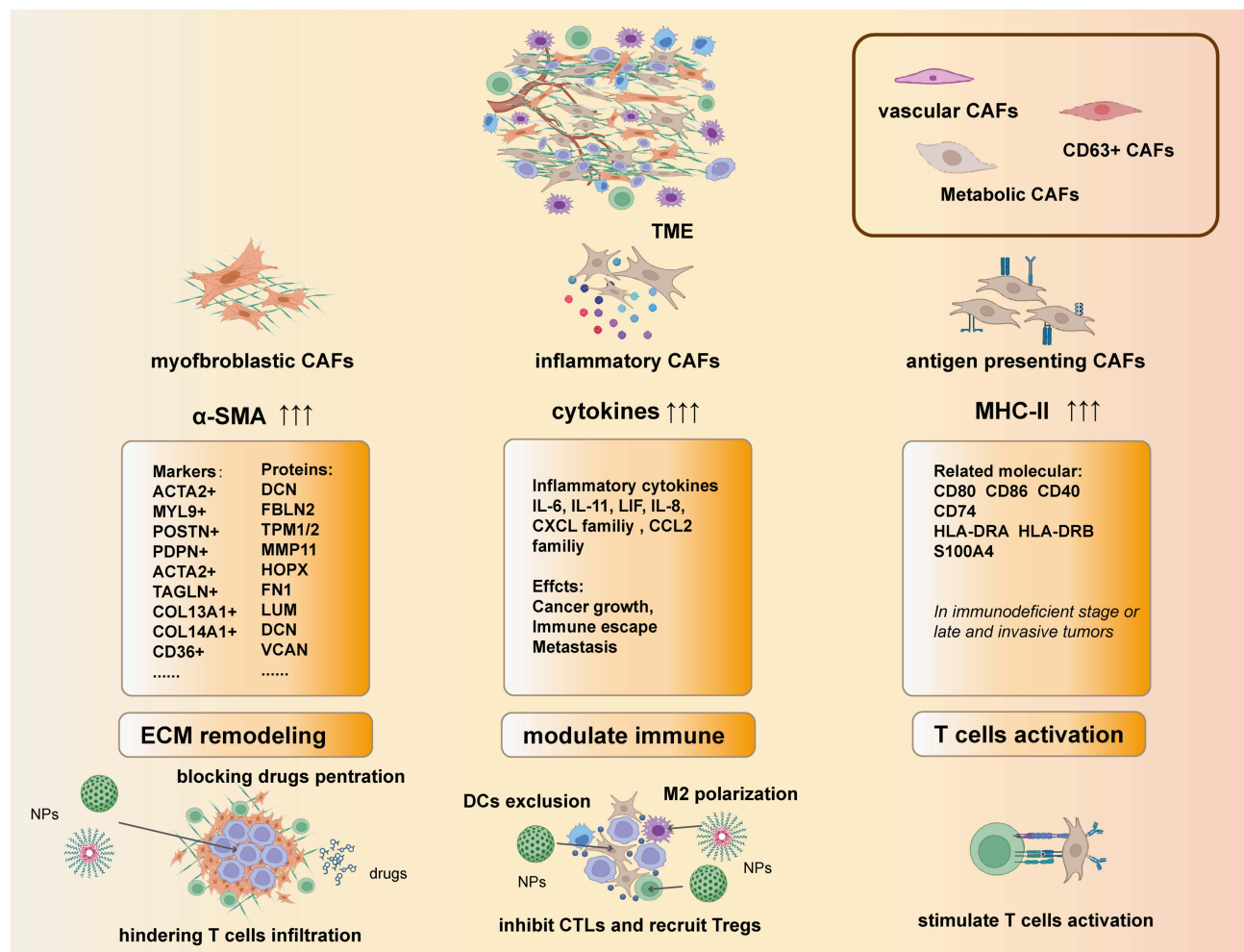
## CAFs Heterogeneity

The heterogeneity of CAFs is influenced by their diverse origins and their spatial relationship with tumor cells within the TME.<sup>58–60</sup> Distinct types of CAFs often vary in their surface markers and biological characteristics.<sup>50</sup> Initially, CAFs were categorized into the myofibroblastic (mCAF) and inflammatory (iCAF) CAF subtypes.<sup>47</sup> However, the advancement of single-cell sequencing technology has facilitated the exploration of CAF subtypes and heterogeneity within the TME in more detail. Current research on the classification of CAFs primarily focuses on connective-tissue-rich tumors, such as those of breast and pancreatic cancers. Despite having different tissue specificities, CAFs demonstrate broad similarities.<sup>61</sup> The three main CAF subtypes are: mCAFs, pro-inflammatory and immune regulatory CAFs (iCAFs), and antigen-presenting CAFs (apCAFs).<sup>59,62</sup> mCAFs remodel the ECM and influence tumor tissue rigidity; they are therefore characterized by the expression of genes associated with collagen formation and smooth muscle contraction (eg,  $\alpha$ -SMA).<sup>63</sup> mCAF subtypes can be further classified based on the surface expression of functional molecules (eg, TPM1,<sup>64</sup> POSTN,<sup>65</sup> ACTA2, and MYL9<sup>66</sup>), which is used to identify their origin and ability to promote tumor growth. Moreover, the different mCAF subtypes can morph into each other during different stages of tumor progression by varying their surface molecule expression. In breast cancer, a significant proportion of CAFs originate from PDPN<sup>+</sup> CAFs (pCAFs), and turn into the Fbn1<sup>+</sup>Mfap5<sup>+</sup> and Acta2<sup>+</sup>Thbs2<sup>+</sup> CAFs in the later stages.<sup>67</sup> Meanwhile, in non-small cell lung cancer (NSCLC), the COL13A1<sup>+</sup> and COL14A1<sup>+</sup> CAFs (with strong epithelial-mesenchymal transition [EMT] characteristics) dominate in the early stages of disease but later transition into the ECM37<sup>+</sup> or ACTA2<sup>+</sup> subtypes.<sup>68,69</sup> The immunomodulatory properties of iCAFs in the TME depend on the organ they reside in and the associated environmental factors. Tumor cells drive iCAFs to perturb certain elements of the TIME. Simultaneously, iCAFs modulate tumor cells through the secretion of their own substances, thereby enhancing immunosuppression and immune evasion.<sup>70,71</sup> iCAFs express relatively low surface  $\alpha$ -SMA levels, and are classified into various subtypes based on specific molecular marker combinations.<sup>47</sup> For instance, iCAF subtypes can be designated as pCAFs or S100A4+CAF (sCAFs).<sup>67</sup> Additionally, CAF subtypes can be distinguished based on the variation in the secretion of certain factors. Moreover, the iCAF and mCAF phenotypes are highly dynamic and can undergo interconversion. Commonly secreted mediators include leukemia inhibitory factor, interleukin (IL)-6, IL-8, IL-11, CXCL, and CCL family members; these mediators are implicated in pathways such as the Hedgehog, IL1R1/JAK/STAT, NF- $\kappa$ B, and classical complement pathways.<sup>59,70,72–74</sup> iCAFs have been reported to inhibit the infiltration of DCs, B cells, and T cells, increase the recruitment of MDSCs and Tregs, and promote the polarization of M2 macrophages.<sup>59</sup> The discovery of apCAFs indicates that not all CAFs have pro-tumor properties;<sup>46</sup> however, the formation of these CAFs largely depends on whether they originate from endothelial or epithelial cells. Moreover, apCAFs typically occur in the late stage of cancer or in individuals with compromised immune function. Overall, few studies have characterized this CAF subtype, and its prevalence, formation, and antigen presenting functions remain unclear. Beyond the three common classifications of CAFs, metabolic CAFs (meCAF, involved in metabolism) and vascular CAFs (vCAFs, involved in angiogenesis) have also been identified.<sup>60,65</sup> Despite a substantial body of research addressing CAF classification, no universally accepted categorization method exists. Therefore, considerable strides still need to be made to comprehensively classify CAF subtypes. The example of the subtypes of CAFs are shown in the [Figure 2](#).

## The Role of CAFs in the TME

### CAFs and Tumor Cells

The interactions between CAFs and tumor cells are mutual. Tumor cells persist creating an education process to recruit and activate CAFs consistently. This process is not only controlled by a wide range of tumor-secreted factors (eg, TGF- $\beta$ , PDGF and IL-6) but also rely on some stimulation and the change of metabolite composition in the TME.<sup>7</sup> For instance, the accumulation of reactive oxygen species triggered by local hypoxia can induce the expression of CXCL12 and hypoxia inducible factor 1 $\alpha$  to promote the transition of mCAFs.<sup>75–77</sup> In the study of Kitamura et al, they found that cancer lactate can be reused by CAFs. CAFs can use monocarboxylate transporter 1 to utilize the lactate to enhance TCA cycle and upregulate the expression of IL-6.<sup>78</sup> Activated CAFs can evolve along with the tumor cells and attach great importance in affecting multiple malignant behaviors. In breast cancer and prostate



**Figure 2** The heterogeneity of CAFs: the main subtypes of CAFs are defined by their features. In the classification of mCAFs, the overexpressed genes such as ACTA2, CD36 and PDPN are define as the subtype of mCAFs. And subtypes of the iCAFs with different functions are define by their secreted cytokines. The way of defining apCAFs are similar to myCAFs but the markers are usually human leukocyte antigens and cluster of differentiations (CD).

cancer, it has been reported that CAFs can boost the tumor growth and induce tumorigenesis and this effect is mainly due to the interaction between CXCL12 (secreted by CAFs) and CXCR4 (expressed on tumor cells).<sup>79,80</sup> Rapid tumor growth demands enough nutrients and oxygen so new vessel formation in the TME is necessary. CAFs-derived amounts of pro-angiogenesis factors including WNT2, VEGF, FGF2 and PDGFA can initiate and stimulate this procedure.<sup>81</sup> Notably, CXCL12 secreted by CAFs can recruit the vesicular endothelial cells into TME and accelerate vessel formation.<sup>7,79,82</sup> CAFs-derived TGF- $\beta$  and CXCL12 can lead EMT in tumor cells, which contribute to invasion and metastasis. Moreover, CAFs can create gaps in ECM, basement membrane and vascular wall which make it favorable for tumor invasion and metastasis.<sup>83</sup> Recent numerous researches have also proved that CAFs are indispensable in the resistance of cancer therapy. CAFs are capable of remodeling ECM by secreting collagen and tenascin C (TNC) to form dense physical barriers which can prevent chemotherapeutic and immunotherapeutic drugs from penetrating into the deep parts of tumor.<sup>7,14,18</sup> Moreover, CAFs-derived factors can increase the resistance ability of tumor cells. For instance, CAFs can cause resistance to cisplatin-based chemotherapy. Cisplatin can increase TGF- $\beta$  level to activate CAFs to produce IL-6, which can induce EMT and avoid apoptosis.<sup>84</sup> And CXCL12/CXCR4 axis mentioned above can activate Wnt/ $\beta$ -catenin pathway to induce cisplatin resistance.<sup>82</sup> Moreover, Xingbo Long et al showed that bladder cancer cells can promote the transformation of CAFs, which then upregulate the IGF1/ER $\beta$  pathway in bladder cancer cells to increase their ability of cisplatin resistance.<sup>85</sup> In general, CAFs and tumor cells can co-evolve along with cancer progression and the mutual interaction may result

in adverse outcomes. This is why CAFs has become the promising target for cancer therapy during recent years. What's more, eliminating CAFs and tumor cells simultaneously is more important and effective than merely targeting CAFs or tumor cells.

