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Drug delivery systems targeting tumor-associated fibroblasts for cancer immunotherapy

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ABSTRACT

Solid tumors especially desmoplastic tumors are complex organ-like structures. Tumor-associated fibroblasts (TAFs), one type of the stromal cells, support the initiation, progression, and metastasis of carcinomas. TAFs also contribute to immunosuppressive tumor microenvironment (TME) and hinder T lymphocytes in killing tumors. Here, the role of TAFs in TME is discussed. In specific, TAFs form barriers for the penetration of T lymphocytes. TAFs also act as negative regulators for T lymphocytes. These findings suggest that targeting TAFs is a promising strategy for improving cancer immunotherapy. Our previous studies have indicated the ability of therapeutic nanoparticles to distribute into, and deplete or inactivate TAFs. This approach is discussed in the context of developing specific and effective immunotherapies for cancer.

1. Introduction

Tumor microenvironment (TME) of solid tumors is a heterogeneous population of cells, consisting of tumor cells and nearby stromal cells including endothelial cells, fibroblasts, as well as various inflammatory and immune cells. The interaction between host stromal cells and tumor cells plays a critical role in tumor growth. In specific, the progress of tumor growth depends on the support of stromal cells with growth factors, blood supply, and mechanical support [1]. In fact, the initiation and evolution of tumors accompany with an alteration of stroma from normal state to malignant state (i.e., with the effect of inflammatory stimulation), exhibiting similarities to that of normal wound healing. This process includes neoangiogenesis, infiltration of fibroblasts and immune cells, and extensive remodeling of extracellular matrices [2]. For instance, transforming growth factor-β (TGF-β) signaling in stromal fibroblasts suppresses the oncogenic potential in adjacent epithelia [3]. In TME, however, stromal cells are kept in an active and tumor-promoting state. It is particularly obvious for tumor-associated fibroblasts (TAFs) that deposit and organize matrices rich in collagen and fibronectin [4]. In healthy tissues, normal fibroblasts and stellate cells generally exist in a quiescent state. In TME, they can differentiate into TAFs under the stimulation of growth factors, cytokines and oxidative stress like TGF-β. Compared to normal fibroblasts and stellate cells, TAFs can be identified by overexpressing various markers which will be summarized in detail in this review. TAFs fail to go back to the resting state with this consistent pathophysiologic response, leading to desmoplastic TME. It commonly appears in pancreatic cancer, prostate cancer, squamous cell cancer and melanoma, although the degree of desmoplasia varies [5–8]. TAFs affect tumor growth, metastasis with the crosstalk with tumor cells, but there are some distinct effects in different cancers. For example, in breast cancer and prostate cancer, TAFs facilitate epithelial cell growth and differentiation via paracrine effects. Besides gene mutation, chromosomal loss of heterozygosity in TAFs could significantly influence breast tumor grade and lymph node metastasis [9,10]. In comparison, in pancreatic cancer, TAFs could be originated from normal pancreatic stellate cells (PSCs) and bone marrow-derived mesenchymal stem cells (BMSCs), and TAFs are densely arranged around tumor cells. Up to 80% of tumor volume is stroma in this highly desmoplastic cancer. In addition, TP53 is one of the key tumor suppressor genes, and its mutation in pancreatic cancer strongly regulate TAFs' functions. Besides paracrine signaling, TAFs-tumor cells interactions could also be mediated by exosomal signal transfer [11]. Moreover, TAFs also play a role in tumors without severe desmoplasia like metastatic colon cancer. In specific, about 170 of 22,000 genes expression is up-regulated in TAFs compared to the skin fibroblasts, especially the genes encoding cyclooxygenase-2 (COX-2) and TGF-β. These two proteins are further confirmed to promote the growth and liver metastasis of tumor cells [12]. During desmoplastic malignancy, tumor stroma especially TAFs contribute to chemoresistance through forming a physical barrier, paracrine crosstalk as well as tumor cell

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transformation [13]. In this case, the synergistic administration of chemotherapeutics and agent targeting TAFs becomes a necessary strategy. For instance, injectable peptide hydrogel loading losartan was applied to inhibit TAFs and potentiate the breast tumor killing effect of doxorubicin [14]. The combined treatment of gemcitabine and Janus Kinases 2 (JAK2) inhibitor which deplete TAFs could greatly inhibit pancreatic tumor growth [15]. TAFs also have direct influences on tumor infiltrating immune cells and play an important role in modulating the tumor immune microenvironment. In this case, TAFs have been identified as a target for cancer immunotherapy to challenge the immunosuppressive TME.

Nanotechnology has attracted much attention and has been widely applied in drug delivery systems. In general, traditional hydrophobic drugs for TAFs treatment encounter barriers including low dosage and short administration interval [16]. In comparison, nanoplatforms are good carriers for hydrophobic drugs in improving their solubility and targeted delivery. Currently, antibodies are also used for treatment against TAFs, and they are often conjugated or modified on the surface of nanoplatforms to maintain their stability in circulation and enhance nanoplatforms targeting efficiency [17–19]. In addition, drug delivery nanoplatforms show advantages in manufacturing methods and the choice of materials, which endow them with flexible configurations regarding size, shape, and payload. It results in a wide range of usage in diagnosis, prevention and therapy in oncology. There are various types of nanoplatforms such as liposomes, polymeric nanoparticles, nanoemulsions and inorganic nanoparticles, with distinct functions [20–23]. In addition to chemotherapy, nanoplatforms are also applied to improve the efficiency of immunotherapy. We have previously designed several cancer vaccines based on nanoparticles, which exhibited improved antitumor immune responses [24,25]. In addition, inflammation regulators such as cytokines can be loaded in nanoparticles to customize TME [26–29]. Nanoparticles can also be conjugated to the surface of transferred T cells, acting as persistent autocrine-like signals [30]. As mentioned above, cancer immunotherapy may benefit much from TAFs-targeted nanoplatforms since nanoparticles are prone to encountering TAFs or TAFs binding sites firstly in TME due to the fact that tumor vessels are usually surrounded by stroma [31].

