Drug targeting to myofibroblasts: Implications for fibrosis and cancer

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Myofibroblasts are the key players in extracellular matrix remodeling, a core phenomenon in numerous devastating fibrotic diseases. Not only in organ fibrosis, but also the pivotal role of myofibroblasts in tumor progression, invasion and metastasis has recently been highlighted. Myofibroblast targeting has gained tremendous attention in order to inhibit the progression of incurable fibrotic diseases, or to limit the myofibroblast-induced tumor progression and metastasis. In this review, we outline the origin of myofibroblasts, their general characteristics and functions during fibrosis progression in three major organs: liver, kidneys and lungs as well as in cancer. We will then discuss the state-of-the-art drug targeting technologies to myofibroblasts in context of the above-mentioned organs and tumor microenvironment. The overall objective of this review is therefore to advance our understanding in drug targeting to myofibroblasts, and concurrently identify opportunities and challenges for designing new strategies to develop novel diagnostics and therapeutics against fibrosis and cancer.

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1. Myofibroblast: biology and function

The wound healing process, in response to an injury, results in the infiltration of inflammatory immune cells to the local tissue. Subsequently, the inflammatory phase progresses to the pro-fibrotic phase leading to release of cytokines and growth factors by infiltrated immune cells which cause the activation and transdifferentiation of quiescent fibroblasts into contractile myofibroblasts. Myofibroblasts secrete vast amounts of extracellular matrix (ECM) proteins mainly collagens to repair the wound [1,2]. These cells are predominantly identified by the expression of alpha-smooth muscle actin (α-SMA), a cytoskeletal protein [1]. During resolution phase, most myofibroblasts undergo apoptosis, while a few revert to quiescent fibroblasts, at the end of the wound healing process. In case of a chronic injury that leads to chronic inflammation, however, myofibroblasts continue producing ECM, resulting in excessive scar tissue deposition, a process known as fibrosis (Fig. 1). Fibrosis not only occurs as a result of chronic injury, but also after a genetic insult to epithelial cells i.e. tumorigenesis. Tumors are generally considered as “wounds that do not heal” [3] and certain tumor types generate abundant fibrotic tissue (so called tumor stroma). Tumor-associated myofibroblasts are the key fibrogenic cells aggravating tumor growth and progression. Intervening into myofibroblast-induced pro-fibrotic and pro-tumorigenic activities using drug targeting technologies can be an interesting approach for developing novel therapeutics against fibrosis and cancer. Moreover, these technologies can be applied to diagnose fibrosis progression in different diseases and tumor. In this review, we discuss the biology of myofibroblasts in relation to liver, kidneys, lungs and tumor and elaborate on drug targeting strategies to modulate myofibroblasts.

1.1. Origin and heterogeneity of myofibroblasts

Since myofibroblasts are the key players in the pathogenesis of organ fibrosis and tumor, considerable research is still focused on understanding the biology of myofibroblasts such as their origin and phenotypic differences. In addition, exploration of different regulatory pathways involved in maintenance of their phenotype is crucial for developing new therapeutic interventions.

To date, the major well-characterized origins of myofibroblasts are tissue-resident fibroblasts, pericytes, and bone marrow (BM)-derived mesenchymal stem/stromal cells (MSCs). Other proposed precursors of myofibroblasts are epithelial, endothelial and mesothelial cells, which undergo epithelial–mesenchymal transition (EMT), endothelial–mesenchymal transition (EndMT) and mesothelial–mesenchymal transition (MMT), respectively [1,2,4–6]. Origin of myofibroblasts in organ fibrosis and tumors is highly diverse and is largely dependent on the pathological site [7–9]. Despite different diseased states and organs, transforming growth factor β (TGF-β) remains the main growth factor causing fibroblast differentiation. Other growth factors and cytokines stimulating myofibroblasts differentiation are platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), monocytic chemotactic protein 1 (MCP1 or CCL2), tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) [4]. In addition, different mechanical forces e.g. matrix stiffness, mechanical tension/shear stress, vascular and interstitial flow, are the important contributing factors regulating myofibroblastic differentiation [10,11]. Herein we discuss the heterogeneous origin of myofibroblasts in different organs during organ fibrosis and tumors.

1.1.1. Myofibroblasts in organ fibrosis

Lately, a lineage tracing study demonstrated that perivascular Gli1 + mesenchymal stem cell (MSC)-like cells markedly give rise to myofibroblasts in heart, lung, kidney and liver fibrosis models in vivo [12]. Targeting perivascular Gli1 + MSC-like cells by genetic ablation strategy markedly prevented the progression of fibrosis in solid organ fibrotic models, suggesting these cells as promising target for therapy in fibrotic diseases (Fig. 2). In line with that, recent animal experiment by the same group showed that pharmacological targeting of Gli1 + MSCS by Gli antagonist 61 (GANT61) attenuated the severity of bone marrow fibrosis [13]. In liver, the primary source of myofibroblasts is the endogenous liver mesenchymal cells which consist of hepatic stellate cells (HSCs) [14] and portal fibroblasts [15]. HSCs, vitamin A and lipid droplets containing cells which are quiescent in the steady-state condition, located in the space of Disse between endothelial cells and hepatocytes [16,17]. Upon injury, they lose their vitamin A and lipid droplets and acquire myofibroblast-like phenotype. Portal fibroblasts are normally present in low numbers in the connective tissue of portal zones around portal vein(s), portal artery(ies) and bile duct(s) to maintain the integrity of portal tract. Upon chronic injury, portal fibroblasts have been shown to proliferate and differentiate into myofibroblasts [18,19]. In addition to HSCs and portal fibroblasts, other sources of hepatic myofibroblasts are BM-derived cells (i.e. fibrocytes and MSCs) [20,21] and mesothelial cells via MMT [22] (Fig. 2). Although EMT of epithelial cells (hepatocytes and cholangiocytes) has also been suggested to actively participate in liver fibrosis [23,24], recent in vivo cell fate-mapping studies negated these findings [25–27]. Hence, the presence and contribution of EMT in liver fibrogenesis seems to be context-dependent and remains a matter of debate [28,29].

In kidneys, the origin of myofibroblasts has also been studied extensively in the past years. Humphreys et al. [30] through cell-fate tracing experiments showed that myofibroblasts in kidneys originate primarily from endogenous stromal cells (pericytes and interstitial fibroblasts) but not from epithelial cells. Also, mesothelial cells have been proposed to transdifferentiate into mesenchymal cells via MMT [31], but further investigations are warranted to confirm these findings. Furthermore, glomerular mesangial cells are shown to acquire the myofibroblast phenotype and produce ECM in renal diseases [32]. LeBlu et al. [33] conducted a comprehensive analysis in genetically engineered mice to track the origin of myofibroblasts in kidney fibrosis. They showed that 50% of the total pool of myofibroblasts arise from resident interstitial fibroblasts through proliferation, while the non-proliferating myofibroblasts derived through transdifferentiation of BM-MSCs (35%), EndMT (10%) and tubular EMT (5%). They also suggested that pericytes probably do not contribute to the emergence of myofibroblasts. In lungs, several candidates have also been identified as potential cell–of-origin of myofibroblasts in pulmonary fibrosis like interstitial fibroblasts [34], circulating BM-derived fibrocytes [35,36], alveolar epithelial cells via EMT [37,38], mesothelial cells [39], capillary endothelial cells through EndMT [40] and pericytes [41] (Fig. 2).