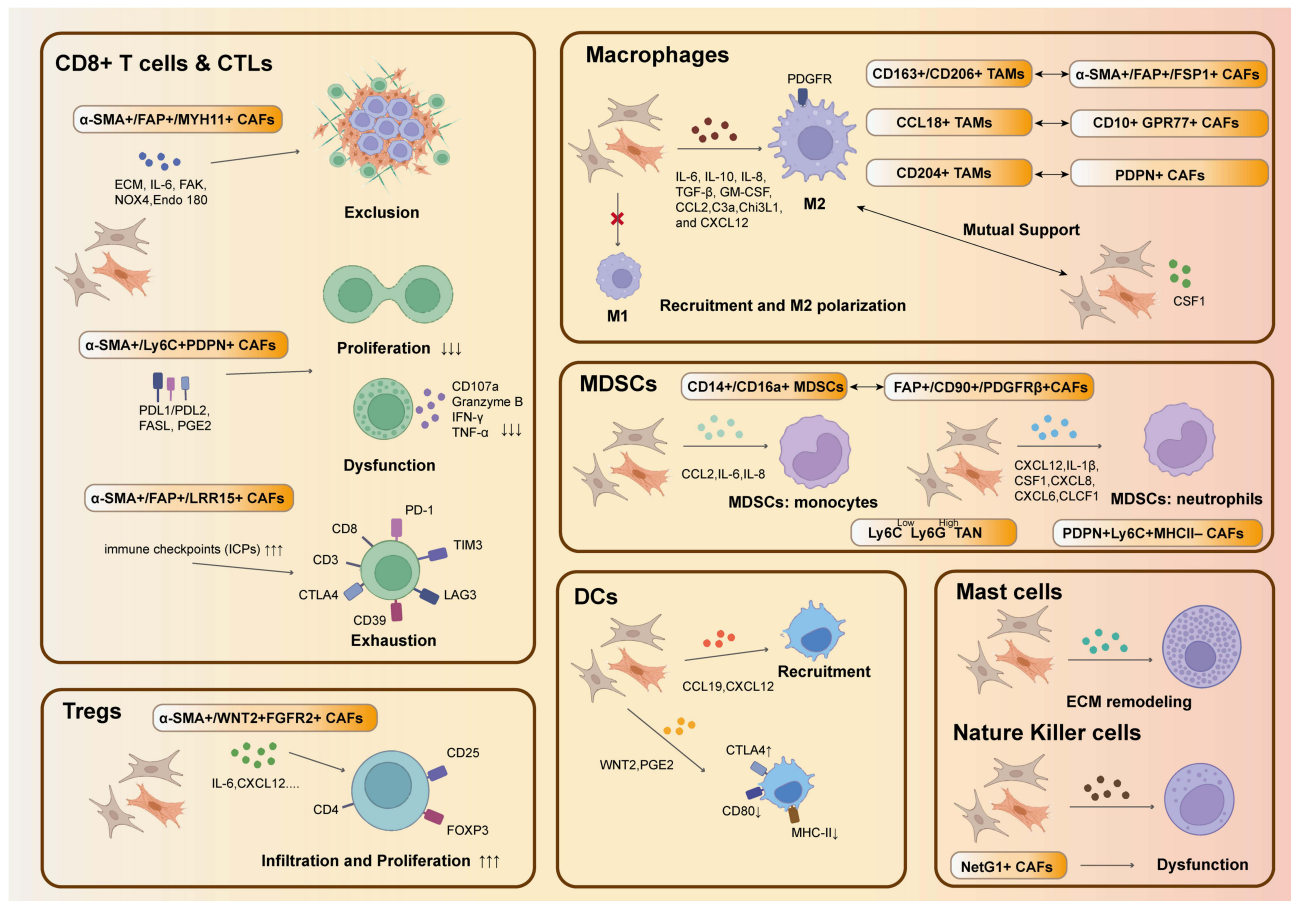
## Crosstalk Between CAFs and Immune Cells

### CAFs and T Cells

T lymphocytes are the most important adaptive immune cells in the TME. They exert an adaptive immune response by recognizing specific tumor-related antigens.<sup>74–77</sup> However, prolonged antigen exposure leads to the upregulation of immune checkpoint proteins (ICPs) on the surface of T cells, which impairs their ability to mount an effective immune response and results in reduced proliferation, loss of memory function, and exhaustion.<sup>78,79</sup> CAFs play a significant role in inducing T cell exhaustion and have a potent capacity to induce the differentiation of Tregs. Notably, Sakai et al observed that the infiltration of Foxp3<sup>+</sup> CD4<sup>+</sup> T cells was accompanied by increased numbers of WNT2<sup>+</sup> FGFR2<sup>+</sup> CAFs.<sup>80</sup> In vitro experiments have also demonstrated a significant increase in Tregs within the peripheral blood mononuclear cell (PBMC) population in the presence of CAFs.<sup>81</sup> Nonetheless, the relationship between CAFs and Treg infiltration in tumors remains unclear, with some studies suggesting that partial CAF depletion actually promotes Treg infiltration into the tumor, thereby promoting tumor progression.<sup>82,83</sup> The influence of CAFs on CD8<sup>+</sup> T cells is also multifaceted. First, CAFs can effectively exclude CD8<sup>+</sup> T cells from the tumor core, causing their marginalization.<sup>84</sup> Second, CAFs can impede the activity and proliferation of T cells. In vitro studies have demonstrated that co-culturing CAFs with PBMCs significantly reduced CD8<sup>+</sup> T cell numbers and their ability to secrete cytotoxic molecules.<sup>81</sup> CD8<sup>+</sup> T cells and CAFs also show great relation in metabolic regulation. Weimin Wang. et al reported that CAFs can produce cysteine and glutathione to lead resistance to platinum-based therapy. While CD8<sup>+</sup> T cells can control the release through IFN- $\gamma$ .<sup>86</sup> Additionally, CAFs themselves can express ICPs such as PD-L1, which induce ICP expression on the surface of CD8<sup>+</sup> T cells and exacerbates their exhaustion. Notably, LRRC15<sup>+</sup> CAFs have been positively associated with the expression of multiple exhaustion molecules, including TIM3, LAG3, and CD39, on T cells.<sup>85,86</sup> (Figure 3)

### CAFs and TAMs

Macrophages are the most abundant and extensively studied innate immune cells in the TME. Two distinct phenotypes of macrophages exist: M1 macrophages have anti-tumor properties, while M2 macrophages exhibit pro-tumor characteristics.<sup>87</sup> CAFs and macrophages behave similarly within tumors, exerting their functions through autocrine and paracrine signaling mechanisms.<sup>88–90</sup> For instance, the KRAS pathway induces M2 macrophage polarization and enhances the capacity of CAFs to secrete IL-6 and CXCL1.<sup>91</sup> mCAF and macrophages can communicate extensively with each other within the TME.<sup>92</sup> Moreover, the presence and functions of tumor-associated macrophages (TAMs) and CAFs appear to be intertwined across various tumor types. In breast cancer, the presence of CD163<sup>+</sup> and CD206<sup>+</sup> TAMs positively correlate with that of  $\alpha$ -SMA<sup>+</sup> CAFs.<sup>89</sup> Similarly, CD204<sup>+</sup> TAMs in lung cancer exhibit concordant infiltration patterns with FDPN<sup>+</sup> CAFs.<sup>93,94</sup> The co-localization of TAMs and CAFs often implies drug resistance and immunosuppression, implying that these cell types can synergistically induce adverse effects. CAF-derived cytokines and chemokines (eg, IL-6, IL-10, GM-CSF, and CXCL12) recruit monocytes into the TME, facilitating their polarization into M2 TAMs.<sup>36,95–97</sup> In gastric cancer, CAFs promote the transformation of M2 macrophages through periostin expression.<sup>98</sup> Moreover, CAFs exacerbate immunosuppression in breast cancer by secreting chitinase-3-like 1, which attracts M2 TAMs and leads to CD8<sup>+</sup> T cell depletion.<sup>99</sup> In turn, TAMs maintain persistent CAF activation, which further contributes to breast cancer tissue fibrosis and sclerosis through the activation of the TGF- $\beta$  signaling pathway.<sup>100</sup> Additionally, macrophages cause MSCs to adopt CAF-like characteristics in vitro by upregulating markers such as  $\alpha$ -SMA, FAP, and vimentin.<sup>53</sup> The intricate interplay among CAF, TAMs, and tumor cells facilitates tumor initiation and progression (Figure 3).



**Figure 3** Crosstalk of CAFs and immune cells: CAFs can inhibit CD8<sup>+</sup> T cells by inducing exclusion, dysfunction and exhaustion and they can also inhibit their proliferation while induce the proliferation of Tregs. CAFs can induce M2 polarization while M2 can also enhance the function of CAFs. This kind mutual support also can be found in the interaction of CAFs and MDSCs. CAFs also can disrupt the functions of DCs and NK cells while recruit mast cells.

## CAFs and MDSCs

MDSCs contribute significantly to the formation of the immunosuppressive TIME. Tumor-associated cytokines drive the differentiation of immature myeloid cells into MDSCs, which share biological similarities and exhibit close associations with CAFs.<sup>101</sup> In lung cancer, a positive correlation has been observed between the expression of MDSC- (eg, CD14 and CD16) and CAF- (eg, FAP and PDGFR-β) associated surface markers, implying the co-infiltration of CAFs and MDSCs.<sup>102</sup> Likewise, CAFs can recruit MDSCs or influence their functions through various pathways. For example, CAFs promote MDSC infiltration and increase the expression of adhesion molecules on MDSCs by secreting IL-6, IL-8, and CCL2.<sup>96,103,104</sup> Moreover, CAFs play a role in MDSC differentiation, for instance, by inducing the expression of APOE and TREM-2 expression in MDSCs, thereby enhancing their ability to suppress CD8<sup>+</sup> T cells<sup>92</sup> (Figure 3).

## CAFs and DCs

As professional antigen presenting cells, DCs prime T cells to recognize and eliminate tumors.<sup>105</sup> CAFs have been found to downregulate the expression of MHC class I and II molecules on the DC surface, thereby impeding T cell priming.<sup>106</sup> In addition, CAFs interfere with the immunomodulatory function of DCs, establishing an immunosuppressive environment. Conversely, CAFs can recruit DCs to the tumor site via the CXCL12 and CCL19/CCR7 axes.<sup>107</sup> In colon cancer, the secretion of WNT2 by CAFs significantly hinders the activation and maturation of DCs.<sup>108</sup> Moreover, CAFs can inhibit DC function by promoting the expression of ICPs, such as CTLA-4, on the DC surface<sup>106</sup> (Figure 3).

## CAFs and Cancer Therapy

As previously mentioned, most CAFs inhibit the pro-tumor immune response within the TME. Therefore, over the past few years, a considerable number of immunotherapeutic approaches targeting CAFs have been developed (Table 1); some of these have successfully entered the preclinical and clinical trial stages.<sup>109</sup> Three main CAF-targeting strategies have been employed: CAF elimination, CAF inhibition, and CAF modulation.<sup>61</sup> Unlike most other immune cells, CAFs do not have a well-defined molecular signature; thus, current studies have mainly targeted the molecules FAP and  $\alpha$ -SMA, which are highly expressed on the CAF surface.<sup>50,110</sup> FAP targeting using FAP inhibitors (eg, PT630<sup>111</sup> and sibrotuzumab<sup>112</sup>), DNA cancer vaccines,<sup>113,114</sup> and CAR-T cells<sup>115</sup> has been relatively well documented. Moreover, a dual-specificity antibody drug targeting CD137 and FAP (RO7122290) is being investigated in clinical trials.<sup>116</sup> These drugs and therapeutic methods have all demonstrated similar tumor-inhibiting effects. However, results from a recent Phase II trial of an FAP-specific monoclonal antibody (PT100) showed no clinical benefits.<sup>117,118</sup> Moreover, FAP-based oncolytic adenoviral vaccines are merely at the preclinical stage of development; this may be due to concerns regarding their low efficacy and stability. Similarly, because CAR-T cell therapy is seldomly used in solid tumor, FAP-specific CAR-T cell therapy is unlikely to be applied to the treatment of all tumor types. Furthermore, not all CAFs express FAP, making it challenging to completely eliminate tumors by targeting FAP-positive cells. Moreover, as FAP is also expressed on healthy cells, targeting this molecule can result in off-target toxicity and adverse effects. Thus, FAP-specific CAR-T cell therapy is likely to induce cytotoxicity,<sup>119</sup> while an  $\alpha$ -SMA-targeting approach will likely increase Treg infiltration.<sup>120</sup>

**Table 1** Drugs for Targeting CAFs in Preclinical and Clinical Trials

Therapy Types	Drugs	Effects	Cancer Models	Stage of Clinical Trials	Source
FAP-expressing cells depletion	PT630	FAP participates in matrix degradation but fails to inhibit tumors	BRCA	Preclinical	[136]
	PT100	FAP-specific monoclonal antibody and inhibitors	COAD	Phase II	[117,118]
	Sibrotuzumab	Kill FAP-CAFs	COAD	Phase II	[112]
	RO7122290	Costimulates T cells for improved tumor cell killing in FAP-expressing tumors.	Solid tumors	Phase I	[116]
	DC/CAF fusion cells-tumor vaccine	Stimulating T cells	HCC	Preclinical	[113]
	FAP $\alpha$ DNA vaccine	FAP $\alpha$ -specific cytotoxic T lymphocyte responses	BRCA	Preclinical	[114]
	FAP based CAR-T	Ablate FAP-expressing cells	Lung cancer	Preclinical	[119]
TGF- $\beta$ Inhibitor	Galunisertib	Inhibit CAFs activation	HCC	Phase II	[123]
Vitamin A analogs	ATRA	Dampening multiple signalling cascades	PDAC	Preclinical	[124]
Vitamin D analogs	Calcipotriol	Reduced markers of inflammation and fibrosis	PDAC	Phase II	[128]
CXCL12-CXCR4 inhibitor	AMD3100	T-cell accumulation and PD-L1 diminished	PDAC	Preclinical	[131]
JAK inhibitors	Ruxolitinib	Alleviate tumor inflammation	PDAC	Phase II	[132]
Hedgehog pathway	Sonidegib	Sensitizes tumors to docetaxel	BRCA	Phase I	[133]
	IPI-926	Depletes tumor-associated stromal tissue	PDAC	Preclinical	[134]
	GDC-0449	Conflicting effects	PDAC	Phase II	[135]



Therefore, restoring the stromal balance in tumor tissue rather than eliminating CAFs altogether has been put forward. And this can be achieved using TGF- $\beta$  pathway inhibitors, such as rizaben and galunisertib.<sup>121–123</sup> However, these inhibitors often induce side effects such as neutropenia, liver toxicity, and thrombosis. In addition, vitamin A (eg, all-trans retinoic acid [ATRA]<sup>124</sup>) and vitamin D (eg, calcipotriol<sup>125</sup>) analogs have been used to interfere with CAF activation.<sup>126,127</sup> When used in combination with chemotherapy and radiotherapy, these inhibitors can effectively enhance the anti-tumor immune response within the TME and achieve tumor growth inhibition. Unfortunately, these approaches are still in the preclinical stage and may harm T cell immunity.<sup>128</sup> Recently, Li et al have investigated the positive role of calcipotriol in tumor metastasis and recurrence after adjuvant photothermal therapy (PTT).<sup>129,130</sup> Targeting important pathways within CAFs is another research focus. Examples of pathway inhibitors include AMD3100,<sup>131</sup> an inhibitor of the CXCL12-CXCR4 pathway; ruxolitinib,<sup>132</sup> a JAK pathway inhibitor; and sonidegib,<sup>133</sup> IPI-926,<sup>134</sup> and GDC-0449 (vismodegib),<sup>135</sup> inhibitors of the Hedgehog pathway. In preclinical studies, AMD3100 has shown promising results when used in combination with ICIs; however, precisely how it modulates the TME remains unclear.<sup>131</sup> In a phase II clinical trial of pancreatic cancer, ruxolitinib has demonstrated expected outcomes, with beneficial effects primarily observed in patients with high C-reactive protein levels.<sup>132</sup> Despite this partial response, ruxolitinib is still regarded as a promising drug. GDC-0449, an inhibitor of the Hedgehog ligand SHH, did not confer benefits when used in combination with gemcitabine for pancreatic cancer; in addition, over half of the patients experienced significant drug toxicity.<sup>135</sup>

As described above, targeting CAFs therapies are currently unresolved challenges in clinical trials. Moreover, the efficacy of individual CAF-targeting methods is limited, while the use of combination therapies often leads to more adverse drug reactions and increases treatment complexity. The emergence of nanotechnology has improved the precision of drug delivery while optimizing drug release mechanisms.<sup>17,20</sup> Additionally, the unique properties of NPs bring forth diverse treatment modalities, such as PTT.<sup>20</sup> Immune therapies utilizing specifically tailored nanomaterials have demonstrated remarkable results in the treatment of various tumors.<sup>16</sup> Therefore, exploring the value of using NPs in targeting tumors and CAFs has been the new research orientation.