In this review, we report the role of TAFs in TME and the crosstalk between TAFs and other cells in the contribution to immunosuppressive TME. Further, we summarize strategies of drug delivery systems to TAFs for immunotherapy especially by using nanoplatforms. These drug delivery systems are used to target TAFs or disturb interactions between these cells and tumor cells or other stromal cells.

2. Basic knowledge of TAFs

2.1. Origins and features of TAFs

TAFs are found in virtually all carcinomas. TAFs are typically prevalent in breast, pancreatic, colorectal, non-small cell lung cancers and prostate cancers, while they are less dominant in brain, renal, ovarian, and head and neck cancers [32]. TAFs present spindle-like fibroblastic appearance morphologically. They can be identified by several markers, including vimentin, desmin, neuron-glial antigen-2 (NG2), podoplanin, fibroblast-associated antigen, platelet-derived growth factor receptor-β (PDGFR-β), fibroblast-specific protein 1 (FSP-1), prolyl 4-hydroxylase, α-smooth muscle actin (α-SMA) and the plasma membrane serine protease fibroblast activation protein (FAP). Among them, α-SMA and FAP are two widely used markers. α-SMA has been the established marker presented on myofibroblasts (MF) and MF-like cells in TME, but it is also expressed on pericytes, perivascular smooth muscle cells, and visceral smooth muscle cells. FAP is a robust marker for TAFs in various tumor types [33].

The multi-source of TAFs contributes to the heterogeneity [34]. As shown in Fig. 1, TAFs can be generated from various cells including resident fibroblasts, local mesenchymal stem cells (MSCs) and BMSCs, *trans*-differentiation of epithelial or endothelial cells, or by hepatic or pancreatic stellate cells. Among them, MSCs and BMSCs can be recruited by tumor cells followed by differentiation into TAFs [35]. In addition, TAFs can also be derived from adipocytes or adipose tissue-associated stromal cells [36]. Regardless of the source, heterogeneity of TAFs raises difficulties to understand their potential effects on tumorigenesis, which indeed provides the complication for the design of stromal cells targeted therapies.

2.2. The role of TAFs in TME

Normal fibroblasts have been reported to prevent the emergence of neoplastic epithelial cells and inhibit tumorigenicity. This result was demonstrated by co-injecting fibroblasts with epithelial tumor cells into nude mice [37]. Fibroblast-derived hypoxia-inducible factor1-α (HIF1-α) was also reported to inhibit tumorigenesis of breast carcinoma [38]. TAFs, on the contrary, contribute to tumor proliferation due to phenotypic transformation. As reviewed by Wernert [39], the growth of tumor cells can be influenced in the following aspects: (a) direct TAFs-tumor cells interactions, (b) TAFs derived soluble factors, (c) growth factors released from extracellular matrix (ECM) which acts as a reservoir, and (d) signals transduced by cell-matrix interactions. Moreover, it has been discovered that TAFs could secrete connective tissue growth factor (CTGF), which could stimulate fibroblast cell growth, matrix production and granulation tissue formation [40]. Recently, it was also proved to contribute to tumorigenesis of prostate cancer [41]. TAFs participate in multiple aspects as mentioned above, and that is why TAFs should be considered as a promising target for tumor therapy. Further, normal fibroblasts could be reprogramed to become TAFs, promoting tumor proliferation in TME [42]. Specifically, TAFs in different TME show distinct properties. For instance, TAFs in breast cancer rarely contain p53 mutations or chromosomal aberrations, while these cells in pancreatic cancer display normal allelotypes [43]. Growth factors are critical in tumorigenesis, for example, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF) can stimulate the formation of blood vessels. TAFs and tumor cells secrete VEGF which is involved in tumor angiogenesis [44]. In this case, new vessels always originate in TAFs abundant sites. In addition, TAFs can produce several growth factors, cytokines, chemokines and matrix-degrading enzymes, which result in tumor favorable TME [1].

TAFs also play a vital role when tumor cells colonize to secondary sites during metastasis and invasion. Metastatic cells could not only home to pre-existing niches created by inflammation or fibrocyte accumulation [45], but also reside in metastatic organs waiting for oncogenic activation [46]. Metastatic cells may alternatively proliferate in blood circulation before they permeate into metastatic organs [47]. It results in intravascular cell clumping and enhances the invasion efficiency. Besides tumor cells, stromal cells, especially TAFs, can contribute in cell clumping [48]. Duyyerman et al. developed a protocol for investigating the role of TAFs in cancer metastasis. They established a spontaneous lung metastasis model in mice in which human TAFs can be selectively depleted once these TAFs colonize the lungs, proved the role of passenger TAFs in tumor metastasis to lungs [49]. Several matrix metalloproteases (MMPs) are reported to be related to tumor metastasis. Interestingly, TAFs secrete MMP3, which stimulate hyperplasia and aberrant branching in the mammary gland. High expression of MMP1 has been used as the diagnosis index for putative breast cancer. MMP1 could regulate invasion via cleaving protease-activated receptor-1 (PAR1) which is expressed on the surface of cancer cells. On the contrary, the invasiveness of metastatic cancer cells could be blocked when knocking down PAR1 in nude mice models. It remind us that exploring drugs targeting MMPs was promising approach to reduce the activity of TAFs [50].