Nevertheless, despite many exciting progress, yet there is no universal consensus on the origin of myofibroblasts in different tissue fibrosis but at the same time it is clear that the proportion of cell sources
Solid tumors are not only consisted of malignant cancer cells but also contain non-malignant cells so-called stromal cells such as myofibroblasts, infiltrated immune cells, adipocytes, pericytes and endothelial cells as well as ECM [42,43]. Dynamic crosstalk between cancer cells and stromal cells stimulate each other and make the tumor microenvironment permissive for supporting tumor growth [44,45]. Myofibroblasts in tumor are generally referred as tumor-associated fibroblasts or cancer-associated fibroblasts (CAFs) [46], whose recruitment and activation is mainly governed by the cytokines released by cancer cells and infiltrated immune cells. For example, IL-1β secreted by immune cells stimulates CAFs to produce pro-tumorigenic secretome in early neoplastic lesions through NF-κB pathway [47]. Evidence from both human and rodent studies indicates the complexity and heterogeneity of CAFs, hence their exact molecular identification is an area of current controversy and remains to be clarified [46,48]. This lack of precise definition of CAFs is attributed to their various cellular origins and the diversity of markers. Myofibroblasts within tumor stroma are both BM-derived and non-BM-derived myofibroblasts [46,48–51]. BM-derived myofibroblasts originate from BM-MSCs and BM-derived circulating fibrocytes. Non-BM-derived myofibroblasts may arise from smooth muscle cells (SMCs), stellate cells, epithelial cells via EMT, endothelial cell via EndMT, pericytes, mesothelial cells via MMT, and adipose tissue-derived stem cells [46,52–67] (Fig. 2).

To identify CAFs, similar to other non-tumoral myofibroblasts, α-SMA is the most commonly used marker [46]. Other CAF markers are fibroblast-specific protein-1 (FSP-1, S100A4), vimentin, fibroblast-activation protein-alpha (FAP-α), platelet-derived growth factor receptor-beta (PDGFR-β), natriuretic peptide B (NPPB), podoplanin, discoidin domain-containing receptor 2 (DDR2) and prol 4-hydroxylase (P4H), and loss of caveolin 1 (CAV-1) and PTEN (phosphatase and tensin homolog). However, none of them are exclusive to specific CAFs and present in all CAFs, rather the expression of some of these markers is highly dependent on their tissue of origin [48,52,53,68–74]. The same degree of diversity is also observed in the gene signature of CAFs in tumor microenvironment [75–77]. Furthermore, recent studies suggest that epigenetic alterations may induce irreversible activation of CAFs. For example, an epigenetic switch in the regulation of leukemia inhibitory factor may result in pro-invasive function of CAFs via JAK-STAT pathway activation [78]. Overall, the existing data suggest that there is a high heterogeneity in CAF population which is largely depending on their origin, microenvironmental stimulus, and epigenetic alterations. For detailed information on fibroblasts in cancer, we refer the readers to the comprehensive review of Kalluri R [79].

1.2. Functions of myofibroblasts

Although myofibroblasts are the master producers of ECM components, they perform number of diverse functions in organ fibrosis and tumor by interacting with other cells in the microenvironment (Fig. 3). Herein, we discuss the role of myofibroblasts in organ fibrosis and in tumor.

1.2.1. Myofibroblasts’ functions in organ fibrosis

Myofibroblast activation occurs in both physiological and pathological conditions, however, persistent activation due to ongoing injury leads to pathological scar formation [1,2]. When activated in response to microenvironmental alterations in injured tissues, myofibroblasts produce several fibrogenic and inflammatory mediators, and ECM proteins such as collagens, laminin, fibronectin, and tenascin. Myofibroblasts not only secrete ECM, but are also capable of remodeling ECM by producing matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [80]. They also acquire specialized contractile features, which result in reorganization and contraction of ECM in wound healing and fibrotic conditions [11]. ECM itself is also a reservoir of growth factors which create a bioactive microenvironment, affecting many cellular behaviors such as cell adhesion, migration, and proliferation [81,82]. Furthermore, the cell cytoskeleton components can modulate transdifferentiation to myofibroblasts as well as affecting myofibroblasts’ reactions to extracellular growth factors and cytokines and biomechanical stimuli/signals from ECM [83].

Myofibroblasts interact actively with the surrounding cells within their fibrotic microenvironment [4] (Fig. 3). They are reported to possess a cytotoxic phenotype that causes apoptosis of epithelial cells in lung fibrosis [84]. Many studies have highlighted the dynamic and mutually interaction between myofibroblasts and immune cells [85,86]. On one hand, myofibroblasts can be activated by components of the innate and adaptive immunity, while on the other hand, they are capable of modifying immune cells’ behavior by altering the microenvironment. Collectively, these myofibroblastic morphological and functional alterations lead to an excessive ECM production and remodeling, which eventually form nonfunctional fibrotic tissue in several vital organs.
Myofibroblasts' functions in the tumor microenvironment

Myofibroblasts are not merely chief actors as ECM-producing cells in fibrotic diseases, but also are strategic effector cells in tumor progression. Tissue fibrosis can be predisposing factor for the development of cancer [87], stressing myofibroblasts as an actual link between fibrosis and cancer. Myofibroblasts are salient promoter of metastasis and invasion in tumor microenvironment [46,88,89] while retaining many similar characteristics of myofibroblasts in other organs. The unique tumor microenvironmental condition such as loss of TGF-β cytostatic effect on stromal epithelial cells upon their mutations, broader variety of cellular differentiation routes, and high levels of reactive oxygen species (ROS) promote CAF functions [88]. These CAF functions result in chronic repair response in tumor microenvironment, known as cancer fibrosis or stroma [79]. In addition to ECM remodeling, CAFs are capable of secreting a wide range of mediators including TGF-β1, hepatocyte growth factor (HGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF1), fibroblast growth factor (FGF), CTGF, the pro-inflammatory chemokine (C-X-C motif) ligand 14 (CXCL14), IL-1, IL-6 and IL-8, MMPs and TIMPs, collagens, tenasin-C, fibronectin and elastin [89]. These secreted mediators are ligands for receptors overexpressed by other cell types in the microenvironment which initiate the cellular crosstalk, leading to tumor progression and metastasis (Fig. 3).

There is a reciprocal interaction between CAFs and other cells in tumor microenvironment. When recruited in the microenvironmental milieu, fibroblasts transdifferentiation and activation are stimulated via a large variety of growth factors and mediators secreted by both immune and cancer cells. One of the most well-characterized regulators of CAFs activation is TGF-β [90]. However, other mediators, cytokines or mechanisms are also shown to have an effect on CAF activation such as PDGF, basic fibroblast growth factor (bFGF), IL-6, IL-1α, EGF, lysosphosphatidic acid (LPA), hypoxia and ROS, cancer-derived exosomes, and NF-κB [46,48,91–94]. When activated, CAFs promote tumorigenesis and metastasis through several phenomena such as metabolic reprogramming of the tumor microenvironment, regulating tumor immunity, and inducing resistance to therapy [79,95]. Moreover, CAFs increase intratumoral interstitial fluid pressure through ECM remodeling and contraction, which results in inefficient uptake of anti-cancer drugs [96]. It is therefore tempting to speculate that targeting myofibroblasts is an effective strategy not only for preventing organ fibrosis, but also in hampering tumor progression [46].