## The Advantages and Functions of NPs

In the past few decades, medical nanotechnology has developed rapidly, and many inorganic biologically inert materials have been applied to nanomedicines. Compared with common drug molecules, nanomedicines have better performance in targeting, stability, long-term efficacy, versatility and safety.<sup>137</sup> The advantages of nanomedicines benefit from their functions. 1) NPs have a stealth effect, that is, they are in a state of immune escape so they will not be quickly cleared in the body. The surface charge of NPs can make them not be cleared in the glomerular filtration membrane, especially for some small molecules. Neutral and hydrophilic polymers can improve the stability and reduce biological interactions in Vivo, and thus prolong the retention time in tissues and blood. The application of polyethylene glycol (PEG) as a modification group is the best proof.<sup>138</sup> Bio-derived NPs can also bring this stealth effect. Nanomedicine based on red blood cell (RBC) membrane can improve the stability owing to the low immunogenicity and similar characteristics to RBC in blood. Albumin has become a good drug carrier because its size and charge characteristics are not filtered by glomeruli. Recently, CD47 has been found as a sign of ‘don’t eat me’ signal to macrophages, which has also led to an increasing number of modified with CD47-modified NPs to achieve stealth effects.<sup>138</sup> 2) NPs provide the basis for the design of multifunctional drugs, which can accurately target tumor. Ph response strategy and redox response strategy are the main methods. They can take advantage of the specific Ph changes in tumor tissue and the specific overactive redox environment. More interestingly, these can bring the cutting effect of PEG, which is conducive to the release and uptake of drugs. At the same time, NPs modified with cell-specific markers can achieve the expected precise targeting effect. For example, nanoparticles modified with macrophage surface marker CD80, CD86 and other molecules can accurately locate macrophages, integrate into the cell.<sup>137</sup> As discussed in this review, NPs modified with CAF surface marker FAP show a high targeting effect on CAF. 3) Some effects of NPs can be triggered in vitro. Light, magnetic field, and ultrasound can trigger effects such as self-luminescence (commonly used in surgical tracers) and photothermal effects (metal nanoparticles, carbon-based nanoparticles, nano-polymers). This feature makes it more versatile, and in the application, doctors can more actively grasp the timing of treatment.<sup>137</sup>

In cancer therapy, nanomedicines need to enter the tissue through blood vessels, then infiltrate into it to release, and eventually be taken up by targeted cells. Macromolecular drugs can infiltrate into solid tumor tissues through blood vessels and accumulate in tumor tissues, which is the enhanced permeability and retention (EPR) effect. This effect is thought to be fundamental for the design of nanomedicine and it is affected by the size, shape, softness and surface properties.<sup>139,140</sup> In the research from Shrey Sindhwani, they thought that the way of entering tumors by processing endothelial cells is more common than EPR effect for NPs<sup>141</sup> And there is also a concern that the EPR effect may cause an increased risk of metastasis.<sup>142</sup> Moreover, EPR effect is limited in clinical applications due to its heterogeneity which is caused by the individual differences between patients.<sup>140</sup> Although there exist some controversies, enhancing EPR effect is still an effective method to improve curative effect in the current. CAF is the barrier for NPs to enter tissue due to its effect on the ECM (CAF makes the tumor become solid, which is not conducive to the penetration of drugs, and impair the diffusion of drugs. CAF can also absorb drugs, which will cause a decrease in drug concentration.<sup>7</sup> Therefore, the depletion of CAFs in cancer therapy can be considered as a way to enhance the EPR effect from the biological perspective. However, this method can bring risks because that eliminating CAFs may promote tumor progress, which is similar to the risk from EPR effect. So, there also exist many researches on disrupting the functions of CAFs and inhibiting its activation. We have summarized the examples to show how NPs are applied for targeting CAFs in Table 2. The types, the targeting sites, the loaded drugs and the targeting strategies are listed. All of these will be discussed thoroughly in the following sections.

**Table 2** Constructed NPs and Their Effects for Targeting CAFs

Materials Type	NPs and Nanomaterials	Targeting Sites	Loaded Drugs	Pharmacological Effects	Source
Liposomes and lipo-based	DSPE-PEG2000	CFH/OM-L (Tenascin C)	Oxymatrine	Reversing EMT, inactivate CAFs, M1 polarization and NK activation	[143]
	DSPE-PEG2000	FH (Tenascin C)	Navitoclax, doxorubicin	CAFs selective apoptosis; facilitating drugs penetration	[144,145]
	LNCs	–	Paclitaxel and acriflavine	Inhibit CAFs and whole tumor	[146]
	DSPE-PEG-2000, DSPE-PEG-NHS, DSPE-PEG-AEAA	–	Fraxinellone	CAFs depletion and remodeling TME	[147]
	PEG-lip	–	Salvianolic acid B	Inhibiting CAFs activation, remodeling TME	[148]
	LPD	–	sTRAIL	Inactivate CAFs, remodeling TME and induce drugs infiltration	[149]
	IR780/DPPC		BMS	Reducing CAFs, inducing CTL activation and blocking PD-1/PD-L1	[150]
	DSPE-PEG2000	HA and GA	Aprepitant, berberine and curcumin	Inhibit CAFs activation	[151,152]
	Nanoliposome	–	Doxorubicin, resiquimod (R848) and losartan (LOS).	Reduces the activity of CAFs	[153]

(Continued)

Table 2 (Continued).

Materials Type	NPs and Nanomaterials	Targeting Sites	Loaded Drugs	Pharmacological Effects	Source
Metallic	SPIONs	–	Sodium citrate	CAFs apoptosis	[154]
	Gold-core silver-shell	–	–	Attenuated CAFs functions	[155]
	Gold NPs		Docetaxel	CAFs as NPs reservoirs	[156]
	SPIONs		FGF2	Inactivate CAFs	[157]
	3nm GNPs	–	–	Attenuated CAFs functions	[158]
	GNPs	–	–	Transform to quiescence; disturb CAFs communication	[159,160]
	Gold shell, SPIOs, PLGA	Her2	Doxorubicin	Reduce CAFs and remodel TME	[161]
	6nm USPOINs	CCK2R	–	Magnetic damage	[162]
	SPOINs	–	Relaxin	Inactivate CAFs	[163]
	GIONF	–	–	CAFs depletion	[164]
Polymeric micelles	TPP, PLGAs	–	Resveratrol	CAFs depletion and inactivation	[165]
	ONP-302	–	–	CAFs dysfunction and apoptosis	[166,167]
	SN38		Triptolide-naphthalene sulfonamide	Reduce CAFs activity	[168]
	Self-assembled Chol-R9	FAP	siCXCL12	Inactivate CAFs	[20]
	Drug self-polymerized		Cyclopamine (CPA) and paclitaxel (PTX)	CAFs depletion and tumor inhibition	[169]
	ZGP-PEOz-PLA, FA-PEOz-PLA, PEOz - PLA	FAP	Doxorubicin	CAFs deletion	[170]
	MLNPs	–	Docetaxel	CAFs deletion	[171]
	Cellax-DTX polymer	–	Docetaxel	CAFs depletion	[172]
	SN38	–	GDC-0449	Reducing CAFs	[173]
	PAMAM	FAP	Doxorubicin	CAFs as NPs reservoirs	[174]
	Self-assembled CAP	FAP	Doxorubicin	CAFs depletion	[175]
	PLGA-PEG	–	Sunitinib	Remolding CAFs	[176]
	PEG-PBLG	–	Tranilast	Remolding CAFs	[177]
	PLGA	–	siRNA for hepatocyte growth factor	Inhibit hepatocyte growth factor secreted from CAFs and prevent CAFs activation	[178]
	mPEG-PLGA	–	Baicalein	Prevent CAFs activation	[179]
Amphipathic hydroxyethyl starch-polycaprolactone	–	LY2157299 and photosensitizer indocyanine green	Enhance CAR-T therapy effects and inhibit CAFs activation	[180]	

(Continued)

**Table 2** (Continued).

Materials Type	NPs and Nanomaterials	Targeting Sites	Loaded Drugs	Pharmacological Effects	Source
Protein	Apo ferritin, ferritin nanocage	FAP	ZnFI6Pc	CAFs depletion, increase CTLs infiltration	[181,182]
	H-ferritin	FAP	Navitoclax	Killing CAFs	[183]
	HSA, CAP-TSL	FAP	Paclitaxel	CAFs depletion and tumor inhibition	[184]
Cell membrane	Fibroblasts membrane	–	SPN	Killing CAFs	[185]
	Tumor cells and fibroblasts membrane	–	Paclitaxel, PFK15	Reprogram TME	[186]
	Tumor cells membrane and thermosensitive LNPs	–	BMS	Disrupt CAFs and ECM, inhibit PD-1/PD-L1	[186]
	Red blood cells membrane, PEG-PLGA	FnBPA5	Retinoic acid, Doxorubicin	CAFs depletion and tumor inhibition	[187]
	Tumor cells membrane	–	Indocyanine green and calcipotriol	Modulate phenotype of CAFs and enhance PPT effects	[129]

## NP Targeting CAFs in Cancer Therapy

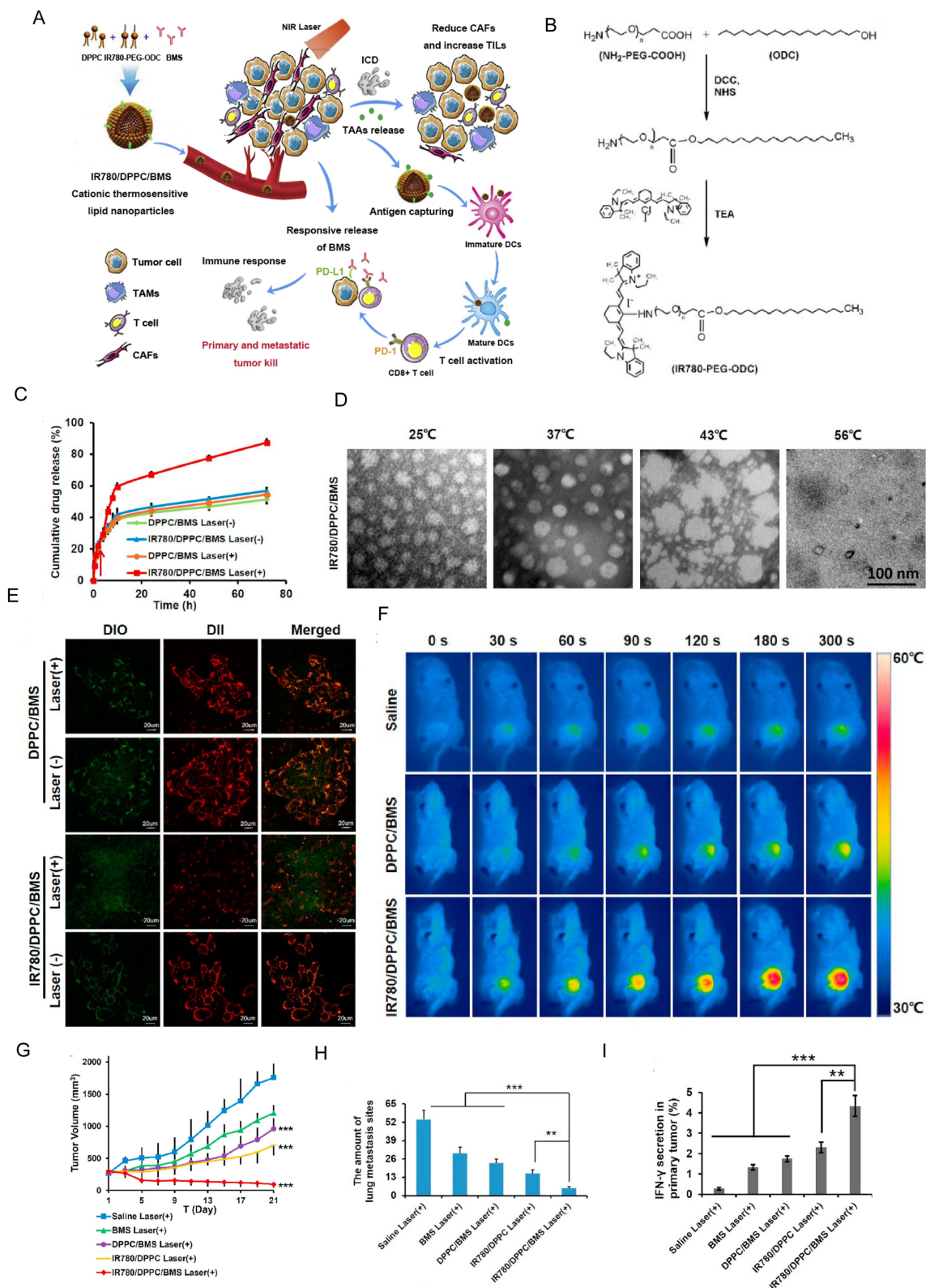
### Drug Delivery Systems for Targeting CAFs