TAFs participate in resistance to chemotherapy induced apoptosis, which may depend on the structure of desmoplastic tumors in TME [51]. This mechanism refers to the adhesion-mediated apoptosis

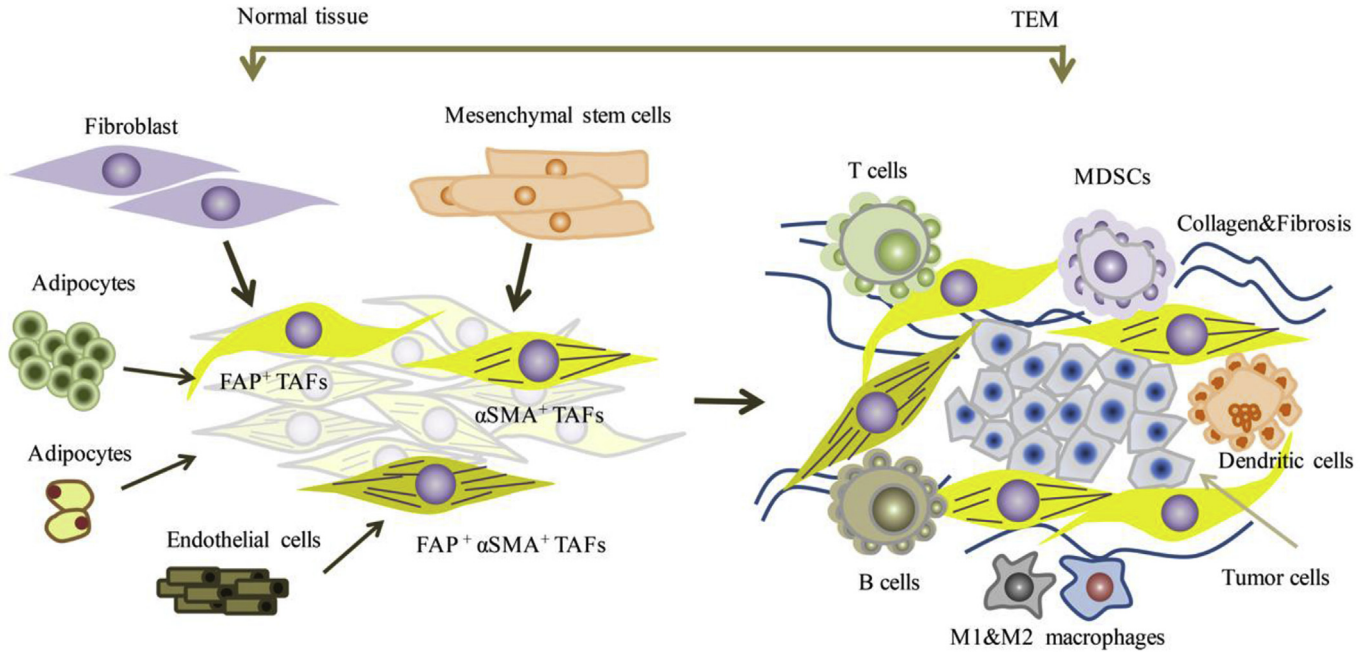

Fig. 1. Sources of tumor-associated fibroblasts (TAFs) and the formation of tumor microenvironment (TME). FAP: fibroblast activation protein. α-SMA: α-smooth muscle actin. MDSCs: myeloid-derived suppressor cells. FAP^+TAFs: TAFs with FAP expression. α-SMA^+TAFs: TAFs with αSMA expression. FAP^+α-SMA^+TAFs: TAFs with both FAP and α-SMA expression.

resistance [52]. In theory, tumor cells interact with adjacent cells via three ways including (a) adhesion to each other, (b) adhesion to stromal cells, (c) adhesion to ECM proteins which are mainly produced by TAFs [53]. ECM is a reservoir of bound proteins or growth factors [54]. TAFs modify ECM with proteases secretion, followed by accelerating resistant TME. Studies have proven that plenty of signaling pathways were involved which could mediate resistance to survival and apoptosis by cell-matrix adhesion, or mitogen activated protein kinase, or extracellular regulated kinase. Detailed regulation mechanisms are summarized by Hao et al. [51]. In particular, TAFs takes part in the resistance to gemcitabine-induced apoptosis in pancreatic ductal adenocarcinoma (PDAC) cells with the effect of nuclear factor-κB (NF-κB) [55]. We previously reported the off-target phenomenon of tumor targeted nanocarriers, and a large percentage of nanocarriers were internalized by TAFs [31]. These results may be part of the reasons of unexpected chemotherapy efficiency in desmoplastic tumors. Therefore, TAFs depletion or modification before the application of cytotoxic agents should be considered as a strategy to improve chemotherapy efficiency.

It is questionable whether the transition from fibroblasts to activated TAFs with a protumorigenic ability is reversible, so it can provide a new therapeutic approach to reprogram TAFs. Although wound repair process accompanies with apoptosis of active fibroblasts, TAFs persist. TAFs may indeed have potentials to reverse to normal fibroblasts. In previous studies, normal stellate cells could be activated to become TAFs, therefore inducing fibrosis in pathological condition. Calcipotriol, a vitamin D receptor agonist, was shown to deactivate TAFs to normal stellate cells via reducing TAFs' response in pancreatitis [56] and hepatic fibrosis [57]. In addition, combined administration of calcipotriol and gemcitabine was a good therapy for reducing markers of fibrosis and inflammation, which therefore inhibited tumor growth and prolonged survival time in mouse pancreatic cancer models [56].