Interestingly, recent independent studies have revealed contrasting findings that CAFs are also capable of inhibiting tumor progression in mouse pancreatic tumor models. They have shown that depletion of α-SMA+ myofibroblasts or hedgehog deletion in pancreatic tumor stroma induced tumor progression instead of tumor suppression [97, Fig. 2. Localization and cell-of-origin of myofibroblasts. The schematic figure shows the origin of myofibroblasts and their localization in lung, kidney, liver and tumor microenvironment. Upon injury, and based on disease etiology, any of these cells can give rise to myofibroblast, and therefore are attractive and potent target for therapy in both fibrosis and cancer. However, for some of them (with question mark), there is both in favor and against evidence, which suggest their importance and contribution as a myofibroblast pool is very much depended on the disease etiology.
98]. However, one needs to consider that these studies were performed with genetic depletion of all myofibroblasts rather than inhibiting their activity. Besides, these observations were only related to pancreatic tumor and the outcome might be different for other cancer types. Considering the complexity and plasticity of the tumor stroma, further studies are therefore warranted to elucidate and delineate the deleterious and beneficial aspects of stroma within tumor.

2. Concept of drug targeting

Targeted delivery of specific therapeutic agents holds great promises due to enhanced therapeutic efficacy of the drugs at lower doses, while preventing or reducing the undesirable off-target effects; these benefits are also largely applicable to develop theranostic agents [99]. Drug targeting strategies are generally categorized as passive or active targeting. Passive targeting is based on the fact that drugs can accumulate into specific organs due to the blood flow, the pathophysiological characteristics of the organ and physicochemical properties of the drug. For example, liver and spleen (reticuloendothelial organs) are actively capable of taking up foreign particles by their resident phagocytic cells. To achieve therapeutic levels via passive targeting, targeted drugs should possess extended blood circulation time and have modifications to avoid uptake and elimination by the reticuloendothelial system (RES). In both drug delivery and imaging applications, the addition of water-soluble polymers such as polyethylene glycol (PEG) reduces RES uptake, increases circulation time, diminish aggregation and association with non-targeted serum and tissue proteins resulting in ‘stealth’ behavior [100]. Active targeting benefits from interactions and communications between the imposed ligand properties on the drug and physicochemical features of targeted cell surface/receptor. In this approach, therapeutic compounds are incorporated into a drug carrier which is decorated with a ligand against a targeting receptor or biomolecule expressed by the target cells. The targeted drug-carrier therefore recognizes and internalizes into the target cells and induces its therapeutic efficacy intracellularly. In addition to the ligand-based targeting which is actively evolving especially in the field of cancer therapy [101], active targeting can also be achieved via modifying the physical properties of the drug (e.g. size, charge, shape) for improved tissue penetration and cellular uptake [99,102]. On the other hand, most of the solid tumors possess leaky and highly permeable blood vasculatures with poor/no lymphatic drainage which allow particles or macromolecules with <200 nm hydrodynamic diameter to extravasate through the tumor blood vasculature and retain within tumors. This phenomenon is commonly known as Enhanced Permeability and Retention (EPR) effect [99,102–104].

3. Biological barriers for drug delivery to myofibroblasts

Since myofibroblasts derivation is a result of an injury to parenchyma, they are localized adjacent to injured epithelial cells and embedded into self-formed complex matrix. Their anatomical localization, however, is largely dependent on the anatomy of the injured organ, site of the injury within the organ and the source of myofibroblasts.
3.1. Liver myofibroblasts

For cell-specific targeted delivery strategies against myofibroblasts, the targeted drugs circumvent several biological barriers to achieve effective drug concentration in the liver. Nevertheless, many of the targeted drug delivery systems do not necessarily be accumulated sufficiently in the intrahepatic target cell-type such as myofibroblasts, because of high non-specific uptake by hepatocytes and Kupffer cells that are mostly active in the uptake of (nano)particles. In the liver, the drug first enters via blood circulation to sinusoids and then reaches perisinusoidal space, where hepatocytes are located. Hepatocytes, comprises of about 80% of the total resident hepatic cells in healthy liver, are strategic key cells in a wide range of liver diseases and are actively involved in the metabolism of numerous drugs entering the liver. In addition to hepatocytes, Kupffer cells (sinusoidal macrophages) accounts for 15% of total healthy liver cell population and constitutes 80–90% of the tissue-resident macrophages in the whole body. They are active in immune defenses by removing not only dangerous foreign compounds, but also many large molecular-weight drugs entering the liver via circulation [105]. Rapid uptake and complete degradation of many biological compounds by Kupffer cells make them challenging barriers to systemic delivery of therapeutics [106].

Also, liver sinusoidal endothelial cells (LSECs), present close to hepatocytes within the space of Disse, like Kupffer cells have high phagocytic ability which has a fundamental role in immune system and host defense. As already mentioned, HSCs are the important fibrogenic cells of liver, playing major role in liver fibrosis/cirrhosis. However, the drugs that enter the liver will not necessarily reach the intended target cell type. Since HSCs are located in the space of Disse between endothelial cells and hepatocytes, the (targeted) drug-conjugates should pass sinusoidal endothelial barriers, and escape Kupffer cells and hepatocytes uptake to be accumulated selectively in HSCs for therapeutic efficacy [107].

During the pathological condition such as liver fibrosis, HSCs proliferate and secrete ECM proteins which altogether can occupy up to 90% of the fibrotic liver. Additionally, increased ECM deposition and remodeling impose extra barriers against successful drug delivery in liver fibrosis. For instance, remodeling and capillarization of sinusoidal cells due to ECM deposition in the space of Disse decrease vascular permeability [108,109] which in result marked reduction in delivery of potent therapeutic to activated HSCs/myofibroblasts. Excessive ECM deposition ultimately results in deterioration of hepatic parenchyma, increased intrahepatic vascular resistance to blood flow and portal hypertension development [16]. Due to these alterations, portal blood flow into the liver may significantly decrease and compensated by arterial supply, which can compromise the effective drug delivery to diseased/fibrotic liver. Consequently, careful and smart drug delivery strategies are desired to effectively target myofibroblasts or their cell-of-origin in fibrotic liver diseases.

3.2. Renal myofibroblasts

Because of the unique anatomical structure and diversity of cells, targeting to myofibroblasts in kidneys is an attractive but challenging task. Renal myofibroblasts are mainly located in kidney interstitium (the intertubular area between nephrons, ureteric epithelial and renal vasculature) in fibrotic conditions, and originate from local fibroblasts, pericytes or infiltrated BM-derived fibrocytes [110]. The first region of kidney exposing to body circulation is the glomerulus which is composed of capillary network, renal capsule, endothelial cells, glomerular basement membrane, podocytes, parietal epithelial cells and mesangial cells. During kidney diseases, the components of glomeruli are shown to be important in inducing kidney injury and damage, and therefore possible potential therapeutic targets in renal diseases [111–114]. Specific targeting to mesangial cells and podocytes have been shown to be a promising approach in both in vitro and in vivo animal models to treat glomerular injury, and consequently hampering the progression of many renal diseases [115,116]. However, drug targeting to tubulointerstitial myofibroblasts in the kidney fibrosis remains a challenge. Considering the biological barriers in kidney (peritubular capillary endothelium, the tubulointerstitium that includes interstitial cells and ECM components), the physicochemical properties of the drug carriers are crucial for targeting renal interstitial myofibroblasts.