#### Liposomes and Lipids

Liposomes, which are characterized by high biocompatibility, sustained release, and effective immune cell targeting, have revolutionized drug delivery.<sup>188</sup> Owing to its exceptional hydrophilicity and hydrophobicity, PEG is frequently employed as a linker to connect drugs and liposomes. Recent studies have explored the potential use of PEG in the development of CAF-targeting NPs.<sup>189</sup> The targeting efficiency of liposomes can be further improved by attaching peptides or antibodies that recognize specific biomarkers.<sup>190</sup> While FAP is commonly used as a CAF biomarker for guiding NPs,<sup>191</sup> TNC,<sup>192</sup> which is expressed in the ECM, has also been increasingly used to target CAFs. The FH and CFH peptides exhibit high binding affinity for TNC,<sup>193</sup> thus, by conjugating these targeting moieties to NPs, the precise targeting of CAFs can be achieved. Moreover, optimized liposome-based NPs, such as lipid nano-capsules, have demonstrated even higher efficiency than conventional liposomes.<sup>194</sup> Additionally, DSPE-PEG2000, known for their stable chemistry, can serve not only as raw materials for liposome synthesis but also as efficient drug carriers. Conjugation of DSPE-PEG2000 to liposomes extends the half-lives of these delivery systems.<sup>144,145</sup> Several traditional Chinese medicines, including oxymatrine,<sup>143</sup> salvianolic acid B (SAB),<sup>148</sup> and fraxinellone (Frax)<sup>147</sup> have also shown great potential as fibroblast modulators when conjugated to DSPE-PEG2000. Multifunctional lipo-based NPs are also developing currently. For instance, thermosensitive lipid NPs can be constructed using the photothermal agent IR-780 and the phospholipid Dipalmitoylphosphatidylcholine (DPPC) (Figure 4).<sup>150</sup> Furthermore, the potential of using liposome complexes with polymeric micelles or biomacromolecules in targeting CAFs has been investigated. Notably, lipid-coated protamine DNA (LPD) complexes exhibit exceptional transfection efficiency, making them suitable for the delivery of drugs that regulate intracellular metabolism and pathways. Indeed, loading LPD complexes with TNF-related factors has been shown to effectively inhibit CAFs and induce their apoptosis.<sup>149</sup>

#### Metals and Metal Oxides

Metallic NPs and their derivatives possess several advantageous properties, including sustained release, local heat generation, high photothermal sensitivity, high affinity, and improved irradiation effects. These unique characteristics enable them to effectively target CAFs and remodel the TME.<sup>162,195,196</sup> Among the types of metallic NPs, gold NPs

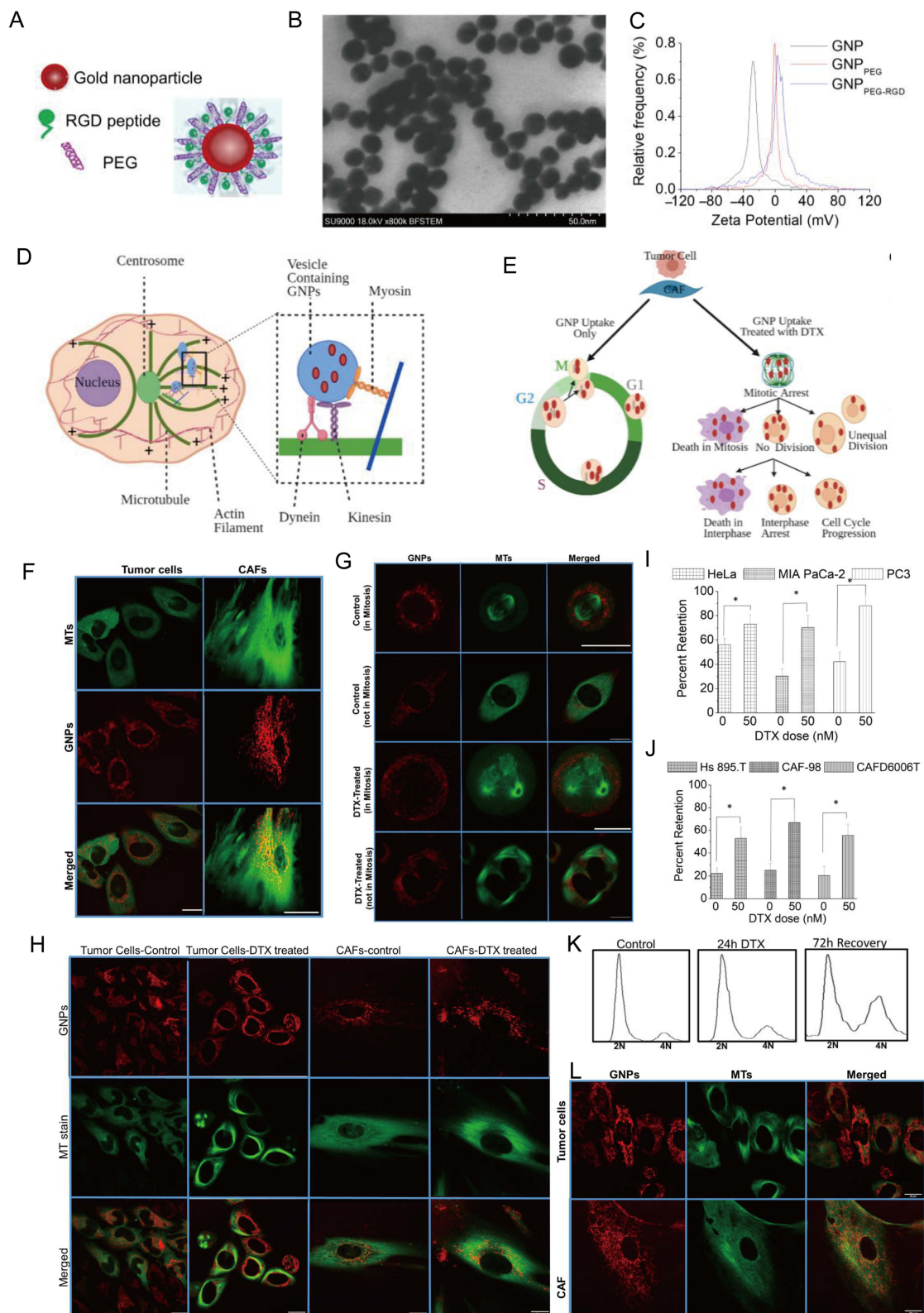


**Figure 4** Design, characteristics and effects of IR780/DPPC/BMS. **(A)** Scheme of IR780/DPPC/BMS. **(B)** Synthetic route of IR780-PEG-ODC. **(C)** In vitro NIR-triggered drug release profiles. **(D)** Transmission electron microscope (TEM) images of IR780/DPPC/BMS at different temperatures. **(E)** Cellular uptake and responsive drug analysis in 4T1 tumor cells. **(F)** In vivo photothermal effect. **(G)** Tumor volumes of 4T1 tumor-xenografted mice in different treatment groups. **(H)** The quantitative analysis of released cytokines in primary tumor after synergistic immuno-photothermal therapy. \*:P<0.05; \*\*:P<0.01; \*\*\*:P<0.001. Reprinted from *Cancer Letters*, volume 522, Tan Y-N, Li Y-P, Huang J-D, et al. Thermal-sensitive lipid nanoparticles potentiate anti-PD therapy through enhancing drug penetration and T lymphocytes infiltration in metastatic tumor. 238–254. Copyright 20221, with permission from Elsevier.<sup>150</sup>

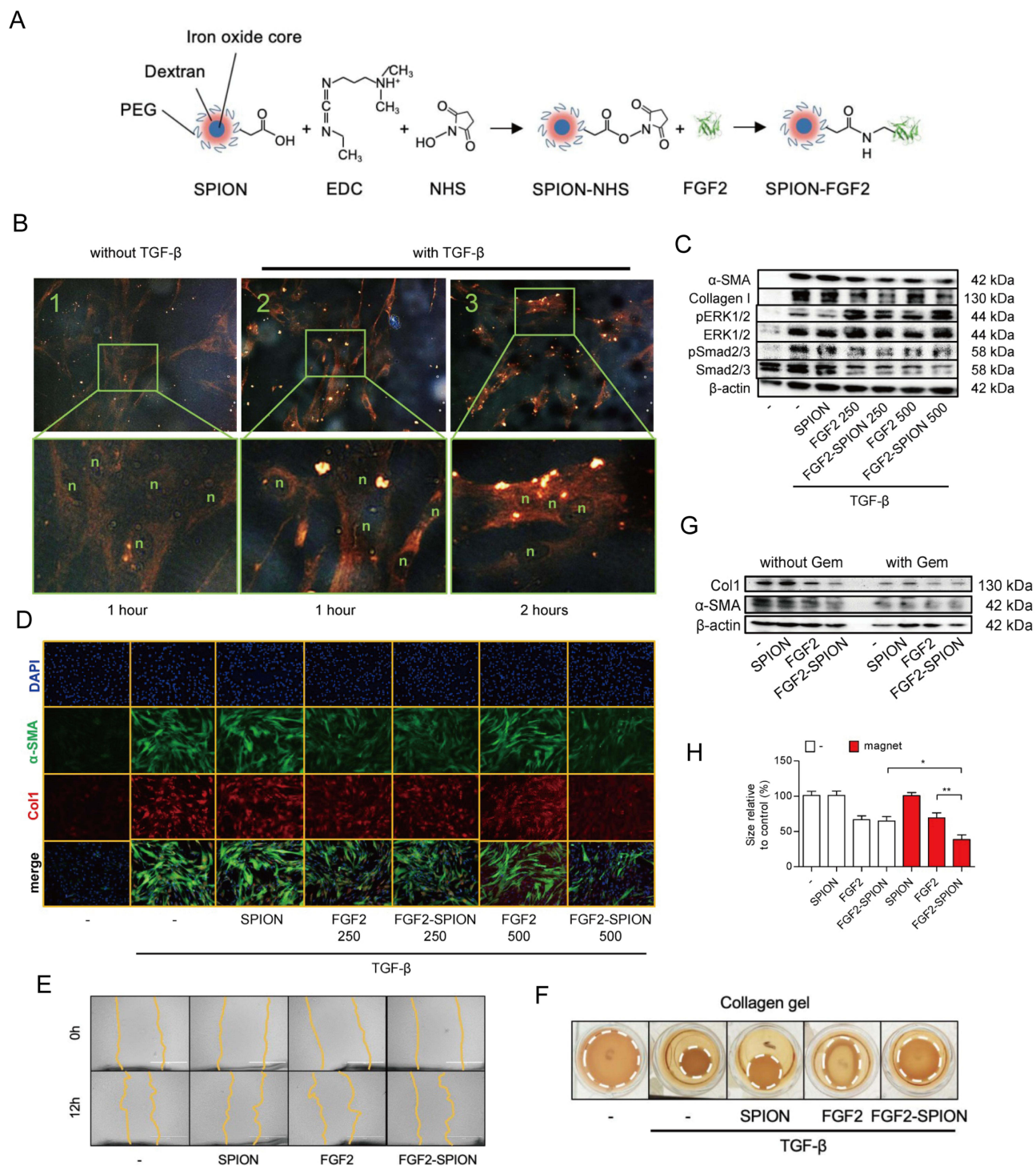
(GNPs) and iron NPs are the most commonly used. CAFs exhibit a 7-fold higher rate of GNP uptake and a 3-fold longer rate of GNP retention than tumor cells (Figure 5).<sup>197</sup> The therapeutic effect of GNPs can be further enhanced by combination with docetaxel (DTX); this may be because GNPs sensitize CAFs to external factors and promote drug absorption. Thus, GNPs not only serve as ideal carriers for CAF-targeted therapies but also remodel CAFs into drug repositories, leading to sustained effects. In vitro studies have demonstrated that GNPs can reduce radiotherapy resistance in tumor and CAF co-culture models by suppressing CAF activity.<sup>198</sup> Additionally, GNPs promote the transition of CAFs from an activated to a resting state; this may occur through the regulation of cellular lipid metabolism and the disruption of communication between tumor cells and CAFs via altered soluble factor secretion.<sup>159,160</sup> The functional properties of GNPs are influenced by their size; 3 nm is considered as the optimal GNP diameter.<sup>158</sup> Furthermore, multifunctional and optimized GNPs-related NPs have recently been developed. Hybrid gold and silver NPs, with a core-shell structure, have been designed to modulate CAFs even more effectively than conventional GNPs.<sup>155</sup> In these particles, gold acts as the Trojan-horse for precise silver delivery, which in turn effectively interferes with CAF-mediated communication pathways.<sup>199</sup> Gold-shell-based photothermal NPs also enhance the CAF killing ability of GNPs and promote targeted drug release.<sup>163</sup> Among the iron-based NPs, superparamagnetic iron oxide nanoparticles (SPOINs) have demonstrated exceptional in vivo compatibility.<sup>156,162,200</sup> Citrate-coated SPOINs can be easily absorbed by CAFs within 15–30 minutes of treatment.<sup>154</sup> Due to their magnetic properties, SPOINs can induce the mechanical destruction of CAFs under low-frequency magnetic conditions,<sup>201</sup> with smaller SPOINs proving most effective. Additionally, when exposed to alternating current magnetic fields, SPOINs can be used as a thermal therapy and induce apoptosis in CAFs.<sup>154</sup> Finally, SPOINs have a large surface area and are richly decorated by various functional groups, which serve as favorable drug attachment sites.<sup>156</sup> As such, this type of NP can enhance the half-life, solubility, and therapeutic effects of drugs. Notably, biomolecules such as relaxin<sup>202</sup> and fibroblast growth factor (Figure 6)<sup>157</sup> have been successfully conjugated to SPOINs to inhibit CAF differentiation and activation, ultimately reducing the extent of fibrosis in the TME. Moreover, the combination of gold and iron architectures enhances the CAF-targeting efficiency of NPs constructed using either metal alone. For instance, the iron-oxide-modified GNPs are preferentially absorbed by CAFs, where they act as a thermal source to effectively kill CAFs, reduce tumor stiffness, and ultimately lead to complete tumor elimination.<sup>163</sup>