2.3. Crosstalk between TAFs and immune cells in TME

Recent studies have demonstrated that TAFs may contribute to immunosuppressive effects on T lymphocytes. Inflammation in TME activates fibroblasts to TAFs. The crosstalk between TAFs and inflammatory cells regulates desmoplasia and further inhibits the accumulation of T lymphocytes in TME [58,59]. It was reported that activated PSCs secreted CXCL12, and this chemokine enhanced chemotaxis of $CD8^+$ T cells towards juxtatumoral stroma. In this circumstance, less $CD8^+$ T cells had access to tumor cells, and their killer ability towards tumor cells was thereby limited. Knockdown of CXCL12 endowed $CD8^+$ T cells with increased proximity to tumor cells due to their quiescent abrogation of chemotaxis to PSCs [2,60]. By secreting a gamut of paracrine factors, TAFs further modulate TME to Th2 phenotype to a large extent, which greatly inhibited tumor killing ability of effector T cells and promoted tumor growth [61]. Various immune cells participated in this process. For instance, TAFs recruited macrophages via the secretion of cytokines such as CXCL1 and CXCL2, further promoting the polarization of macrophages to M2 phenotype called tumor-associated macrophages [62,63]. M2 macrophages not only presented poor antigen-presenting ability, but also suppressed the Th1-adaptive antitumor immune responses. In pancreatic cancer, for example, thymic stromal lymphopoietin (TSLP) secreted by TAFs contributed to the maintenance of Th2 phenotype by the maturation of myeloid dendritic cells (DCs). These DCs were able to migrate into lymph to activate $CD4^+$ T cells to Th2 phenotype [64]. Interleukin (IL) 6 produced by TAFs also accelerates inflammation response [2]. TAFs could further bolster the immunosuppressive TME by recruiting MDSCs and regulatory T cells (Tregs). When these cells came into TME, Tregs were able to expand in the presence of factors secreted by TAFs like TGF-β. Myeloid cells became a suppressive phenotype under the influence of Th2 cytokines such as IL-10, IL-4 and IL-13. These suppressor cells also secreted immunosuppressive cytokines, nitric oxide NO [65]. Conversely, suppressive immune cells can regulate TAFs as well, which exacerbates desmoplasia in TME. For instance, M2 macrophages secreted CXCL12, which made it easier for fibroblasts to activate and differentiate [66].

When T lymphocytes infiltrate into TME, they frequently encounter TAFs which defunctionalize T lymphocytes [67]. TAFs also act directly on T lymphocytes through expressing programmed death-ligand (PD-L) 1 and PD-L2 which are ligands of programmed cell death protein-1 (PD-1) expressed on T lymphocytes [68]. In addition, interferon-γ (INF-γ) facilitates the expression of PD-L1 on TAFs, which aggravates dysfunction of T lymphocytes and promotes tumor evasion [69]. The