Based on physicochemical properties and design of a drug-carrier conjugate, it may pass through glomerular basement membrane and reaches urinary space. Glomerular basement membrane is a specialized extracellular matrix of about 300 to 350 nm thickness, constituting a sophisticated network of glycosaminoglycans and fibrous proteins, which together with podocytes and endothelial cells form glomerular filtration barrier in the kidney glomerulus, a critical element in glomerular permeability. Glomerular filtration barrier works as a size- and charge-selective barrier against molecules/particles, and should be considered in designing drug-carrier for kidney drug delivery [117]. After successful passage across of glomerular filtration barrier, the designed drug-conjugate enters the lumen of proximal epithelial tubules. By accessing the tubular cells, the conjugate can bind to specific receptor (e.g. megalin receptor) expressed at the luminal side (apical) of proximal tubules [118]. Then, the carrier system can either be degraded or transported to the basolateral side via transcytosis process and thereby enters into the tubulointerstitial space. When the targeted delivery system is not filtered in the glomerulus due to its large size or highly negative surface charge, and then alternative route to reach tubulointerstitial myofibroblasts is via the peritubular capillaries. The drug delivery carrier should pass across the vascular capillary wall to end up at the tubulointerstitial area. It has been reported that the endothelial cells of peritubular capillaries contain fenestrations (60–70 nm) [119], although the effect of ECM deposition on the diameter and availability of these fenestrations in renal fibrotic condition remains unclear. Another barrier is the tubulointerstitial space itself which contains remodeled ECM and several cell types such as immune cells, fibroblasts, fibrillar collagen, as well as interstitial fluid. The scar tissue, particularly in advanced form of kidney fibrosis, is the biggest challenge in delivering carriers to myofibroblasts. Direct injection in the subcapsular area of kidney has been also utilized as another drug delivery method [120], however, whether this strategy is effective in cell-targeted therapies in kidney diseases such as renal fibrotic conditions is currently unknown and warrants further investigations.

3.3. Lung myofibroblasts

The lung is also an interesting organ for non-invasive systemic delivery of drugs because of its anatomical and physiological characteristics such as large absorptive surface area, high epithelial permeability and thin blood-alveolar barrier, rich vascularization and blood supply, and lower drug-metabolizing enzymes [121,122]. Nevertheless, the complex morphology of respiratory tract with around 40 different cell types put forward several challenges for successful lung drug delivery, e.g. targeting lung myofibroblasts. In addition to cellular barriers, there are also non-cellular barriers such as mucus and surfactant. The conductive regions (trachea, bronchi, and bronchiole) largely consist of ciliated monolayer epithelium covered by a thick mucus layer with an aqueous hypophase constituting a highly efficient system for fast removal of detrimental trapped pathogens and pollutants [123,124]. Surfactant, a surface-active lipid-protein material, synthesizes by type-II pneumocytes and covers the alveolar surface. Pulmonary surfactant plays a crucial role in decreasing the surface tension at the air-liquid interface, preventing pulmonary collapse during expiration and lowering the workload required for inhalation. Despite their vital protective roles, both viscous mucus secretion and surfactant layer are the most important hindrances in drug delivery to the respiratory airways. The local immune system has also a key role in maintaining the normal function of pulmonary system. However, regarding pulmonary drug delivery, both cellular immune components consisting of macrophages, dendritic and mast cells, and the humoral components like lactoferrins, surfactant...
proteins, defensins, mannose binding lectin and lysozyme, are other types of barriers [124–126]. Lung myofibroblasts are usually located in the alveolar interstitium in the healthy lung, but presence of distinct subset so-called ‘subepithelial myofibroblasts’ has also been reported in the alveolar septa of lung adenocarcinomas beneath or around tumor cells [127]. Delivery of potent drug to the lung myofibroblasts (or their precursor cells), which normally accumulate in the lung interstitium in fibrotic conditions, via intravascular systemic route is also challenging. While in the blood circulation, the designed drug-carrier conjugate should pass endothelial cells to end up in interstitial area of the lung. However, in pathological conditions such as lung fibrotic diseases, the permeability of blood vasculature within pathological lesions may change due to the injury to endothelial cells and vascular remodeling. This blood capillary permeability is essential for proper accumulation of systemically administered drug-conjugate, especially for nanoparticles drug delivery to target cells (myofibroblasts) in lung stroma [128]. In addition, the dense fibrotic tissues due to collagen deposition and remodeled ECM in chronic conditions may cause low diffusion of the drugs. Due to unique pulmonary blood circulation, large particles (micrometer size) can be entrapped within lung capillaries. This property has been used to not only deliver passively large particles to the lung, but also to enhance the pulmonary retention of designed microparticles [129,130]. This passive delivery strategy might be applied also for targeting lung myofibroblasts.

Overall, for targeted delivery of potential therapeutic compounds to myofibroblasts in pulmonary fibrosis, the intended delivery system should be designed to overcome all the significant barriers while retaining its therapeutic activity.

3.4. Tumor myofibroblasts

Highly complex and heterogeneous pathophysiology of tumors poses many limitations on delivering proper and effective dose of drugs to the site of tumor microenvironment to CAFs. The physiologically abnormal structure of tumor microenvironment compromises the major and most important routes of molecular transports which are highly efficient in healthy organs: vascular, trans vascular and interstitial transport, and cellular uptake [131]. The major abnormalities in tumor microenvironment are: solid stress, which is induced by tumor growth, can effect vascular and interstitial transport and consequently increase interstitial fluid pressure; tortuous and leaky blood vessel networks which hamper the normal blood flow in tumor microenvironment; collapsed and nonfunctional lymphatic vessels that in combination with leaky and heterogeneous blood vessels give rise to interstitial fluid retention leading to further increase in interstitial pressure; and finally highly dense ECM accumulation that makes additional barrier to interstitial transport [132]. Because of these transport barriers, most drugs exhibit low efficacy in cancer therapy as they have poor penetration and distribution into tumors [133], hence present unique challenges against targeted delivery to CAFs.

For targeted delivery of potent drug-conjugates to CAFs, the first challenge is to overcome the substantial barrier of morphologically abnormal blood vessels’ basement membrane in tumors [134]. The specific characteristics of remodeled basement membrane in tumor vasculature can affect/limit the extravasation of the designed drug-carrier (e.g. nanoparticles) from intratumoral blood vessels into the interstitium, where CAFs are localized. In addition, infiltrated immune cells accumulated in perivascular area can be another significant barrier for efficient delivery of the drugs to CAFs due to their high phagocytic capacity, especially for nanoparticle-drug delivery systems [135].

Several approaches have been proposed to improve the delivery and therefore the potency of therapeutics to tumor microenvironment like solid stress attenuation, improving the function of tumor vasculatures, dampening the interstitial fluid pressure, and improving the physicochemical properties of therapeutic agents [132,136]. These strategies improve the pharmacokinetic of the drugs, and concurrently keep the drugs pharmacodynamically active [136]. Despite that, emerging evidence highlights the complexity and heterogeneity of tumor microenvironment milieu. This underlines the pressing need for further detailed knowledge on cancer biology to allow for designing new therapeutic agents to selectively target not only tumor cells, but also stromal cells such as CAFs.

4. Potential targets in myofibroblasts

The most important part of the targeted therapy development is to identify potential molecular targets that are involved in the pathological processes that, upon alternation, can lead to the disease resolution/reversion. The therapeutic targeting strategies to inhibit myofibroblasts functions can be categorized into (i) small molecule drugs/inhibitors e.g. receptor tyrosine kinases inhibitors such as RhoA kinase, ERK, JNK etc.; signaling pathways inhibitors such as TGF-β, PDGFR-β, Hedgehog, Notch, Wnt, endothelin-1, and siRNA and microRNA (for details, refer to reviews [137–143]). (ii) Monoclonal antibodies that can identify and bind to the targets on the cell surface or outside the cells. (iii) Targeted delivery systems consisting of the targeting moiety such a delivery vehicle or protein carrying therapeutic agent conjugated to targeting ligands. Also, increasing investigations substantiate the advantage of applying dual-targeting strategy, especially for simultaneous targeting of different cells/components in tumor microenvironment using nanomedicine [144].