### Polymeric Micelles

In recent years, polymeric micelles have been rapidly adapted as NPs for use in cancer therapy. Their significant advantage lies in their ability to penetrate into cells independently of the properties of their drug cargo.<sup>164,203,204</sup> Poly (lactic-co-glycolic acid) (PLGA) is one of the commonly used nanomaterials.<sup>205</sup> An example is ONP-302, a PLGA-based NP specifically designed to target TAMs and CAFs. With a diameter ranging from 400 to 800 nm and a negative surface charge of 35 to 50 mV, ONP-302 selectively targets CAFs, inhibits their cancer-promoting function, and induces their apoptosis.<sup>166,167</sup> PLGA-like polymers are commonly employed to construct multi-response and sequential delivery NPs (Figure 7).<sup>165,206</sup> FAP serves as the molecular target, while pH-responsive groups like folic acid, benzimidazole, and  $\beta$ -cyclodextrin are conjugated to PLGA-like polymers to form the complete CAF-modulating agents.<sup>170</sup> Certain molecules, such as amphiphilic peptides, 9-arginine, and PEG5K-P(MMESSN38)5K (PSN38) (Figure 8), possess both hydrophilic and hydrophobic properties.<sup>168,173</sup> These molecules have the ability to self-assemble into carrier cages when exposed to the appropriate medium. While these NPs exhibit excellent drug-loading properties, the addition of cell localization groups, such as FAP, is often necessary. Other polymers that have been reportedly used for CAF targeting include a complex of carboxymethylcellulose and DTX, which preferentially accumulates in CAFs and causes their depletion.<sup>172</sup> Micellar-like NPs synthesized from poly (ethylene glycol)-*b*-poly(lactide)-*co*-poly(N3- $\alpha$ - $\epsilon$ -caprolactone) demonstrate similar effects.<sup>171</sup> Furthermore, the positive surface charge of cationic poly(amidoamine) (PAMAM) enables better drug penetration into CAFs.<sup>174</sup> Finally, the self-assembly of anti-CAF agents and anti-neoplastic drugs into NPs has been identified as a promising approach for cancer therapy.<sup>175</sup>



**Figure 5** Docetaxel (DTX)-Mediated Uptake and Retention of Gold Nanoparticles (GNPs) in Tumor Cells and in CAFs. **(A)** Schematic diagram of functionalized GNP. **(B)** TEM of GNPs. **(C)** Zeta Potentials for pure GNPs. Scheme of transportation of GNPs in cells **(D)** and effects of DTX on cell division **(E)**. Confocal microscopy images of GNPs transportation **(F)** and effects of DTX on cell division **(G)**. **(H)** Effect of DTX on GNP Retention of NPs in the presence of DTX in tumor cells **(I)** and CAFs **(J)**. **(L)** Retention of GNPs in tumor cells and CAFs **(K)** post recovery. \* $P < 0.05$ . Reprinted from Alhussan A, Bromma K, Perez MM, et al. Docetaxel-mediated uptake and retention of gold nanoparticles in tumor cells and in cancer-associated fibroblasts. *Cancers (Basel)*. 2021;13(13):3157. Creative Commons.<sup>197</sup>

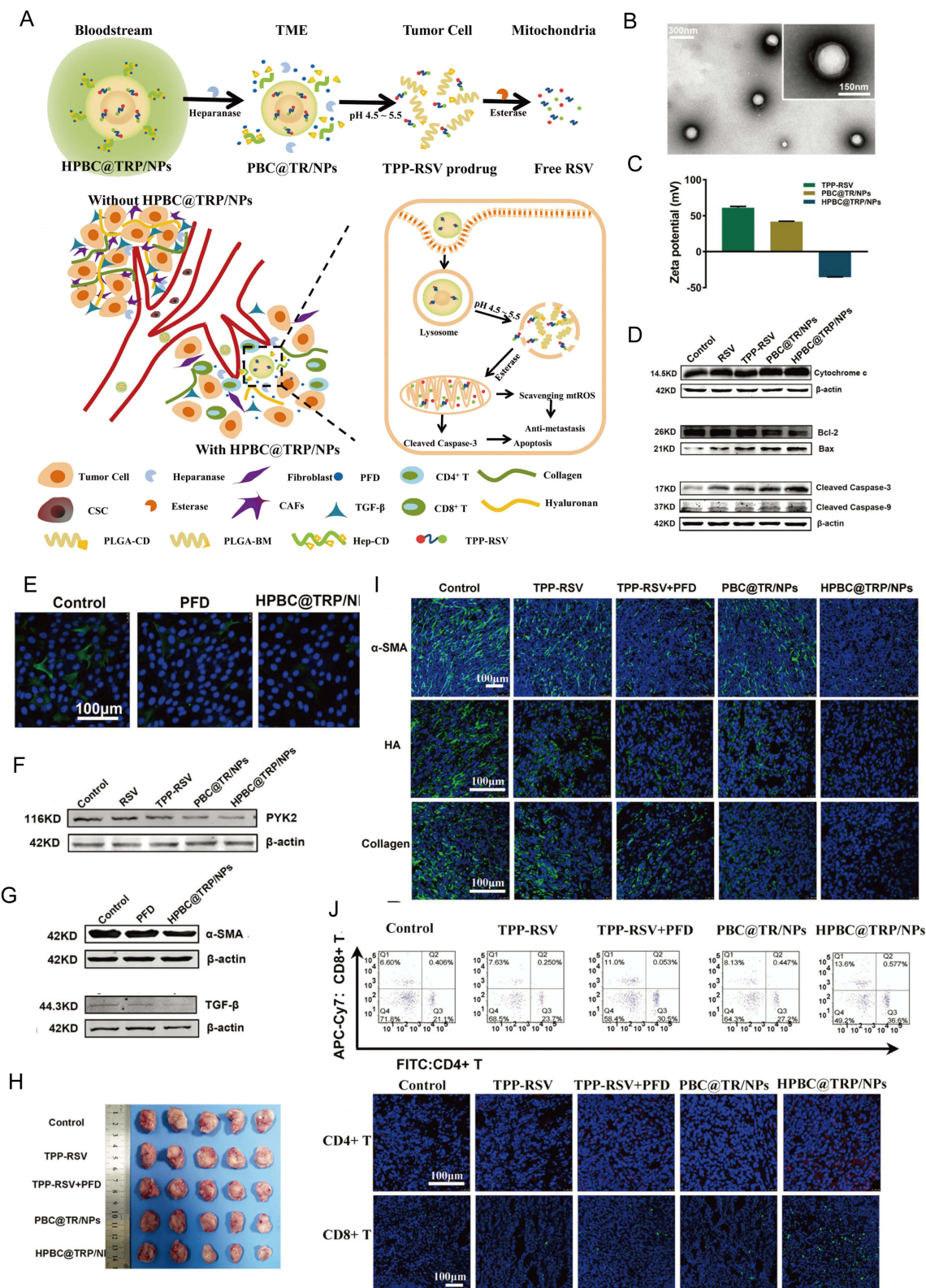


**Figure 6** Fibroblast growth factor 2 (FGF2) engineered SPIONs attenuate tumor stroma and potentiate the effect of chemotherapy in pancreatic tumor. **(A)** Schematic representation of conjugation of FGF2 to SPION. **(B)** Binding of FGF2-SPION on non-activated human pancreatic stellate cells (hPSCs) and activated hPSCs. Western blots demonstrating the differentiation-related biomarkers **(C)** and representative immunofluorescence images showing the effect of FGF2 and FGF2-SPION on the protein expression of  $\alpha$ -SMA and col-I in TGF- $\beta$ -activated hPSCs **(D)**. Effect of FGF2-SPION on hPSCs migration **(E)** and contractility **(F)**. **(G)** Effect of FGF2-SPIONs on the tumor stroma and gemcitabine efficacy in 3D heterospheroids. **(H)** Magnetic driven iron oxide accumulation. \*P<0.05; \*\*P<0.01. Reprinted from Mardhian DF, Vrynas A, Storm G, et al. FGF2 engineered SPIONs attenuate tumor stroma and potentiate the effect of chemotherapy in 3D heterospheroidal model of pancreatic tumor. *Nanotheranostics*. 2020;4(1):26–39. Creative Commons.<sup>157</sup>

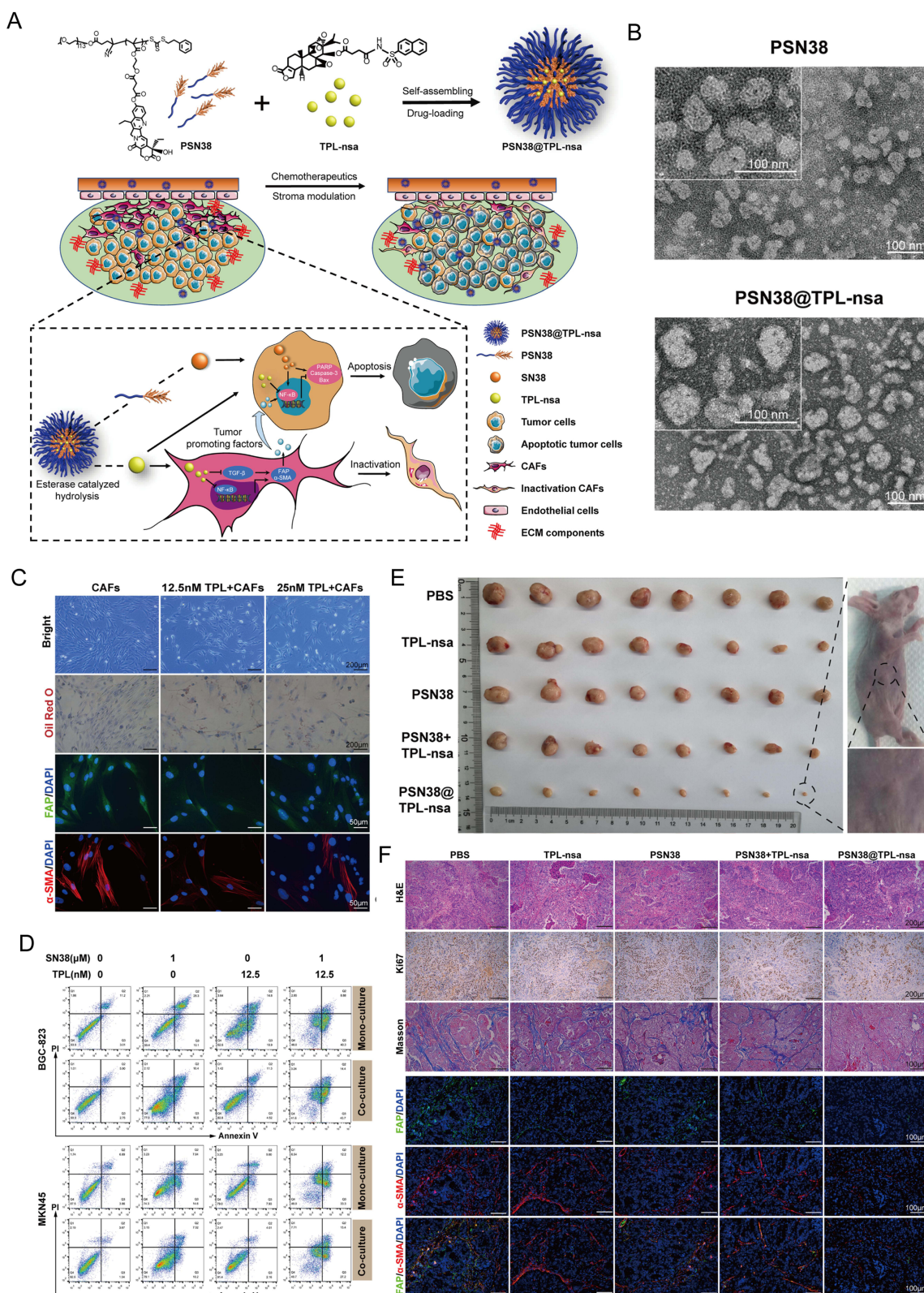
**Proteins**

Ferritin and albumin are the primary protein types utilized in the construction of NPs for CAF targeting. These proteins possess similar chemical characteristics and are natural biomacromolecules that elicit minimal immunogenic