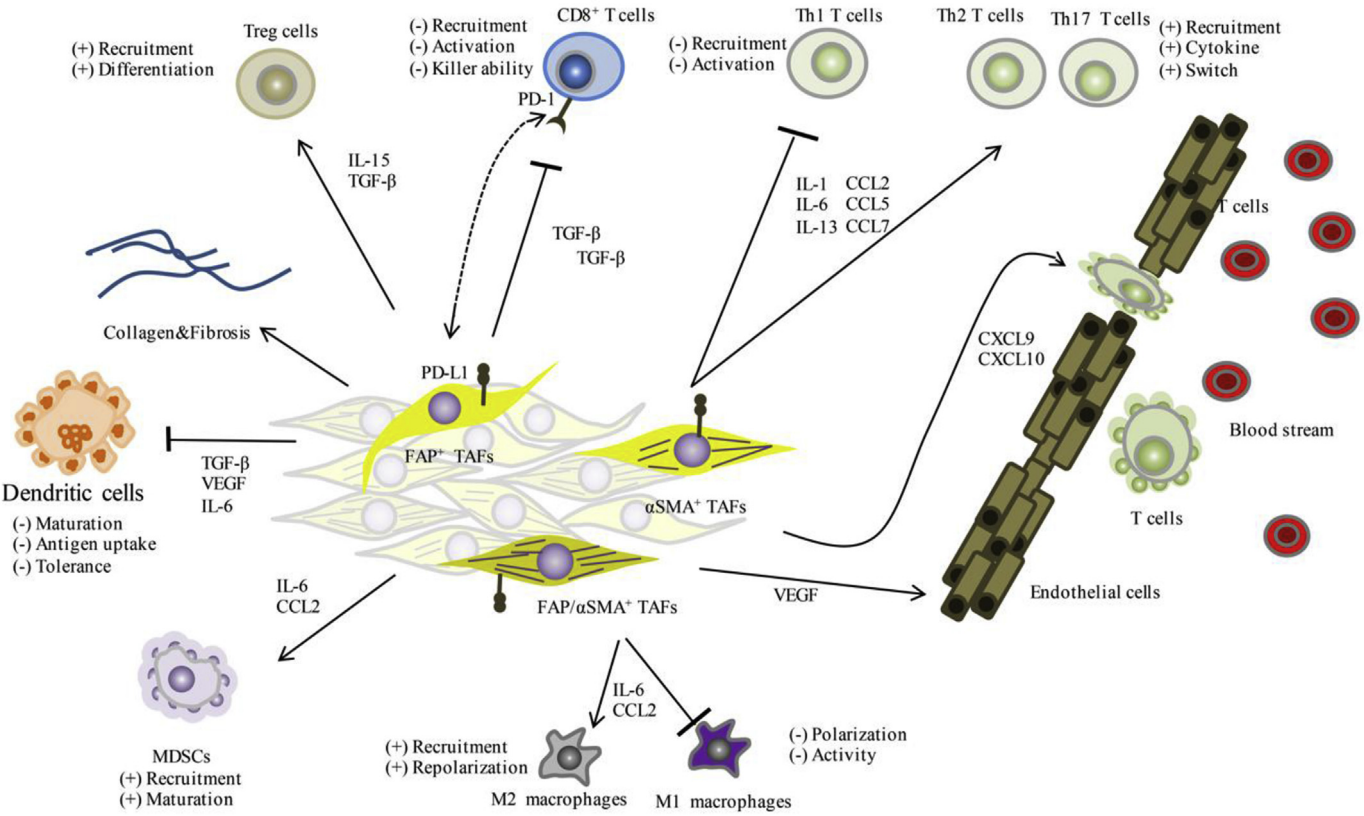

Fig. 2. Crosstalk between tumor-associated fibroblasts (TAFs) and immune cells in TME. Tregs: regulatory T cells. Th cells: T helper cells. IL: interleukin. TGF-β: transforming growth factor-β. VEGF: vascular endothelial growth factor. PD-L1: PD-1 ligand.

crosstalk between TAFs and various immune cells is shown in Fig. 2 [70,71]. TAFs inhibit the activity of tumor killer T cells, leading to the accumulation of immunosuppressive cells.

Taken together, the crosstalk between TAFs and other cells contributes to immunosuppressive TME. It further demonstrates that targeting TAFs or TAFs-related pathways should be considered as a powerful strategy to attenuate stromal barriers and promote immunotherapy in cancer [50,72,73].

3. Nanoscale drug delivery systems for TAFs targeting

As mentioned above, TAFs are a vital target of immunotherapy especially for desmoplastic tumors, and nanoparticles are excellent drug delivery systems. There are various types of nanoparticles used in delivering drugs for TAF targeting [23]. In our lab, we have developed several nanoplatforms including lipid-coated calcium phosphate (LCP) and lipid-protamine-DNA (LPD) nanoparticles as drug delivery systems [74]. These nanoparticles are small, stable, and easy to be functionalized with targeting ligands. For instance, by optimizing compositions of the surfactant systems, the mean size of LCP could be turned between 18 and 70 nm with a clear core/membrane structure [31]. LCP can be loaded with genes or hydrophobic drugs such as quercetin and cisplatin, enhancing the targeted accumulation and normalizing immunosuppressive TME [74–76]. LPD are versatile and stable gene delivery systems, protecting genes from degradation in blood circulation [77]. The proposed mechanism of TAFs-targeting nanoparticles for TME modulation is shown in Fig. 3 by one of our studies. A secretory form of TNF-related apoptosis-inducing ligand (TRAIL) (sTRAIL) was delivered by LPD, and TAF-targeted gene delivery and expression was observed [78].

Ligand modification on the surface of nanoparticles can improve their targeting abilities. Receptors like sigma receptors expressed on TAFs always correlate with metabolic rates of these cells. Although sigma receptors have been reported to express on many different types of cancer cells, recent studies have indicated the up-regulation of sigma receptors on TAFs, which is correlated with α-SMA upregulation [79]. These results are also consistent with our previous research that most of aminoethylanisamide (AEAA, ligand of sigma receptors) modified nanoparticles accumulated in TAFs instead of pancreatic tumor cells with a strong binding affinity. The expression of sigma receptors on TAFs is also confirmed in desmoplastic bladder cancer samples [31]. Thus, AEAA is a convenient targeting ligand for TAFs-targeted therapies.

4. Nanoscale drug delivery systems targeting TAFs for cancer therapy

Since TAFs are a critical target for cancer immunotherapy, either targeting TAFs or destroying their interactions with other cells should theoretically work to improve the therapy. In specific, these strategies contains (a) inhibiting the expression of FAP, TGF-β, PDGFR-β, CXCL12, etc., (b) disturbing the crosstalk between TAFs and tumor cells through blocking CXCL12/CXCR4 axis, hepatocyte growth factor (HGF), TGF-β, cyclooxygenase-2 (COX-2), (c) destroying interactions between TAFs and endothelial cells through VEGF, PDGF, FGF, etc., and (d) aiming at TAFs induced immunosuppression or inflammation such as a blockade of CXCL12, CXCL13, and NF-κB. As shown in Table 1, genes, small molecules and proteins etc. [80] targeting the above factors can be loaded in various nanoplatforms for immunotherapy via TAFs-targeting strategies.

4.1. Gene-loaded drug delivery systems

Genes including DNA, small interfering RNA (siRNA), and micro RNA (miRNA) are good mediators for TAFs modulation. In our lab, we have worked on targeted delivery of gene nanoparticles for decades. With TAFs as a target, we formulated LPD for the encapsulation of

