Asian Journal of Pharmaceutical Sciences 16 (2021) 47–61

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/AJPS

Review

Progress in drug delivery system for fibrosis therapy

Lei Xing[a,1], *Xin Chang*[a,1], *Lijun Shen*[a], *Chenglu Zhang*[a], *Yatong Fan*[a], *Chongsu Cho*[b,*], *Zhiqi Zhang*[c,*], *Hulin Jiang*[a,*]

[a] *State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China*

[b] *Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea*

[c] *Department of General Surgery, Shanghai Fourth People's Hospital Affiliated to Tongji University School of Medicine, Shanghai 200081 China*

ARTICLE INFO

Article history:
Received 3 December 2019
Revised 22 March 2020
Accepted 22 June 2020
Available online 17 July 2020

Keywords:
Fibrotic disease
Liver fibrosis
Lung fibrosis
Kidney fibrosis
Anti-fibrosis
Drug delivery system

ABSTRACT

Fibrosis is a necessary process in the progression of chronic disease to cirrhosis or even cancer, which is a serious disease threatening human health. Recent studies have shown that the early treatment of fibrosis is turning point and particularly important. Therefore, how to reverse fibrosis has become the focus and research hotspot in recent years. So far, the considerable progress has been made in the development of effective anti-fibrosis drugs and targeted drug delivery. Moreover, the existing research results will lay the foundation for more breakthrough delivery systems to achieve better anti-fibrosis effects. Herein, this review summaries anti-fibrosis delivery systems focused on three major organ fibrotic diseases such as liver, pulmonary, and renal fibrosis accompanied by the elaboration of relevant pathological mechanisms, which will provide inspiration and guidance for the design of fibrosis drugs and therapeutic systems in the future.

1. Introduction

Fibrosis is similar to many cancers that typically survive only a few years after diagnosis [1]. Fibrosis is a pathological process characterized by an excessive accumulation of extracellular matrix (ECM), such as collagens, fibronectin and hyaluronic acid, that occurs in chronic inflammation [2]. Fibrosis can occur anywhere in the body, such as liver, lung, kidney, heart, blood vessel, eye, pancreas, skin, bone and so on. Further progress of fibrosis will lead to function decline and even failure and death in organs. Apart from organs damage, fibrosis also occurs on many chronic autoimmune diseases as a major pathological feature, including scleroderma, rheumatoid arthritis, Crohn's disease, ulcerative colitis, myelofibrosis and systemic lupus erythematosus [3]. The early-stage of fibrosis can be treated and reversed to avoid serious health problem. Therefore, how to reverse fibrosis has become the focus and research hotspot in recent years.

Among various type of fibrosis, liver, pulmonary and renal fibrosis have widely studied because of their high incidence and mortality. Firstly, the end-stage of liver

* Corresponding authors.
E-mail addresses: chocs@snu.ac.kr (C. Cho), zzq72@163.com (Z.Q. Zhang), jianghulin3@gmail.com (H.L. Jiang).

[1] These authors contributed equally to this work.

Peer review under responsibility of Shenyang Pharmaceutical University.