Many efforts have gone into unraveling the detailed mechanisms involved in transdifferentiation of precursors to myofibroblasts as well as determining the potential targets expressed on activated myofibroblasts. However, most of these proposed targets have been identified based on in vitro experiments, and therefore their expression profile and therapeutic value in in vivo situation need to be further validated. Moreover, the majority of those candidate targets are also expressed in other cells in the body, and even their extent and pattern of expression alter much among different disease pathology. Furthermore, more specific targets are needed that are universally expressed on myofibroblasts, as some targets are present on certain precursors of myofibroblasts. Hence, non-targeted systemic therapies to treat myofibroblasts may not be a successful approach and exerts unwanted side effects in off-target tissues or cells. It would be desirable to define moieties that are exclusively expressed or over-expressed by myofibroblasts during pathological conditions such as fibrosis and carcinogenesis. Consequently, we elaborate herein on cell surface targets which have been used in vivo as well as potential candidates for in vivo drug delivery to myofibroblasts, considering the translational potential of the target/delivery strategy towards clinical practice.

4.1. Platelet-derived growth factor (PDGF) receptors

PDGF receptors are the tyrosine kinase receptors (PDGFR-α and PDGFR-β) with common domain structures, including five extracellular immunoglobulin (Ig) loops and an intracellular tyrosine kinase (TK) domain. PDGFR-α and -β exist in homo- or hetero-dimeric forms and bind to PDGF dimeric subunits [145]. Until now, PDGFR-α, -BB, -AB, -CC, and -DD forms are reported to exist [146]. Binding of the PDGF to their receptors leads to autophosphorylation of tyrosine residues that activate several signaling molecules regulating cell growth, proliferation, differentiation, and development in many diseases including fibrosis and cancer [145]. The expression of PDGF receptors on myofibroblasts has been shown to be tissue specific in different fibrotic diseases. For example, PDGFR-α expression on lung myofibroblasts has been induced by or suppressed by different stimuli in pulmonary diseases, while they express PDGFR-β constitutively. In contrast, PDGFR-β expression on liver myofibroblasts is extremely inducible and upregulated during liver injury, and is a hallmark of early HSCs activation [147]. PDGFR expression has also been correlated with myofibroblasts differentiation and proliferation, and subsequent ECM deposition, both in experimental models
and human diseases [147]. PDGFR antagonism and pharmacological inhibition of PDGFR-β has shown to be a promising therapeutic approach and is therefore a potential target in organ fibrosis as well as in tumor growth and metastasis [147–152]. We and others have utilized the myofibroblasts’ PDGFR receptors for targeted delivery of compounds to treat organ fibrosis or tumor growth [153–156], demonstrating the potential of targeting PDGFR receptors as clinically feasible therapeutic approach in fibrosis and cancer [157].

4.2. Integrins

Integrins are 90 to 160 kDa transmembrane heterodimeric receptor proteins that bridge the ECM to the intracellular cytoskeleton, playing a crucial role in ECM-cell adhesion and also cell-cell adhesion [158]. They exist in α/β non-covalently associated heterodimeric forms consist of 18 α and 8 β subunits, that assemble into 24 different receptors with different binding properties and distinct tissue and cellular distribution. Integrins mediate the crosstalk between the epithelia, tissue myofibroblasts and immune cells and thereby are involved in the initiation and progression of tissue fibrosis. Integrin receptors are reported to be overexpressed on the myofibroblasts in different fibrotic diseases and tumor stroma [159]. Recently, a crucial role of αv integrins in tissue fibrosis in multiple organs has been highlighted [160]. An extensive study by Henderson et al. [161] has demonstrated that HSCs express several αv-containing integrins (αvβ1, αvβ3, αvβ5 and αvβ8) and their expressions are markedly increased in fibrotic livers, as shown in mice models and human patients. Furthermore, using Pdgfrb-Cre murine model, they demonstrated that selective αv integrin depletion in PDGFR-β-expressing myofibroblasts significantly inhibited the progression of hepatic, pulmonary and renal fibrosis. These interesting findings suggest that strategies to manipulate αv integrins could be potentially attractive therapeutic targets to prevent the progression of fibrosis.

Integrin αv is well-reported to be overexpressed in angiogenic blood vasculature and has been targeted using RGD sequence-containing peptides to tumors [162,163]. Lately, Chen et al. [164] reported higher expression of αv6 integrin in lung myofibroblasts both in vitro and in vivo. Genetic ablation and pharmacological inhibition of αv6 integrin attenuated bleomycin-induced experimental lung fibrosis. Also, integrin α11 has been also identified to be overexpressed by CAFs of non-small-cell lung carcinoma (NSCLC), and head and neck cancer [165, 166]. Earlier findings have indicated that stromal integrin α11 has a crucial role in both primary tumor growth and in the metastatic process, highlighting integrin α11 as a unique therapeutic target in tumor stroma [167,168]. We have recently identified α11 as a key target overexpressed on myofibroblasts within different fibrotic tissues including liver, kidneys, and lungs, while its expression is very low in normal organs [169], and showed that α11 knockdown in myofibroblasts inhibited their differentiation and pro-fibrotic functions. These data highlight α11 integrin as both cell surface and therapeutic target in myofibroblasts. In addition to integrin α subunits, recently Martin et al. [170] has illustrated upregulation of integrin β1 in myofibroblasts and its important role in liver fibrogenesis. Integrin β1 interacts with several α subunits and is broadly expressed on number of cells and tissues which makes it less interesting cell-specific target. Nevertheless, targeting β1 integrin or its downstream intracellular pathways in myofibroblasts still remains a promising therapeutic approach.

4.3. Mannose-6-phosphate/insulin-like growth factor-II receptor (M6P/IGF-IIIR)

M6P/IGF-IIIR is a multifunctional receptor, also known as cation-independent M6P receptor, involved in the transport of cellular proteins from the cell surface or trans Golgi network to lysosomes [171]. This receptor has four distinct binding sites for M6P-containing molecules and other sites for non-M6P-containing ligands. M6P-containing ligands are latent TGF-β and lysosomal enzymes, whereas non-M6P-containing ligands are IGF-II and retinoic acid [172]. This receptor is highly efficient for intracellular delivery as it rapidly internalizes after binding to its ligands. M6P/IGF-IIIR is particularly expressed on activated HSCs during liver fibrosis [173], and was explored for HSC-specific drug delivery [174,175]. This receptor is also shown to be selectively expressed by various cancer cells and fibroblasts in vitro and in B16 and C62 tumor models in vivo [176]. Apart from liver, M6P/IGF-IIIR expression was demonstrated in arteries, glomeruli and tubular epithelial cells of TGR(mRen2)27 rat kidneys, but not specifically on renal fibroblasts/myofibroblasts [177]. Collectively, these data suggest that M6P/IGF-IIIR is a suitable target for drug targeting to HSCs, but more studies are warranted to establish it as a pan-myofibroblast target.