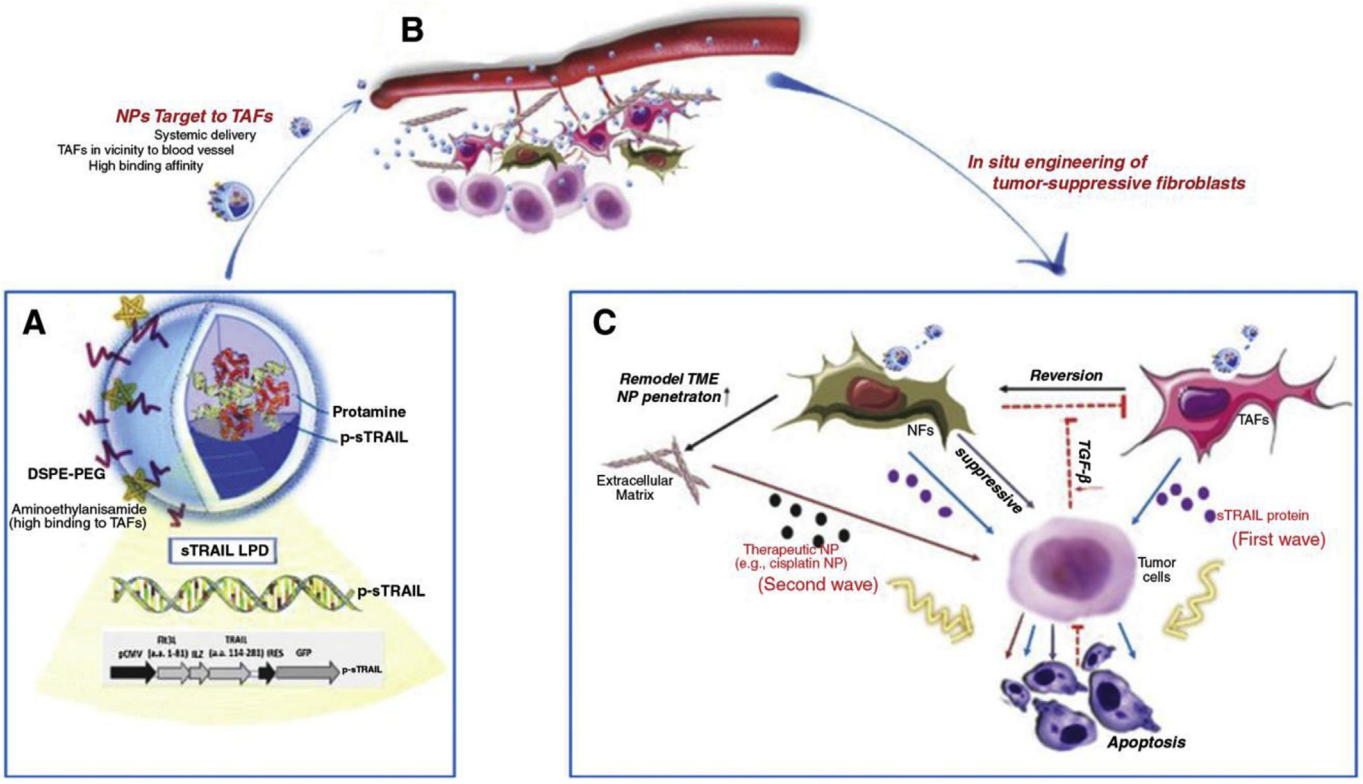

Fig. 3. Proposed mechanism of TAFs targeted LPD. (A) Plasmid encoding sTRAIL protein is condensed by protamine, which is further encapsulated into PEGylated liposomes coating with aminoethylanisamide-targeting motif (LPD). (B) LPD is systemically delivered into tumors. Blood vessels are frequently surrounded by TAFs in desmoplastic tumors. (C) TAFs take up LPD, produce sTRAIL which diffuses and kills neighboring tumor cells. Apoptotic tumor cells fail to secrete TGF-β to keep TAFs activated. TAFs return to normal fibroblasts which suppress tumor growth, and remodel TME to allow penetration of immune T lymphocytes or chemotherapeutic nanoparticles. Adapted by permission from American Association for Cancer Research [78].

plasmid DNA encoding sTRAIL in a stroma-vessel type desmoplastic tumors (bladder transitional cell line UMUC3/embryonic fibroblast cell lines NIH3T3) model. High gene expression was found in TAFs, and finally these secreted sTRAIL proteins from TAFs induced apoptosis of tumor cells in the nests adjacent to TAFs [78]. Feig et al. proved that FAP^+ TAFs secreted CXCL12, and targeting CXCL12 synergizing with anti-PD-L1 immunotherapy could greatly diminish tumors in a pancreatic cancer model [81]. In addition, we designed a plasmid encoding a fusion protein, named trap, as a novel approach for TAFs based immunotherapy. We formulated LPD with a plasmid encoding CXCL12 fusion protein (CXCL12 trap), combining with PD-L1 trap in LPD. In an orthotopic pancreatic cancer model, CXCL12 trap facilitated the penetration of T cells into TME, while PD-L1 trap allowed infiltrated T cells to kill the tumor cells [77]. TAFs contributed to tumor metastasis, and specifically activated hepatic stellate cells (HSCs) played a critical role in tumor liver metastasis. We therefore administrated LCP loading a plasmid encoding CXCL12 trap to mice. It resulted in enhanced infiltration of T lymphocytes, while it caused drop in the expression of CXCL12 and immunosuppressive cells in the liver. After four injections of LCP, fibrosis and tumor metastasis were reduced in the liver, and

Table 2
List of miRNAs which played a role in TAFs from various tumors.

miRNA	Cancer type	Target gene	Functions	Ref.
miR-149	Gastric	IL-6	Differentiation, anti-stromal effects on tumor cells	[90]
miR-338-3p	Gastric	SSX2IP	Tumorigenesis	[91]
miR-106b	Gastric	PTEN	Migration, invasion	[92]
miR-21	Colorectal	RECK	Differentiation	[93]
miR-15/-16	Prostate	FGF2	Migration, proliferation	[94]
miR-31	Endometrial	SATB2	Migration, invasion	[95]
miR-148a	Endometrial	WNT10B	Migration	[96]

Table 1
Summary of TAFs targeted drug delivery nanoplatforms.