**Figure 7** On-demand responsive nanoplatform mediated targeting of CAFs and down-regulating mtROS-PYK2 signaling for antitumor metastasis. **(A)** Disintegration of the on-demand successively responsive HPBC@TRP/NPs and its therapeutic mechanism. **(B)** TEM images of HPBC@TRP/NPs. **(C)** Zeta potential reversal characteristic. **(D)** Evaluation of the relative cell viability and mechanism of the apoptosis signal pathway. **(E)** CLSM images of  $\alpha$ -SMA activated CAFs in 4T1/NIH3T3 cells treated with PFD and HPBC@TRP/NPs. Down-regulation of the mitochondrial oxidation system and cancer associated fibroblasts in vitro: Western blot protein expression levels of PYK2 **(F)**,  $\alpha$ -SMA **(G)**, and TGF- $\beta$  **(G)** in 4T1/NIH3T3 co-cultured cells. **(H)** Enhanced in vivo therapeutic effect. **(I)** Normalization of the tumor ECM to remodel TME. **(J)** Modulation of TIME in 4T1/NIH3T3 tumors after treatments. Reprinted from Zuo T, Zhang J, Yang J, et al. On-demand responsive nanoplatform mediated targeting of CAFs and down-regulating mtROS-PYK2 signaling for antitumor metastasis. *Biomater Sci.* 2021;9(5):1872–1885. 2021 © Royal Society of Chemistry.<sup>165</sup>



**Figure 8** Prodrug polymeric micelles integrating CAFs and synergistic chemotherapy for gastric cancer (GCs). **(A)** Schematic overview of the composition and synergistic mechanisms. **(B)** TEM images of PSN38 and PSN38@TPL-nsa NPs. **(C)** Low dose of TPL inactivated CAFs and inhibited CAFs. **(D)** Apoptotic analysis of BGC-823 and MKN45 cells after the treatment of TPL, SN38 or a combination of SN38 and TPL for 24 h in the co-culture and mono-culture model respectively. **(E)** The antitumor efficiency of PSN38@TPL-nsa in GC PDX model. **(F)** PSN38@TPL-nsa remodeled GC microenvironment in vivo. Reprinted from Zheng S, Wang J, Ding N, et al. Prodrug polymeric micelles integrating cancer-associated fibroblasts deactivation and synergistic chemotherapy for gastric cancer. *J Nanobiotechnology*. 2021;19(1):381. Creative Commons.<sup>168</sup>

reactions.<sup>207–209</sup> The presence of amino acid residues within these protein-based NPs provides multiple binding sites for drug loading, and their amphoteric nature allows for pH-responsive behavior within the TME.<sup>210</sup> Ferritin and albumin have been employed as nanocarrier cages, to which FAP -specific single-chain variable fragments have been conjugated to target CAFs (Figure 9).<sup>181,184</sup> These NPs have been recently modified to encapsulate ZnF16Pc, a common photosensitizer, for use in photodynamic therapy (PDT).<sup>181,182</sup> These NPs not only selectively eradicate CAFs to halt tumor progression but also activate T cells and enhance the efficacy of ICI therapy.

### Cell Membranes

Cell membranes have recently gained significant attention as NP biomaterials as they closely resemble the cellular components of the TME. Cell membrane surfaces exhibit a greater diversity of receptors and target molecules than the surfaces of synthetic NPs, allowing them to more effectively simulate the biological characteristics and functions of their source cells.<sup>211–213</sup> Currently, the application of CAF-targeting cell membrane NPs is not widespread, and the main sources of cell membranes include RBCs, tumor cells, and fibroblasts. RBC-based NPs can protect peptides used in cell targeting from degradation.<sup>187</sup> For instance, the FnBPA5 peptide exhibits a strong affinity for CAFs and the ECM, but its rapid metabolism poses a challenge. Encapsulating the external peptide segment of PLGA-PEG-FnBPA5 within RBC-based NPs enhances its targeting ability.<sup>187</sup> The homology between the CAF membranes of NPs and those of their target cells makes them ideal for modulating CAFs.<sup>185,214</sup> Moreover, when used as adjuvants, tumor cell membranes can activate immune responses to enhance the efficacy of anti-fibrotic drugs.<sup>186</sup> Furthermore, the hybridization of the two types of cell membranes was more effective than using a single type, as it resulted in the targeted damage of both CAFs and tumor cells in the TME (Figure 10).<sup>215</sup>

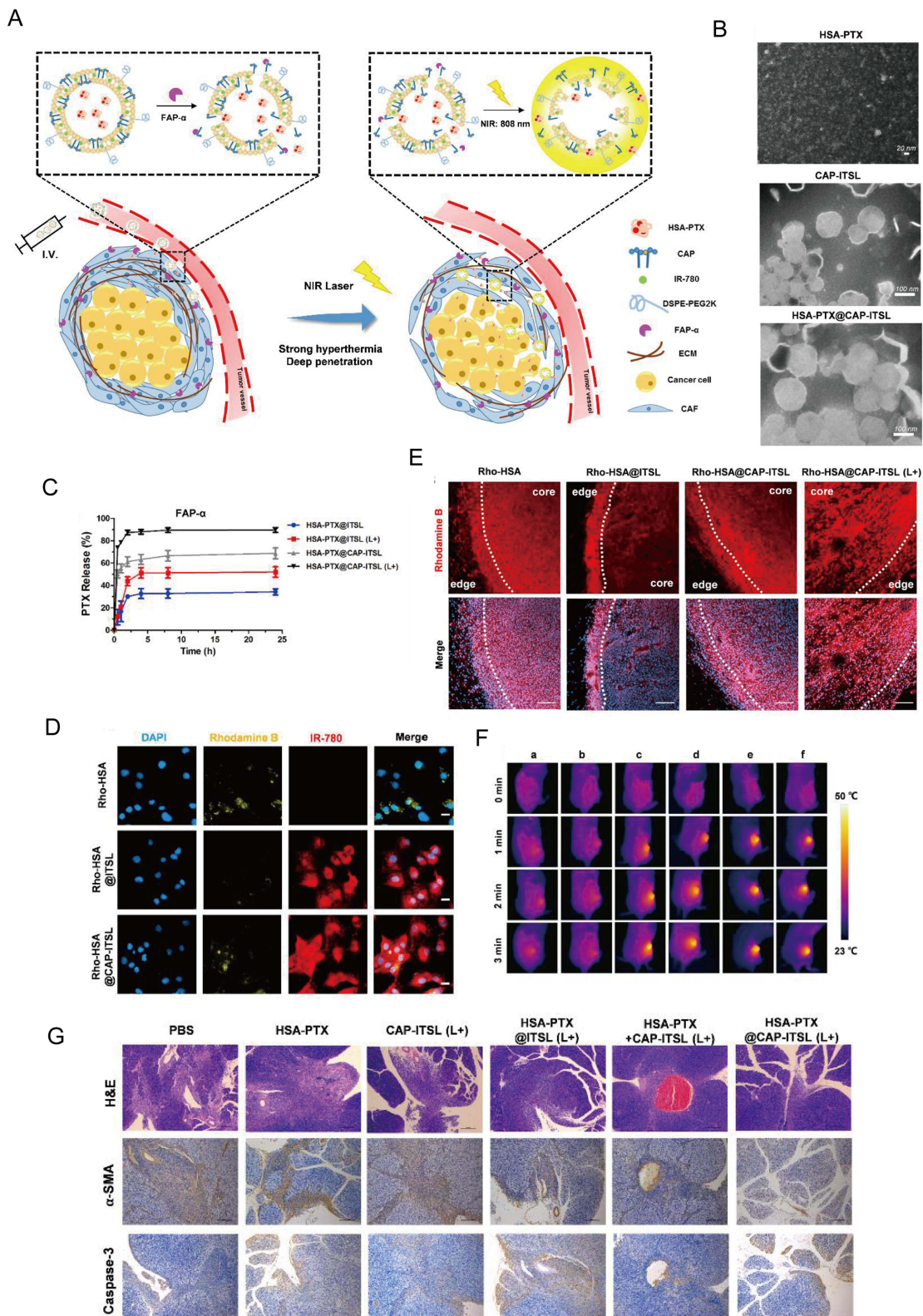
## Strategies of NPs Construction for Targeting CAFs

### CAF Depletion

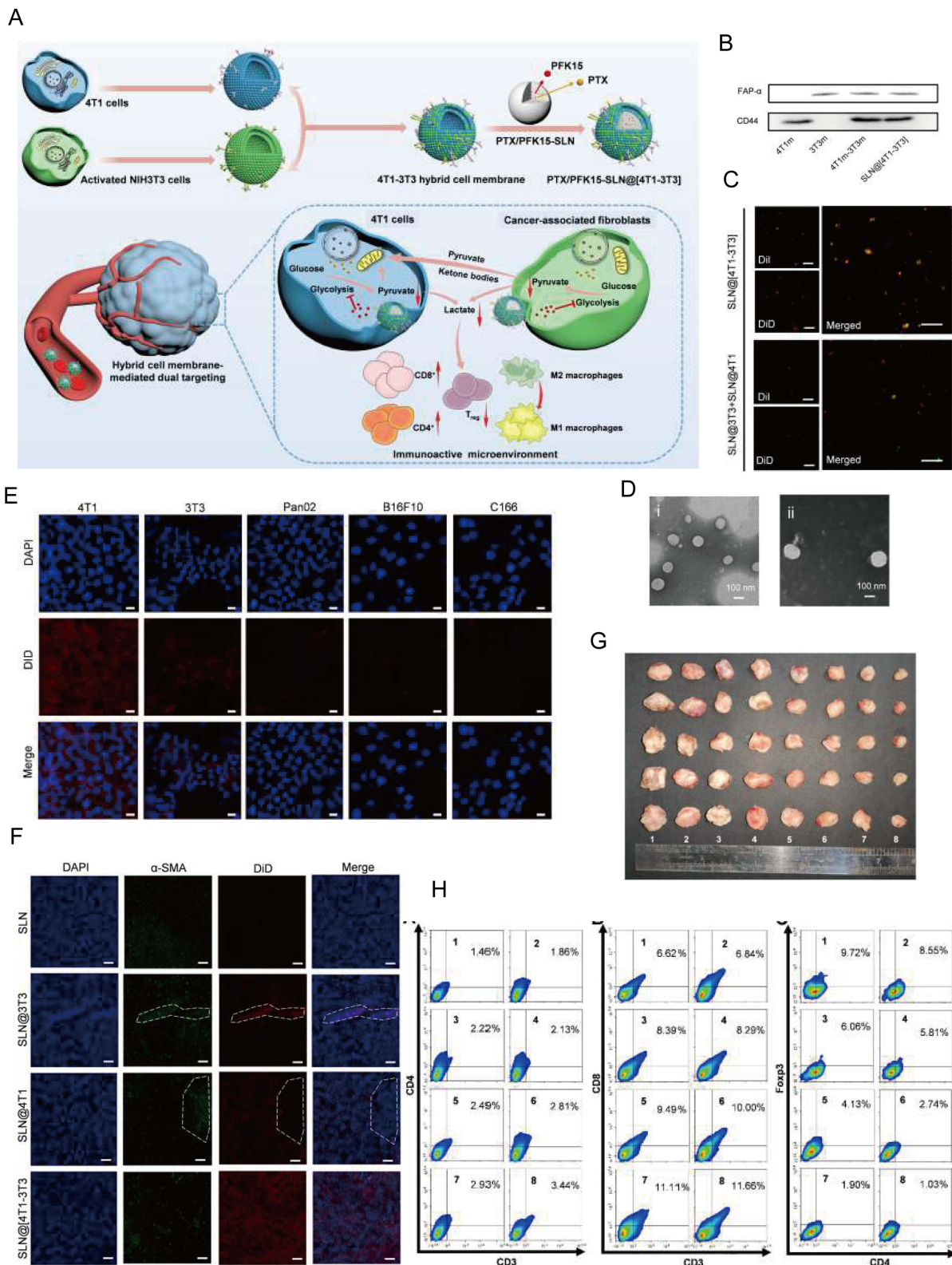
Modifying the NPs surface with molecules specific for CAF surface markers represents the most common method of CAF targeting. For instance, targeting FAP, a surface marker expressed by 90% of CAFs, to deliver drug payloads to CAFs has been shown to significantly inhibit tumor growth.<sup>216</sup> In a recent study, Shin, H designed antigenic peptide epitopes derived from the FAP protein and conjugated them to lipid NPs containing CpG adjuvants which is FAPPEP-SLNPs and it can be acted as a nano-vaccine, effectively activating T cell immunity and eliminating FAP-positive CAFs.<sup>217</sup> And in the research of Guo et al, they synthesized dipeptide Z-glycine-proline to target FAP to eliminate CAFs barriers.<sup>218</sup> Additionally, TNC, a component of the ECM expressed by CAFs,<sup>144,192</sup> has been targeted using a stable peptide called FH.<sup>143</sup> Some studies have also proposed targeting  $\alpha$ -SMA. Depletion of  $\alpha$ -SMA<sup>+</sup> CAFs in an  $\alpha$ -SMA-thymidine kinase hybrid transgenic mouse model resulted in the inhibition of tumor angiogenesis but also increased hypoxia, subsequently inducing EMT and stemness. Furthermore, targeting  $\alpha$ -SMA has been shown to increase Treg infiltration into the tumor and exacerbate immunosuppression in the TME.<sup>219,220</sup> Although the direct use of  $\alpha$ -SMA-targeting immunotherapeutic drugs has not achieved the desired results, their value should not be dismissed in the context of NPs-based therapeutics.<sup>169,219</sup> NPs can be used to deliver CAF-sensitive drugs rather than directly targeting  $\alpha$ -SMA.<sup>169</sup> Furthermore, NPs can accommodate a complex formulation of both anti-tumor and anti-CAF drugs<sup>169</sup> to remove the side effect of merely targeting CAFs.<sup>221</sup>

The direct NP-mediated elimination of CAFs typically uses one of two strategies. The first strategy is using NPs to load non-specific cytotoxic drugs, and then conjugate the targeting molecules such as CAF surface biomarkers to NPs surface. The second strategy encompasses anti-fibrotic drugs, which exhibit a high affinity for CAFs but require modification and encapsulation within NPs due to their pharmacokinetic limitations. In some solid tumors with high infiltration of CAFs, the EPR effect will decrease. Depletion of CAFs can “loosen” the TME to assist nanoparticles enter the deep tumor tissue. Both mentioned strategies can improve their effect through the enhanced EPR effect provided by CAF depletion.