4.4. Fibroblast activation protein (FAP)

FAP is a type II membrane serine protease with an extracellular catalytic domain with dipeptidyl peptidase IV (DPPIV)-like fold. FAP expression is shown to be mainly restricted to the reactive stroma of many tumors including breast, colorectal, skin and pancreatic tumors, while no expression was seen in the neighboring healthy cells. FAP expression has been also observed in non-tumoral diseases such as liver cirrhosis and arthritis [178–180]. Bremenn et al. [181] generated a thapsigargin (TG)-based FAP-activated prodrugs which after CAF interaction became proteolytically activated by FAP and releasing TG analog in the tumor microenvironment, resulting in significant inhibition of tumor growth in breast and prostate xenograft cancer models. FAP has also been used for disassembling cleavable amphiphilic peptide (CAP)-based nanoparticles or activating promelittin-containing FAP-cleavable sequences attached to phospholipid and reduced graphene oxide nanosheets for efficient and rapid release of encapsulated drugs at the tumor sites [182,183]. Using transgenic lung and xenograft CT26 colon cancer mouse models, Santos et al. [184] showed that genetic deletion and pharmacologic inhibition of FAP inhibited tumor growth which attributed to an indirect inhibition of tumor cell proliferation, marked reduction of myofibroblasts content, and tumor angiogenesis. These findings highlight FAP as a promising therapeutic cell surface target for designing drug delivery strategies.

4.5. Retinol binding protein (RBP) receptor

Retinol (vitamin A) receptor, also known as “stimulated by retinoic acid 6” (STRA6) or RBP receptor, is a membrane-bound cell surface receptor which acts as a transporter for retinol. Retinol binds to RBP in the blood, and the complex is transported into HSCs through STRA6 and stored as retinyl palmitate in cytoplasmic lipid droplets [185]. STRA6 is highly expressed by HSCs and plays an important role in uptake and storage of retinol, and used as a promising target for HSCs-specific drug delivery as shown in experimental models of liver fibrosis [186,187]. However, more studies in other fibrotic diseases and cancer are warranted to establish RBP receptor as pan-myofibroblasts target.

5. Targeting systems for myofibroblasts

Herein we describe the drug targeting systems for delivering therapeutic agents to myofibroblasts, which utilize the cell surface targets, described in Section 4. As a general targeting strategy, ligands such as peptides, antibodies, aptamers or other moieties are designed against receptors overexpressed by myofibroblasts. These ligands are either directly conjugated to the therapeutic molecule or to a (nano)carrier incorporating a therapeutic agent. If a ligand is therapeutically active by itself, then such strategy may act as “dual targeting”. The major targeting systems reported in literature are summarized in Fig. 4 and described as follows.
targeting. Receptor, vitamin A-coupled liposomes have been designed and used to deliver siRNA against heat-shock protein 47 to HSCs in liver fibrosis, kidney fibrosis and tumor stroma. A cyclic peptide so called PPB has been used to target this receptor after conjugating to albumin or a nanocarrier, as illustrated here. For example, (1) PPB-modified sterically-stabilized liposome (SSL) loaded with IFNγ (PPB-SSL-IFNγ); (2) PDGFRβ-specific IFNγ construct (PPB-PEG-IFNγ) and (3) PPB-modified human serum albumin (PPB-HSA). Another receptor mannose-6-phosphate (M6P)/insulin like growth factor-II receptor (M6P/IGF-IIIR), which is overexpressed specifically on HSCs but not reported on general myofibroblasts, was targeted with M6P(28)-HSA (albumin chemically modified with 28 M6P moieties) or M6P-HSA conjugated liposomes. Furthermore, retinol binding protein (RBP) receptor, expressed by HSCs, has the main role in vitamin A uptake and storage. To target RBP receptor, vitamin A-coupled liposomes have been designed and used to deliver siRNA against heat-shock protein 47 to HSCs in liver fibrotic models. Integrin αv receptor which is available in various forms (e.g. αvβ3, αvβ5, αvβ6, αvβ8) has been shown to be expressed on myofibroblast in different organ fibrosis which makes it a potential candidate for targeting. “?” indicates that there is a lack of integrin targeting approaches to target myofibroblasts.

5.1. Modified albumin-based systems

Since during liver fibrogenesis myofibroblasts largely originate from HSCs, the differentiated forms of HSCs become the key target cells. Therefore, several targeted drug delivery systems have been designed against HSCs to inactivate them using ligand modified albumin [188]. In this system, human serum albumin (HSA) was modified by conjugating targeting ligands such as sugar molecules or peptides which have specific binding to a cell surface target receptor on the differentiated HSCs. In addition, therapeutic drug molecules or imaging agents could be conjugated to HSA for therapeutic efficacy or diagnosis, or both. Use of HSA has several benefits as a carrier: (i) high immunocompatibility being the most abundant blood protein; (ii) the presence of many functional groups (e.g. 44 lysines, free cysteine) providing enormous possibilities for modification; (iii) long circulating half-life; (iv) optimal molecular size preventing renal filtration but avoiding recognition by the RES. For targeting HSCs, albumin has been modified with mannose-6-phosphate and PDGFRβ-binding peptide.

M6P/IGF-IIIR, overexpressed on HSCs, was explored for HSC-specific drug delivery using M6P-HSA (albumin chemically modified with 28 M6P moieties) [174] (Fig. 4). Using in vitro cell culture, animal models of liver fibrosis and liver slices from normal and cirrhotic human livers, it was demonstrated that M6P-HSA construct was effectively taken up and internalized in activated HSCs via receptor-mediated endocytosis. Two cytostatic and anti-proliferative drugs: doxorubicin (DOX) and pentoxifylline (PTX) were coupled to M6P-HSA carrier and showed HSC-specific accumulation and anti-fibrotic effects in vitro and in vivo in bile duct ligation (BDL) rat model [189,190].

Subsequently, apoptotic drug 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) was delivered using two carriers: M6P-HSA, and peptide-modified albumin (PPB-HSA) that has affinity for PDGFRβ [175]. It was shown that 86% of 15dPGJ2-M6P-HSA and 63% of 15dPGJ2-PPB-HSA accumulated predominantly in the HSCs (64%) and Kupffer cells (39%) in the BDL rat fibrotic liver 15 min post-intravenous injection [175]. Gliotoxin (GTX), another pro-apoptotic drug, was also delivered to HSCs using M6P-HSA delivery carrier and M6P-HSA-GTX construct induced apoptosis of activated HSCs in vitro, in vivo and in fibrotic liver slices [191]. These results showed the potential of both carriers for delivering pro-apoptotic agents to the myofibroblasts in the fibrotic liver.

Angiotensin II inhibitors and several other kinase inhibitors, e.g. rho-kinase inhibitor, impede key signaling pathways in HSCs and myofibroblasts. Thus, attempts were made to deliver these inhibitors (Losartan, Angiotensin II inhibitor and Y27632, rho-kinase inhibitor) specifically to HSCs using previously tested M6P-HSA carrier. The results showed HSC-specific accumulation, and improved therapeutic efficacy with reduced off-target effects in several models of liver fibrosis. Both carrier-coupled inhibitors lowered portal pressure without affecting mean arterial pressure, while free untargeted drugs induced severe systemic hypotension [192–195]. Similarly, ALK5 inhibitor LY-364947 (TGF-β signaling pathway inhibitor) was also delivered via M6P-HSA, and conclusively showed HSC-specific uptake and inhibited ECM.
deposition in acute liver fibrosis model while prevented unwanted adverse effects in other cells [196].