Type	Therapeutic agent	Animal/Cell tumor model	Targeted cell	Nanoplatform	Ref.
Gene	sTRAIL plasmid DNA	UMUC3/NIH 3T3 bladder cancer model	TAFs	LPD	[78]
	CXCL12 trap plasmid	KPC pancreatic cancer model	TAFs	LPD	[77]
	CXCL12 trap plasmid	CT26-FL3 liver metastasis cancer model	HSCs	LCP	[82]
	Wnt16 siRNA	UMUC3/NIH3T3 stroma-rich bladder cancer model	TAFs	LPH	[97]
Small molecules	Fraxinellone	BPD 6 desmoplastic melanoma	TAFs	Nanoemulsion	[29]
Protein	Gemcitabine monophosphate	UMUC3 stroma-rich bladder cancer model	TAFs	Lipid bilayer modified nanoparticle	[76]
	Quercetin	UMUC3 stroma-rich bladder cancer model	TAFs	LCP	[74]
	Docetaxel	OCIP19 and OCIP23 Pancreatic cancer model	TAFs	Carboxymeth-ylcellulose based nanoparticle	[102]
	Doxorubicin	TAFs/Pc3 prostate cancer model	TAFs	Peptide-assembled nanoparticle	[98]
	Single-chain Fv	FAP-expressing fibrosarcoma cell	PSCs	Liposome	[99]
	Relaxin-2	Panc-1/hPSCs pancreatic cancer model	TAFs	Super-paramagnetic iron oxide nanoparticle	[103]

mice showed a prolonged survival time [82]. FAP is a specific marker for TAFs, and can be used as a target for TAFs inhibition [83]. Upon delivering FAP siRNA to TAFs, the growth of ovarian cancer tumors was inhibited together with a decreased level of TAFs [84]. Further, the aberrant expression of miRNAs was proved to happen in TAFs compared to that in normal fibroblasts. Their deregulation facilitated fibroblast reprogramming and pro-tumor ability of TAFs [85–87]. Various TAF-related miRNAs were listed in Table 2, the effects of which were demonstrated by transfecting them into normal fibroblasts or knockdown them from TAFs [88]. Encapsulation of miRNAs into nanoparticles is an effective way for protecting miRNAs from degradation when administrated systemically [89]. Unfortunately, the number of studies for delivering miRNAs for TAFs targeting therapy is still limited. Therefore, it could be considered as an promising strategy to delivery these elucidated miRNAs via nanoparticles for TAFs regulation.

4.2. Small molecules loaded drug delivery systems

Small molecules with antifibrotic effects have been explored for the treatment of fibrosis in TME. In our lab, we have paid attention to natural compounds especially traditional Chinese medicines which present limited toxicity as TAFs-targeting candidates. For example, we recently developed an AEAA-targeted nanoemulsion (NE) system to deliver fraxinellone (Frax), an anti-fibrotic medicine, to TAFs. Desmoplastic melanoma was used as a tumor model. After the treatment of Frax loaded NE, the amount of α-SMA$^+$ TAFs significantly reduced by about 4-fold. This NE inhibited tumor growth and remodeled TME. Level of CTL and natural killer cells infiltration into TME was increased, while the accumulation of MDSCs and regulatory B cells (Bregs) showed a significant decrease. Combination of NE and a peptide vaccine further exhibited superior anticancer and TME remodeling effects even accompanied with activated memory immune response [29]. We have found that cisplatin delivered to TAFs induced activation of fibroblasts and overexpression of Wingless-type MMTV integration site 16 (Wnt16), followed by tumor resistance to cisplatin. The delivery of Wnt16 siRNA by liposome-protamine-hyaluronic acid nanoparticles (LPH) to TAFs could reduce the crosstalk between tumor cells and TAFs [97]. We further investigated the synergistic effect of combined gemcitabine monophosphate (GMP) nanoparticles and cisplatin nanoparticles on TAFs. These dual drug-loaded nanoparticles dramatically abrogated TAFs [76]. In addition, quercetin, a natural antifibrotic compound, was also showed to downregulate Wnt16 expression. We thereby formulated LCP-quercetin phosphate (LCP-QP) for TAF treatment. LCP-QP (~35 nm in size) decreased the expression of α-SMA and collagen in TME. This nanoparticle also reduced the expression of Wnt16. Combination administration of LCP-QP and cisplatin-loaded nanoparticles not only limited the cisplatin induced chemoresistance and TAFs reconstruction, but also improved cancer immunotherapeutic effects via TAFs degradation and TME remodeling in a stroma-rich tumor model [74]. In another study, Ji et al. developed a cell-penetrating peptide-assembled nanoparticle composed of FAP targeted antibodies and doxorubicin (DOX). Through TAFs targeting and increased cellular uptake, the nanosystem resulted in efficacious amelioration of the xenograft prostate tumor [98].

4.3. Other TAFs-targeted strategies for immunotherapy

FAP is also a promising target for TAFs-targeting strategy. For instance, single-chain Fv (scFv) immunoliposomes were prepared to target TAFs. In detail, scFv was modified with an additional cysteine residue at C-terminus, which allowed a site-directed conjugation [99]. In addition, Fang et al. engineered a novel immunotoxin, Afap-PE38, for FAP$^+$ TAFs targeting within melanoma, which was further combined with three antigens based vaccines for cancer immunotherapy. This synergetic treatment killed TAFs, facilitated T lymphocyte infiltration and activated cytotoxic CD8$^+$ T cells to destroy tumors cells [100]. Loeffler et al. constructed an oral DNA vaccine targeting FAP. Through TAF killing mediated by CD8$^+$ T cells, this vaccine significantly suppressed the growth of primary tumor cell as well as metastasis. Accompanied with reduced collagen expression, this treatment also revealed up to 70% greater uptake of chemotherapeutic drugs compared to the untreated group [101]. Chemotherapeutic agents loaded in nanoparticles were also reported for TAFs targeting. For example, Ernsting et al. prepared a carboxymethylcellulose-docetaxel nanoparticle which named Cellax-DTX. Greater than 90% of Cellax-DTX accumulated in TAFs, which decreased collagen intensity and enhanced TAF apoptosis. This nanoparticle dramatically regulated TME and inhibited tumor progress and metastasis [102]. A most recent study showed that relaxin-2 (RLX), an endogenous hormone, was able to inhibit PSCs into TAF-like myofibroblasts. Therefore, they designed a RLX nanoparticle system by conjugating RLX to superparamagnetic iron oxide nanoparticle system, and resulted in effective treatment against pancreatic tumor [103].