In the first strategy, the CAF-targeting NPs can reduce fibrosis, improving the penetration of anti-tumor drugs into the tumor tissue and suppressing CAF function. Additionally, the anti-tumor drugs can themselves eliminate CAFs, leading to favorable therapeutic outcomes. Several promising candidates, which achieve both tumor inhibition and CAF



**Figure 9** Targeting CAFs by dual-responsive lipid-albumin NPs to enhance drug perfusion for pancreatic tumor therapy. **(A)** Diagram of dual-responsive lipid-albumin NPs (HSA-PTX@CAP-ITSL) **(B)** TEM images of HSA-PTX, CAP-ITSL and HSA-PTX@CAP-ITSL. **(C)** The PTX release profiles of different formulations incubated with FAP- $\alpha$ . **(D)** cellular uptake after TGF- $\beta$  activated NIH 3T3 cells incubated with different formulations. **(E)** Rhodamine B distribution of different treatment groups. **(F)** IR thermal images of subcutaneous tumor-bearing mice in different treatment groups. **(G)** H&E staining,  $\alpha$ -SMA staining and Caspase-3 staining of tumor tissues. Reprinted from *J Control Release*, volume 321, Yu Q, Qiu Y, Li J, et al. Targeting cancer-associated fibroblasts by dual-responsive lipid-albumin nanoparticles to enhance drug perfusion for pancreatic tumor therapy. 564–575, Copyright 2020, with permission from Elsevier.<sup>184</sup>



**Figure 10** Metabolic Reprogramming by Dual-targeting Biomimetic NPs for Enhanced Tumor Chemo-Immunotherapy. **(A)** Scheme of PTX/PFK15-SLN@[4T1-3T3] NPs. **(B)** Western blot assay of 4T1 membrane (4T1m), 3T3 membrane (3T3m), 4T1-3T3 membrane (4T1m-3T3m), and SLN@[4T1-3T3] NPs for 4T1 membrane marker CD44 and 3T3 membrane marker FAP- $\alpha$ . **(C)** CLSM images of the SLN@[4T1-3T3] NPs and a physical mixture of SLN@4T1 NPs and SLN@3T3 NPs. **(D)** Representative TEM images of PTX/PFK15-SLN and PTX/PFK15-SLN@[4T1-3T3] NPs. **(E)** CLSM images of 4T1, 3T3, B16F10, Pan02 and C166 cells stained with DAPI and cultured with DiD-labeled SLN@[4T1-3T3] NPs. **(F)** Representative CLSM images in tumor sites. The dotted line inside indicated CAFs. **(G)** Antitumor effect evaluation in vivo. **(H)** Representative flow cytometric images of the tumor CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and Treg cells (gated by CD25). Reprinted from *Acta Biomater*, volume 148, Zang S, Huang K, Li J, et al. Metabolic reprogramming by dual-targeting biomimetic nanoparticles for enhanced tumor chemo-immunotherapy. 181–193, Copyright 2022, with permission from Elsevier.<sup>215</sup>

depletion, have been identified; these include acriflavine (ACF),<sup>146</sup> paclitaxel (PTX) (Figure 10),<sup>215</sup> DTX,<sup>172</sup> and navitoclax (Nav).<sup>183</sup> LNC-encapsulated PTX and ACF have been shown to inhibit tumor growth in vitro.<sup>146</sup> Moreover, FAP-guided thermosensitive liposome-coated human serum albumin-PTX NPs can induce hyperthermia in the tumor stroma to enhance the release of chemotherapy drugs to eliminate CAFs (Figure 9).<sup>184</sup> Self-polymerizing micelles containing cycloamine (CPA) and PTX can reshape the TME by regulating Hedgehog signaling.<sup>169,222</sup> NPs generated using the cellax-DTX polymer (with a size of 120 nm), specifically accumulate in  $\alpha$ -SMA<sup>+</sup> CAFs, leading to their depletion. This strategy reduces the density of the TME, consequently increasing tissue perfusion. Furthermore, cellax-DTX NPs have been shown to deplete TAMs, thereby disrupting the lines of communication between TAMs and CAFs, subsequently inducing CAF depletion.<sup>172</sup> Nav promotes the apoptosis of CAFs by preventing the binding of the pro-apoptotic molecules BAX and BAK to BCL-2.<sup>223,224</sup> Although the application of naked Nav is limited by its toxicity and hydrophobic nature,<sup>225</sup> the use of Nav encapsulated in FAP-labeled H-ferritin (HF<sub>n</sub>) cages has demonstrate promising anti-CAF effects in vivo.<sup>183</sup> The action of such FAP-labeled HF<sub>n</sub> cages has now been validated in various studies. For instance, ZnF16Pc can be encapsulated within these cages to induce PDT.<sup>181,182</sup> In another study exploring the nano-drug loading of Nav, a TNC-linked peptide was used as a targeting ligand, resulting in similar levels of efficacy.<sup>144</sup> Moreover, although the co-delivery of Nav and doxorubicin (DOX) does not enhance therapeutic efficacy, it allows for a reduction in the dosage of DOX, thereby alleviating its side effects. The use of FAP- and folate-conjugated dual-responsive NPs loaded with DOX has shown excellent therapeutic efficacy by facilitating endolysosomal escape<sup>170</sup> and removing stromal barriers via CAF elimination; the mechanism of CAF depletion may rely on the downregulation of  $\alpha$ -SMA expression on CAFs and their subsequent inhibition. The absence of side effects may be attributed to the fact that NP-mediated anti-tumor effects may indirectly influence  $\alpha$ -SMA expression on CAFs. In breast cancer, Her2-DOX-SPIONs@PLGA@Au induces a photothermal effect, which reduces CAF infiltration, promotes DOX release, and enhances immunogenic cell death.<sup>161</sup> Retinoic acid (RA), a vitamin A metabolite, has been shown to disrupt Golgi apparatus function in CAFs.<sup>226</sup> Encapsulation of the RA-DOX complex within F<sub>n</sub>BPA5-RBC-PEG-PLGA promotes CAF targeting, inhibits the Golgi functions of CAF, and facilitates DOX penetration.<sup>187</sup> DOX NPs loaded with sulfated hyaluronic acid and mifepristone operate via a similar mechanism to the previously mentioned NPs.<sup>227</sup> The incorporation of triptolide (TPL), an active ingredient of *Tripterygium wilfordii*, in PSN38@TPL-nsa can inhibit CAFs, reversing their pro-tumor functions and increasing their sensitivity to chemotherapy by inhibiting the NF- $\kappa$ B pathway<sup>168</sup> (Figure 8). PFK15, an inhibitor of glycolysis, was loaded into hybrid membrane NPs composed of tumor and fibroblast cell membranes. The resulting NPs could interfere with glucose metabolism in both cell types, thereby promoting the infiltration of CD8 T cells into the tumor (Figure 10).<sup>215</sup> Additionally, fibroblast membrane NPs can be loaded with photosensitive materials to achieve similar effects.<sup>185</sup> For instance, in the case of IR780/DPPC/BMS, the IR780 component induces PTT, DPPC facilitates thermosensitive release, and BMS inhibits the PD-1/PD-L1 pathway. When exposed to radiation, this system can disrupt ECM integrity and CAF function. Of note, this effect does not specifically target CAFs, but rather facilitates the capture of tumor-associated antigens and enhances the sustained release of PD-1 inhibitors via the PTT-induced degradation of the ECM (Figure 4).<sup>150,228</sup>

In the second strategy, the NPs or the encapsulated drugs themselves exhibit strong CAF-targeting and -modulating abilities. For example, 3-nm GNPs are easily absorbed by CAFs and downregulate key CAF markers (eg, N-cadherin,  $\alpha$ -SMA, vimentin, FSP-1, and S100 family proteins) to inhibit CAF activation and motility. Additionally, GNPs can reduce the CAF-mediated secretion of HGF, IL-6, IL-8, TGF- $\beta$ 1, and PDGF- $\alpha$ , thereby disrupting the communication between CAFs and tumor cells.<sup>158</sup> Additionally, the use of GNPs modified to contain iron oxide (GOINFs) has been validated. These NPs function as efficient nano-heaters and outperform GNPs in the near-infrared (NIR) spectral range.<sup>163</sup> Similar to gold, iron exhibits a pronounced thermal effect and can be effectively delivered to CAFs. Following a brief exposure to an alternating magnetic field, SPIONs can induce caspase 8-dependent apoptosis in CAFs.<sup>154</sup> Furthermore, a relatively novel, promising approach using ultra-small iron oxide NPs exploits the magnetic properties of iron to mechanically damage both tumors and CAFs.<sup>201</sup> Other non-metallic materials with similar CAF-eliminating capabilities also exist. For instance, PLGA-derived ONP-302 can delay tumor growth and reverse the exhausted and immunosuppressive phenotypes of CD8<sup>+</sup> T cells.<sup>166</sup> In addition, ONP-302 induces the polarization of macrophages toward the M1 subtype, inhibits the expression of pro-tumor genes in CAFs, and promotes CAF apoptosis. The NP-mediated delivery of small interfering

RNA has also emerged as an effective strategy for targeting CAFs. CAFs secrete CXCL12 to retain their immunosuppressive properties and facilitate tumor growth and migration via the activation of the CXCL12/CXCR4 pathway. Therefore, utilizing the PNP/siCXCL12/monoclonal antibody approach to effectively block CXCL12 expression in CAFs could be a promising strategy to reduce tumor growth and angiogenesis.<sup>20</sup>

### Inhibiting CAF Activation

Using NPs to revert activated CAFs back to a resting state represents a feasible CAF-modulating strategy. However, the presence of cytokines, such as TGF- $\beta$ 1, IL-10, and IL-6, within the TME can persistently activate CAFs and significantly reduce the efficacy of CAF-targeting drugs.<sup>9</sup> Therefore, it is crucial for NPs formulations to maintain optimal drug concentrations in the tumor and elicit long-lasting effects. Many studies have focused on targeting TGF- $\beta$ 1, the main inducer of fibroblast activation.<sup>12</sup> In the recent research of Zheng et al, they found that mPEG-PLGA loaded baicalein could inhibit CAFs activation through blocking TGF- $\beta$  signaling pathway.<sup>179</sup> Moreover, in another study by Mardhian et al, the SPOIN-mediated delivery of fibroblast growth factor 2 has been shown to inhibit the pSmad2/3 pathway and activate ERK1/2 signaling, thus, effectively interfering with TGF- $\beta$ 1-induced CAF activation. This in turn reduced  $\alpha$ -SMA and collagen-1 expression in CAFs and inhibited the formation of new CAFs via the differentiation of stellate cells (Figure 6).<sup>157</sup> Moreover, the liposomal delivery of oxymatrine can restore the ECM and collagen content of the TME to normal levels, downregulate HMGB1, and inactivate stellate cells, consequently reducing the TGF- $\beta$ 1 concentration.<sup>143</sup> Similarly, PEG-encapsulated SAB inhibits TGF- $\beta$ 1 secretion, which may increase the expression of tumor suppressor factors such as CXCL9 and CXCL10, recruit CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and M1 macrophages, while reducing the infiltration of MDSCs, Tregs, and M2 macrophages.<sup>148</sup> CAFs may be the cause of limited therapeutic effects of CAR-T in solid tumors. So, Tang et al utilized amphipathic hydroxyethyl starch-polycaprolactone to load TGF- $\beta$  inhibitor (LY2157299) and photosensitizer indocyanine green. This co-loaded NPs can enhance the CAR-T effects and maybe a promising application.<sup>180</sup>