Beljaars et al. [197] showed HSC-specific delivery using HSA-conjugated RGD-sequence containing cyclic peptides which selectively binds to collagen type VI receptor (via integrins), the receptor that is eminently expressed on activated HSCs. Both in vitro and in vivo results verified that this carrier can be used for the targeted delivery of potent anti-fibrotic drugs to HSCs, and simultaneously it can also be utilized as receptor antagonists while delivering the drugs to target cells (dual targeting) [198].

5.2. Peptide-modified cytokines

 Knowing the central importance of PDGFR-β in liver fibrosis [199, 200], novel peptide-based targeting approaches were developed for the delivery of anti-fibrotic cytokine IFNγ to PDGFR-β-expressing HSCs in fibrotic liver [201]. IFNγ was coupled to PDGFR-β-recognizing peptides (either monocyclic PPB or bicyclic dimeric BiPPB) via bifunctional PEG linkers or HSA drug-delivery carrier (Fig. 4). Targeted IFNγ showed HSC-specific uptake and improved therapeutic efficacy, while reducing systemic side effects as compared to untargeted free or PEGylated IFNγ using different targeting approaches in carbon tetrachloride (CCL2)-induced liver fibrosis mouse models. These results demonstrate the impact of cell-specific targeted therapies as compared to untargeted free biologicals. For clinical translation, targeted IFNγ was further miniaturized by synthesizing a chimeric molecule (mimy-mimy-BiPPB) composed of mimetic IFNγ (IFNγ signaling domain) and PDGFR-β-binding bicyclic peptide either chemically (via chemical conjugations) or biotechnologically (in E.coli) [202]. This designed IFNγ peptidomimetic conjugate (mimy-BiPPB) substantially prevented the progression of fibrosis in CCL2-induced mouse models of liver fibrosis, offering novel therapeutic approaches not only for the treatment of liver fibrosis, but also in curing other fibrotic diseases.

In another study, we reported the beneficial effects of targeted IFNγ delivery to kidney myofibroblasts to halt fibrosis both in vitro and in vivo [154]. By conjugating the PEGylated IFNγ to PDGFR-β-recognizing peptides (PPB-PEG-IFNγ) (Fig. 4), myofibroblasts-selective PPB-PEG-IFNγ construct inhibited the progression of renal fibrosis in unilateral ureter obstruction (UUO) mouse models, while at the same time prevented the unwanted systemic side effects of untargeted IFNγ. This novel targeted delivery approach, by increasing the efficacy and reducing the off-target systemic side effects of IFNγ, holds a great promise for the future of drug targeting to renal myofibroblasts. These studies highlight the potential of myofibroblasts targeted therapeutics in the treatment of renal fibrosis.

In addition, we have also shown the specific stromal targeting of IFNγ using PDGFR-β expressed on pericytes and CAFs. We synthesized PDGFR-β-specific IFNγ construct (PPB-HSA-IFNγ) and revealed that specific PDGFR-β-binding cyclic peptides can be used to deliver IFNγ to pericytes and fibroblasts to inhibit angiogenesis and tumor growth [203]. In another study, we examined the effect of conjugated doxorubicin to PPB-HSA in targeting PDGFR-β-expressing cells in C26-tumor bearing mice (Fig. 4) which markedly diminished the C26 tumor growth, compared to non-conjugated, free doxorubicin treated mice [156].

5.3. Nanoparticle-based systems

 Although some conventional drugs have good therapeutic index, their systemic delivery at effective dose may induce severe side effects in healthy cells. To avoid these unwanted effects, while simultaneously increasing the concentration of the drugs at specific location or target cell, and overcoming many of the inadequacies in drug development and delivery, e.g. difficulties in crossing biological barriers, nano-carriers systems for drug delivery purposes have been advanced technologically. This pronounced advancement substantially improved the efficacy of established therapeutics and successively increased the therapeutic index of drugs, both via ‘site-specific’ and/or ‘site-avoidance’ drug delivery [204,205].

Targeting peroxisome proliferator-activated receptor-γ ligand (rosiglitazone) using M6P-HSA conjugated liposomes showed promising therapeutic results in CCl4-induced rat model of liver fibrosis [206] (Fig. 4). Also, Li et al. [207] by using M6P-modified bovine serum albumin nanoparticles encapsulating anti-fibrotic drug sodium ferulate demonstrated HSC-specific uptake and improved efficacy as compared to free drug both in vitro and in vivo. Similar studies illustrated improved anti-fibrotic property with reduced systemic effects by using a targeted PPB-modified sterically-stabilized liposome (SSL) loaded with IFNγ [208,209].

Another promising target is the retinol binding protein receptor (RBP receptor or STRA6) expressed by HSCs. Vitamin A-coupled liposomes loaded with siRNA against heat-shock protein 47 that acts as a collagen-specific chaperone, were shown to inhibit hepatic fibrosis in different experimental fibrotic models [186]. Duong et al. [187] showed promising results by using vitamin A-nanoparticles to deliver nitric oxide (NO) into HSCs that attenuated the progression of liver fibrosis and portal hypertension.

Recently, Zhang et al. [210] aimed at examining the effectiveness of nanoparticle carrier which assists corona formation in drug delivery to HSCs. They meticulously validated retinol-conjugated polyetherimine (RCP) nanoparticles that could attract plasma proteins such as the retinol binding protein 4 (RBP4), and thus formed corona on the surface of nanoparticles. Usually the corona formation poses a negative effect on the modified ligands and may impede the targeting properties of the designed conjugate by interfering in ligand-receptor interaction. This modified corona formation composed of RBP/retinol complex was able to be directed to HSCs as a retinol-storing cells. Using this delivery system, they delivered antisense oligonucleotide (ASO)-loaded RCP carrier to activated HSCs in CCl4 and BDL models of liver fibrosis [210]. By this strategy, they could effectively increase the therapeutic potential of the compound in treating the disease. This study highlights the practical importance of modifying corona formation as a new promising approach for targeted delivery, which needs to be further evaluated for its clinical translation. Du et al. [211] designed IFN-α1b-encapsulated RGD-coupled sterically stable liposomes that specifically bind to HSC-expressing collagen VI receptor. cRGD-liposomes showed increased HSCs accumulation (10-times higher than unlabelled liposomes) and improved therapeutic efficacy in BDL model of liver fibrosis. Also, Thomas et al. [212] by means of hyaluronic acid (HA) micelles delivered angiotensin type I receptor blocker losartan to activated HSCs which markedly reduced the development of liver fibrosis. These studies highlight the potential of drug delivery strategies in targeting myofibroblasts by taking advantage of effective and potent drugs or biologicals for the treatment of fibrotic diseases, though mainly in hepatic fibrosis.

Increasing evidence indicate that nanoparticles are capable of combating several barriers and difficulties faced using conventional drugs. Various studies have shown potential applications of nanoparticles especially in drug delivery and targeting, as well as in diagnostic field such as imaging in kidney diseases [213]. A well-designed study by Choi et al. [214] examined the renal distribution of 10 to 150 nm nanoparticles by intravenous injection. They showed the intra-renal distribution and size-dependent deposition of gold-loaded nanoparticles, with 80 to 100 nm preferentially accumulated in mesangium, while smaller particles were able to reach the peritubular capillaries of the kidney. The data emerged from this and other studies offers the possibility of developing targeted nanoparticles for effective treatment of glomerular disease [215]. In addition to therapeutic options, the non-invasive imaging techniques using nanoparticles for kidney diseases have been developed [213]. Recent advances in MRI-detectable magnetic nanoparticles revealed very promising results in order to monitor functional and histological parameters of the kidney. For example, in patients progressing to chronic kidney disease (CKD) as an early detection approach such as
measuring/evaluating the numbers and the function of glomeruli/nephron and/or in assessing renal inflammation [117,121,26].