5. Clinical significance and future directions

Over past decades, the studies of TAFs have been fruitful. The essential roles of TAFs in tumorigenesis, metastasis and the contribution to immunosuppressive TME are evident. The knowledge leads to the birth of TAF-targeted immunotherapies for desmoplastic tumors. A list of therapeutic targets of TAFs are shown in Table 3, and many of which have entered into clinical trials. JAK2 is considered as a potential target in TAFs, and it contributes to the activation of STAT3. JAK2 inhibitors such as SAR302503 [104] and Pacritinib [105] are developed to deactivate TAFs and deplete stroma, leading to diminished collagen structure. Sonic hedgehog (SHH) is also overexpressed in TAFs and activated STAT3 upregulates it as well. Therefore, SHH inhibitor is considered as an excellent strategy to suppress TAFs [106]. FAP is a

Table 3
Therapeutic targets in TAFs.

Target	Function	Drug	Mechanism	Preclinical or Clinical trials	Ref.
JAK2	Promote progression	SAR302503	JAK2 inhibitor	Phase III	[104]
		Pacritinib	JAK2 inhibitor	Phase III	[105]
SHH	Activation of TAFs	Vismodegib	SHH inhibitor, decrease stroma expansion	Conflicting results	[106]
FAP	Serine protease	PT-100	Activity inhibitor	Phase I	[119]
		Sibrotuzumab	Anti-FAP antibody	Phase I	[107]
VEGF	Angiogenesis	IMC-IC11	Anti-VEGFR-2 antibody	Phase I	[108]
		RPI.4610	Anti-VEGFR-1 ribozyme	Phase II	[109]
		Bevacizumab	Neutralization VEGF	Phase II	[110]
CTGF	Promote progression	FG-3019	Anti-CTGF antibody	Preclinical	[111]
MMPs	Metalloproteinases	Marimastat	Activity inhibitor	Phase III	[112]
		Tanomastat	Activity inhibitor	Phase III	[113]
uPA	Serine protease	PAI-2	Activity inhibitor	Preclinical	[115]
Growth factor	Promote progression	Pasireotide	Somatostatin analog	Preclinical	[120]

TAFs specific marker, and could be targeted by monoclonal antibody named Sibrotuzumab [107]. Its anti-tumor efficiency against colorectal cancer patients is under a clinical trial. In addition, several VEGF inhibitors are under investigation in clinical trials, and they deplete endothelial cells which are one of sources of TAFs, thus decreasing the amount of TAFs [108–110]. As mentioned above, TAFs are sources of CTGF and MMPs, and some drugs of their inhibitors were under clinical trials for cancer therapy [111–113]. Besides, urokinase type plasminogen activator (uPA) is an indicator of tumor metastasis [114]. It could be secreted by TAFs, therefore drugs targeting uPA are under preclinical investigation [115]. We have discussed above that abnormal gene expression happen in TAFs, therefore, more targets would be explored in TAFs based cancer treatment.

Although the role of TAFs in TME is clear and both TAFs themselves and their interactions with other immune cells or tumor cells can be promising targets for immunotherapy, strategies of drug delivery with high specificity to TAFs are limited. Nanotechnology contributes to enhanced targeting efficiencies, which has been increasingly applied in various treatments such as chemotherapy, radiation therapy and immunotherapy. We have summarized the TAF-targeted nanoplatforms for tumor treatment. Since geno-damaged TAFs secrete Wnt16 to protect the remaining tumor cells, direct killing of TAFs can be a troublesome approach [97,116]. Previous studies have reported that monoclonal antibodies or small molecule inhibitors were used to functionally block Wnt-canonical β-catenin pathway. However, it induced severe safety concern due to the off-target effect [117,118]. Instead, targeted deactivating Wnt16 by drugs, siRNA or genes based on nanoplatforms may be a smart idea. It has been demonstrated that cisplatin loaded nanoparticles showed enhanced antitumor effects with synergistic administration of either quercetin loaded LCP or Wnt16 siRNA loaded LPH [74,97]. Using TAFs as a factory to locally produce anti-tumor or immune modulating proteins is another worthy approach. We have shown that locally produced checkpoint inhibitor did not induce systemic immunodeficiency [75]. Further studies will be required to test these hypotheses. Despite the tremendous advantages of using nanoplatforms for TAFs targeted cancer therapy, great efforts still need to be made to translate nanoplatforms into clinic. Issues which need to be considered including the safety and toxicity of nanomaterials, as well as the stability and biocompatibility of nanocarriers. As we mentioned above, plenty of miRNAs act as excellent regulators in TAFs, but current miRNA loaded nanoparticles mainly aim at tumor cells. It is a promising approach to fabricate TAFs targeted nanoplatforms for miRNA delivery. Of course, it is not the optimal therapy only based on TAFs inhibition, nano-delivery of drugs and genes can always be synergized with other approaches such as radiation, checkpoint inhibitors and vaccines for tumor treatment.

Conflicts of interest

The authors declare no conflict of interest. LH is a co-founder of OncoTrap, Inc.

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