Moreover, anti-tumor drugs are often used to reduce CAF activation. Pirfenidone, a TGF- $\beta$ 1 antagonist, is commonly used in the treatment of idiopathic pulmonary fibrosis. In a recent study, Zuo et al developed pirfenidone-containing HPBC@TRP/NPs, which allow pirfenidone to effectively reach the TME without degradation in the peripheral tissues and rapidly exert its therapeutic effects. Simultaneously, another core drug, resveratrol, enters the cells and is released through lysosomes, where it activates the mtROS-PYK2 pathway to induce mitochondria-driven apoptosis (Figure 7).<sup>165</sup> In the study of Li et al, they used glycyrrhetic acid (GA) to target hepatocellular carcinoma and HA to target hepatic stellate cells, and then constructed GA&HA-modified liposomes to load aprepitant (APR) and curcumin (CUR), which is CUR-APR/HA&GA-LPs. In vivo and in vitro, CUR-APR/HA&GA-LPs can prevent hepatic stellate cells transforming into CAFs.<sup>151</sup> In another study from Wu et al, they utilized the same construction methods in Li et al study and showed that the berberine also presented the same effects of targeting CAFs as APR.<sup>152</sup>

NPs can improve drug delivery into cells. This approach not only enables the targeting of CAFs but also ensures optimal drug doses are delivered to the tumor site.<sup>198</sup> GNPs serve as a prime example in this regard. GNPs (with a size of 20 nm) not only suppress the expression of CAF markers (specifically  $\alpha$ -SMA and fibronectin), diminishing their responsiveness to activators, but also disrupt intercellular communication by interfering with TGF- $\beta$ 1, TSP1, PDGF, and uPA release from tumor cells.<sup>159</sup> Additionally, GNPs promote the expression of FASN, SREBP2, and FABP3 in their target cell, thereby increasing the cellular lipid content and causing the cell to adopt a resting phenotype.<sup>159</sup> Another noteworthy example involves the use of an LPD-coated secretable TNF-related apoptosis-inducing ligand (sTRAIL). This construct exhibits stable expression and release within CAFs, inducing apoptosis in adjacent cancer nests while concurrently inhibiting CAF activation.<sup>149</sup>

### Disrupting CAF Function

This type of NPs mainly targets CAF indirectly, causing non-specific cellular damage, which affects CAF functions and products. To date, this approach has proved more effective than others in reversing the immunosuppressive state of the TME. For instance, Frax (with a diameter of 145 nm) is absorbed by both tumor cells and CAFs. Once in the cells, Frax reduces the expression of CUGBP1, thereby interfering with the TGF- $\beta$ 1 and IFN- $\gamma$  pathways and attenuating tumor

fibrosis.<sup>147</sup> Additionally, Frax reduces the amounts of immunosuppressive cells such as MDSCs in the TME and enhances the infiltration of CD8 T cells. Moreover, the action of Frax can be further boosted by combining it with tumor vaccines. The presence of surface molecules, such as CXCR4 and CD44, on PLGA NPs coated with tumor cell membranes inhibits the migration of tumor cells toward CAFs and promotes the infiltration of T cells, particularly IFN $\gamma$  +CD8<sup>+</sup> T cells into the tumor.<sup>229</sup> By utilizing hyperthermia, FAP-guided, thermosensitive, liposome-coated human serum albumin -PTX NPs selectively target the tumor stroma and eliminate CAFs, facilitating the release of chemotherapy drugs (Figure 9).<sup>184</sup> In the study of Panagi et al, they developed tranilast-loaded micelles, which can be uptake by CAFs thus inhibiting the expression of TGF- $\beta$ . In the breast cancer model constructed by 4T1 and EO771, tranilast-loaded micelles can reduce the content of HA and collagen, which induce CD8 T cells penetrate.<sup>177</sup> Another study reported the development of a NIR-responsive, membrane-coated PD-1/PD-L1 inhibitor; its primary function was to enhance the capture of tumor-associated antigens by DCs and activate T cells, while its secondary function was to eliminate CAFs via PTT.<sup>186</sup> Polymer-coated, sunitinib-containing NPs enhance the infiltration of T cells into the tumor while reducing the proportions of Tregs and MDSCs in the TME. They also remodel the TME by exerting effects on CAFs, collagen, and blood vessels, as well as altering the cytokine composition of the TME by facilitating the transition from a T helper (Th)2 to a Th1 immune response.<sup>176</sup> In the recent study from Li et al, they chose losartan to reduce the activity of CAFs. And they used nanoliposome to co-load resiquimod, DOX and losartan to construct the multifunctional NPs to remodel TME.<sup>153</sup>

CAFs contribute to tumor development and drug resistance by activating the Hedgehog signaling pathway in a paracrine manner. In a study by Wang et al the use of SN38-coated NPs containing the Hedgehog pathway inhibitor GDC-0449 effectively suppressed the expression of key drug resistance molecules (eg, GLI-1 and UGT1A) while reducing collagen content within tumor tissues.<sup>173</sup> Additionally, Kovacs et al have discovered that gold-silver-gold composite NPs possess potent anti-metastatic properties and can inhibit the pro-tumor effects of CAFs, ultimately reducing the migration and invasiveness of tumor cells.<sup>155</sup> In colorectal cancer, CAFs can secrete hepatocyte growth factor to promote tumor progression. Based on this, Shen et al designed siRNA to interfere the expression of hepatocyte growth factor and used PLGA to deliver the siRNA. When combined with chemotherapy, the novel PLGA-siRNA NPs can overcome the chemotherapy-induced hepatocyte growth factor upregulation and then inhibit the CAFs activation. This loop showed great potential of targeting CAFs to alleviate chemotherapy resistance.<sup>178</sup> There also exist an approach which is inserting molecules for disrupting CAFs function on the surface of NPs rather using NPs to load them. Liu et al inserted integrin  $\beta$ 1 inhibiting peptide FINIII4 on their PLGA loaded DOX NPs. This made the NPs can respond to FAP and induce the combination of FINIII4 and integrin  $\beta$ 1 to disrupt the functions of CAFs to decrease the density of tumor.<sup>230</sup>

## Conclusions and Future Perspectives

Although we had gained a considerable understanding of CAFs, many questions remain unanswered. The incomplete understanding of CAF biology may be a contributing factor to the occurrence of many unexpected outcomes in clinical studies targeting CAFs.<sup>7</sup> Consequently, many studies investigating the use of NPs as CAF-targeted therapies are still somewhat away from being adopted into the clinic.<sup>18</sup> Currently, there is no explicit consensus on the tumor models used to study CAFs. The construction of these models faces challenges such as the selection of an appropriate cellular source for CAF generation (the most commonly used cells are embryonic fibroblasts<sup>231</sup> and fibroblasts from original tissue sources<sup>232</sup>), the method of model establishment (eg, xenograft models or spontaneous tumor models),<sup>9</sup> and the most physiologically relevant tumor cell to CAF ratios (existing studies suggest tumor cells to fibroblast ratios ranging from 1:1 to 1:10).<sup>9,231–233</sup> The cancer type is also an important consideration in studies of NPs-mediated CAF targeting. It is therefore crucial to choose research models that are optimized for the study of a specific cancer type. During the process of tumor development CAFs exhibit considerable heterogeneity, particularly in terms of their cellular origins and surface marker expression.<sup>9</sup> Thus, the optimal approach involves inducing tumor formation in situ, as it better aligns with the requirements of studying CAFs and replicates the natural occurrence of tumors. Furthermore, there is room for improvement in the way the cellular CAF sources are handled during the study of cell-membrane-encapsulated NPs. For instance, in the research of Li et al and Zang et al, the source of CAFs current is TGF- $\beta$ -treated NIH/3T3 cells, which



is evidently inaccurate.<sup>185,215</sup> The primary purpose of cell membrane encapsulation is to mimic the target cell, thereby increasing the targeting efficacy of the therapeutic approach. However, using highly artificial cell membranes may impact the therapeutic efficacy of NPs.

Currently, the main CAF-targeting NPs are constructed using lipids, metals, and various polymers. These materials have been extensively studied and have shown considerable success in CAF-targeted therapy studies. However, in recent years, emerging materials such as hydrogels,<sup>234</sup> nanoenzymes,<sup>235</sup> and metal or carbon rods have gained increasing attention.<sup>236</sup> These nanomaterials offer certain advantages over existing ones and have shown promising results in targeting tumor cells. Future research will explore whether these more optimized nanomaterials meet the diverse therapeutic requirements. Increasingly, specific targeting ligands (eg, FAP<sup>20,154,164</sup>), nanomaterials, and drugs are being combined to form complexes. However, this singular approach resembles that of drug-modified nanomaterials and does not fully exploit the unique properties of nanomaterials. Consequently, these nanoscale particle drugs eventually enter systemic circulation and undergo hepatobiliary metabolism, highlighting the importance of addressing how NPs can be engineered to evade rapid clearance from the body.<sup>165</sup> Recently, Zuo et al developed the HPBC@TRP/NPs system, which effectively protects the core drug-loaded NPs, thereby achieving improved therapeutic efficacy.<sup>165</sup>

Current strategies for NPs construction primarily focus on eliminating CAFs. However, previous basic and clinical trials have shown that normalizing the tumor stroma and TME may limit tumor occurrence and progression more effectively than direct CAF elimination.<sup>112,117,118</sup> Therefore, the future design of NPs targeting CAFs should place more emphasis on inhibiting CAF activation and interfering with CAF functions.<sup>15</sup> Consequently, compounds such as vitamin A/D and CXCL12-CXCR4/JAK pathway inhibitors are promising CAF-modulating drug candidates, as these drugs have already shown considerable efficacy in targeting CAFs in the clinical trials setting.<sup>124,125,131,132</sup> Additionally, strategies that simultaneously target CAFs and kill tumor cells have been proven more effective than drugs that target each cell type individually. Therefore, in the future development of NPs targeting CAF, the principle should be centered around killing tumor cells, while using CAF-targeting as complementary approach. Targeting CAFs inhibits tumor progression, while killing tumor cells promotes the normalization of the tumor stroma and the TME; thus, targeting both cell types simultaneously represents a synergistic approach. Currently, the main approach for targeting CAFs is through the surface modification of NPs with FAP- or TNC-binding molecules.<sup>143,157</sup> However, FAP is not a universal CAF marker; moreover, CAF markers can vary at different stages of tumor development. Therefore, a better approach would be to use NPs encapsulated in a CAF membrane. Additionally, the therapeutic benefits of multifunctional NPs that eliminate tumor cells and target CAFs would be more significant than those derived from combination therapies. The development of a more specialized repertoire of CAF-targeting NPs will undoubtedly lead to superior therapeutic outcomes.<sup>197</sup>

In summary, the NPs-mediated targeting of CAFs has achieved remarkable outcomes, indicating that their application will likely form an essential component of tumor immunotherapy. However, before CAF-targeting NPs enter clinical studies, important aspects, including the toxicity and metabolic properties of the nanomaterials used, need to be considered.<sup>237</sup> In the future, the development of CAF-targeting drugs will be driven by advancements in cutting-edge research techniques such as single-cell sequencing, bispecific antibodies, flow cytometry, and high-resolution imaging. Concurrently, nanomaterials used for the construction of CAF-targeting NPs will continue be optimized in accordance with the therapeutic requirements. This highlights the need for more extensive collaboration between experts in the fields of materials science and biomedicine.

## Abbreviation

CAFs, cancer-associated fibroblasts; rCAFs, cancer-restraining CAF; myCAF, myofibroblastic CAFs; iCAF, inflammatory CAFs; apCAF, antigen-presenting CAFs; meCAF, metabolic CAFs; vCAF, vascular CAFs; EMT, epithelial-mesenchymal transition; TME, tumor microenvironment; TIME, tumor immune microenvironment; ECM, extracellular matrix; NPs, nanoparticles; NK, natural killer cells; DCs, dendritic cells; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor;  $\alpha$ -SMA,  $\alpha$ -smooth muscle actin; MSCs, mesenchymal stem cells; LIF, Leukemia Inhibitory Factor; ICPs, immune checkpoint proteins; ICI, immune checkpoint inhibitor; PBMCs, peripheral blood mononuclear cells; TAMs, tumor-associated macrophages; BM, bone marrow; NSCLC, non-small cell lung cancer; IL, interleukin; ATAR, all-trans retinoic acid; PTT, photothermal therapy; PDT, photodynamic

therapy; PEG, polyethylene glycol; FAP, fibroblast activation protein- $\alpha$ ; TNC, tenascin C; SAB, salvianolic acid B; Frax, fraxinellone; LPD, lipid-coated protamine DNA; GNP, Gold nanoparticle; DTX, docetaxel; SPOIN, superparamagnetic iron oxide nanoparticle; PLGA, Poly(lactic-co-glycolic acid); EPR, enhanced permeability and retention effect; PSN38: PEG5K-P(MMESSN38)5K; PAMAM, the positive surface charge of cationic poly(amidoamine); RBC, red blood cells; ACF, acriflavine; PTX, paclitaxel; Nav, navitoclax; CPA, cyclophosphamide; DOX, doxorubicin; TPL, triptolide; NIR, near-infrared; sTRAIL, secretable TNF-related apoptosis-inducing ligand; DPPC, dipalmitoylphosphatidylcholine; APR, aprepitant; CUR, curcumin.

## Data Sharing Statement

No data was used in the research and all related researches were referenced.

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## Disclosure

The authors report no conflicts of interest in this work.

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