In a recent study, Ji et al. [217] designed a dual-mode nanomaterial which enhanced the efficacy of anti-cancer drug doxorubicin. Doxorubicin-loaded nanoparticles (PNP-D) were synthesized via encapsulation by peptide nanoparticles (PNP). PNP-D-mAb construct was then engineered through electrostatic binding of monoclonal mouse antibody (mAb), which recognizes FAP-α on CAFs. These modifications enabled PNP-D-mAb construct to bind FAP-α-expressing CAFs (Fig. 4), resulting in release of cell-penetrating peptide (CPP) in tumor microenvironment for increased cellular uptake and therapeutic efficacy of DOX.

Ernst et al. [218] benefitted from taxane nanoparticles in order to target stroma in an animal model of pancreatic tumor. They prepared a construct (Cellax-DTX polymer) consisting of docetaxel (DTX), PEG, and acetylated carboxymethylcellulose. By injecting this compound, they showed that these nanoparticles accumulated in CAFs and subsequently reduced metastatic potential of the tumor and increased the survival in mice bearing pancreatic cancer.

5.4. Antibody-based systems

Antibody-mediated delivery of therapeutic modalities to selective antigens has caught growing interest mainly in cancer therapy as an effective strategy. The encouraging results and emerging evidences of e.g. antibody-drug conjugates in tumor field hold lots of promise for treating patients [219,220]. Schuster et al. [221] developed FAP antibody-modified immunoliposomes encapsulating antifibrotic drug deferoxamine and showed substantial attenuation of collagen deposition in activated fibroblasts in vitro. In another study, targeted lipid-coated nanoparticles were designed using TNF nanocites (tumor necrosis factor was covalently attached to the surface of polymeric nanoparticles) which incorporated with a single-chain Fv (scFv) molecule targeted to FAP-expressing cells [222]. Although the in vivo practical applicability of this approach awaits future studies, these results showed the potential of this multifunctional lipid-nanoparticle composite system in targeting FAP-expressing cells, which may have future applications in treating fibrotic conditions and tumor. Also recently, biphasic immunoliposomes which target concomitantly endoglin (CD105) and FAP were designed, and tested in vitro with promising results [223].

The role of PDGF system in fibrotic condition and tumor stroma, particularly liver fibrosis, has been well-documented. There are several promising therapeutic antibodies and aptamers for targeting the PDGF receptors in liver fibrosis which are currently in advanced preclinical studies or clinical trials [224].

5.5. Polymer-based systems

The recent advancements in polymer-based drug delivery underscore the great potential of this technology in targeted delivery of both therapeutics and theranostics [225,226]. The polymer-drug conjugates gained significant attention especially in cancer therapy in order to improve the efficiency of targeted delivery to tumor microenvironment [227]. However, the application of this delivery system in the field of organ fibrosis is still very limited. Yang et al. [228] constructed a polymer-based compound by conjugating collagen type I specific triplex forming oligonucleotide (TFO) to HPMA, as polymer carrier, accommodated with M6P and GFLG peptidyl linkers. These conjugates markedly increased the delivery of TFO to HSCs in rat model of liver fibrosis, underlying the potential of this polymer-based delivery strategy in treating liver (or possibly other) fibrotic diseases. Future investigations are indeed warranted to confirm the advantages of the polymer-based drug delivery over other delivery strategies in organ fibrosis and tumor.

5.6. Aptamer-based systems

Due to the capacity of these single-stranded oligonucleotides to bind to their targets with high specificity and affinity, both RNA- and DNA-based aptamers have been shown to possess enormous potential for therapeutic applications [229]. The specific characteristic features of the aptamers make them also favorable candidates for drug delivery strategies such as aptamer drug conjugates [230]. Aptamers are also called chemical antibodies on account of their functional similarities with antibodies. However, because of several unique properties such as specificity, stability, penetration efficiency, etc., aptamer nanomedicines have great potential and more advantages compare to common antibody-based therapeutics, particularly in oncology field [231,232].

Using osteopontin-directed RNA aptamer (OPN-R3), Hunter et al. [233] could significantly block the osteopontin-induced signaling activity in human dermal fibroblasts in vitro, suggesting the potential possibility of this aptamer as an anti-fibrotic therapeutic. In a rat model of glaucoma filtration surgery, administration of aptamer S58 (which targets TGF-βRII) in conjunction with chitosan-based hydrogel (CS/S58) showed superior anti-fibrotic effects than chitosan alone [234]. In a recent well-conducted study, Kato et al. [235] convincingly demonstrated the beneficial effects of modified anti-ATX RNA aptamer ROB14 in reducing markers of lung fibrosis in an experimental mouse model of bleomycin-induced pulmonary fibrosis. Although, to best of our knowledge, there is no published study yet available using aptamer-based delivery strategy to specifically target myofibroblasts in vivo, the encouraging findings of recent investigations using aptamers pose as promising clinically feasible approach in treating fibrotic diseases.

The promising therapeutic concepts emerging from these recent advancements in the field, although awaiting further validation in future, suggest that novel drug delivery strategies have potential to open a new era in treating patients suffering from cancer. However, there are several challenges before such advanced therapies can be advanced to clinic. For example, for drug delivery to the fibrotic microenvironment the challenges are: (i) Physiological challenges: crossing different biological barriers including extravasation and penetration through the complex fibrotic tissue; (ii) Formulation challenges: the complexity of the formulation (combining different components such as ligand, carrier and drug), multiple products within a formulation, batch-to-batch variations, physicochemical characterization and scale up issues; (iii) Translational challenges: a regulatory roadmap, lack of clinically relevant animal models leading to clinical failures despite strong preclinical data, clinical study designs and lack of defined clinical endpoints for chronic fibrotic diseases.

Despite the above-mentioned challenges, recent advancements in the targeted drug delivery approaches and nanomedicines have delivered promising results and have achieved many preclinical and clinical successes in the field of cancer therapy. Because of the ample available information, reader is referred to some comprehensive reviews [236–240] for further details on the topic.

6. Conclusion and future perspectives

Emerging studies underline the central role of myofibroblasts in organ fibrosis and tumorigenesis much beyond their traditional ECM producing role. There are increasing efforts towards unmasking the complexity of myofibroblast phenotypes revealing that myofibroblasts are not a single cell population but a mixture of different cell populations with contrasting functions. These new insights on the diverse origin and multitudinal functions of myofibroblasts will help in discovering the novel therapeutic targets to design new interventions. At the same time, many developed therapeutic agents resulted in a lack of efficacy in vivo due to their premature degradation or inability to enter cells such as the cases of siRNA and miRNA. Drug targeting technologies have demonstrated great potential by protecting their
degradation and specifically delivering them to myofibroblasts, thereby enhancing the therapeutic efficacy and reducing side effects. In future, drug targeting to subpopulations of myofibroblasts will be a highly appealing approach to specifically hamper the disease-inducing functions of myofibroblasts while leaving the rest unaffected. However, the challenges to achieve this appear to be at a very early stage, as the surface markers delineating these populations are not yet identified. Nevertheless, the coherent efforts from the biologists, and pharmacologists and drug targeting scientists will entail this complex issue.

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Conflict of interest

Jai Prakash is the founder and stakeholder of ScarTec Therapeutics BV, Enschede, The Netherlands.

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