https://doi.org/10.1016/j.ajps.2020.06.005

fibrosis, liver cirrhosis, is a significant cause of global health burden [4]. At present, epidemiological data show that cirrhosis affects hundreds of millions of people around the world and have become the 14th common cause of disease death worldwide [5] The mortality rate of liver cirrhosis is high, therefore it is particularly important to implement effective therapeutic treatment in the stage of liver fibrosis and prevent the progression to the stage of liver cirrhosis. Current treatment drugs for liver fibrosis include hepatocyte-protecting drug [6,7], inhibitors of hepatic stellate cells (HSCs) activation and proliferation [8-10], ECM degradation agents [11,12] and traditional chinese medicine (TCM) [13,14]. Secondly, pulmonary fibrosis (particularly idiopathic pulmonary fibrosis, IPF) is a chronic progressive interstitial lung disease (ILD) of unknown etiology, of which the median survival is only three years [15]. Its mortality was significantly higher with increasing age, and males have consistently higher mortality than females. To date, pirfenidone and nintedanib are the only two drugs approved by Food and Drug Administration (FDA) for pulmonary fibrosis therapy [16]. Finally, renal fibrosis is the main process of progression from chronic kidney disease (CKD) to end-stage of renal disease with high morbidity and mortality [17,18]. Renal fibrosis is characterized by glomerular sclerosis and tubulointerstitial fibrosis, and is a dynamic process consisting of four phases: initiation, activation, execution and progression. A number of epidemiological studies have shown that the prevalence of CKD worldwide is about 8%–16%, the prevalence of CKD is increasing year by year [19,20]. Treatment drugs for renal fibrosis cover inhibitor of pro-fibrotic factors [21-23], inhibitor of collagen synthesis [24,25] and TCM [26,27]. Although there are many anti-fibrosis drugs used clinically and preclinically to treat fibrosis, they can only relieve the symptoms of fibrosis. Until now, there still are no therapeutic drugs that can completely cure fibrosis due to the complex fibrosis mechanism and traditional formulation inefficiently delivering drugs to the lesion [28-31]. The nano-delivery system is an emerging pharmaceutical preparation that has received widespread attention. Importantly, the nano carrier can accurately deliver the drug to the target tissue and the target cell, thereby improving the therapeutic effect and reducing the side effect. Although the application of nanotechnology provides new methods and ideas for the treatment of fibrosis, however, these nano-delivery systems still have some defects, such as the industrial production and the safety problem of carrier materials. In this review, we focused on the most recent progress in the liver, pulmonary and renal fibrosis as well as related treatment strategies and also discussed the development of anti-fibrotic drug delivery system (Fig. 1).

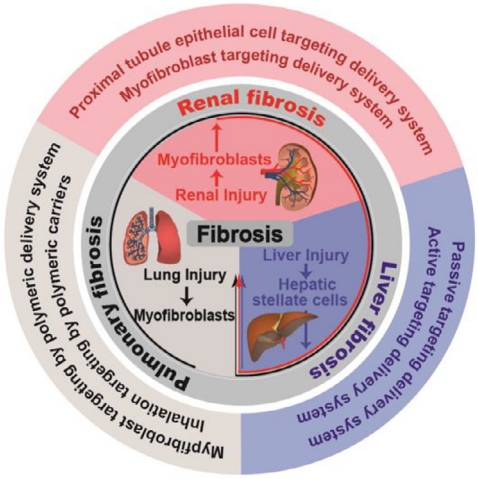

Fig. 1 – Schematic illustration of drug delivery systems for liver, pulmonary and renal fibrosis therapy.

2. Liver fibrosis

2.1. *Mechanism of liver fibrosis*

Liver fibrosis is the result of chronic liver damage caused by a variety of factors, including viral infection, drug toxicity, alcohol abuse, autoimmune disease, nonalcoholic fatty liver disease and dysplasia [32]. The occurrence of liver fibrosis is a dynamic and continuous process, which means the continuous necrosis of hepatocytes and progressive accumulation of ECM. It reflects the imbalance of repair and scar formation of the liver when facing damage [33]. If left uncontrolled, it may develop into the end-stage liver disease or cirrhosis. And the loss of liver function, ascites, portal hypertension, esophageal varices and increased risk of hepatic carcinoma are the most serious complications and are often fatal [34].

HSCs are resident perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells, accounting for 5%–8% of hepatocytes [35]. The HSCs are the primary effector cells in fibrogenesis and also the major ECM producing cells. Once hepatocytes are damaged or apoptotic, they release reactive oxygen species and other fibrotic mediators along with recruiting immune cells, which results in further release of cytokines and chemokines [34,35]. It mediates the transformation of quiescent HSCs in the normal liver into myofibroblast-like phenotype and stimulates collagen secretion. The activated myofibroblasts produce excess abnormal ECM as well as matrix metalloproteinase (MMP) inhibitor, which finally lead to liver fibrosis. In addition to HSCs, fibroblasts (portal fibroblasts and other populations), bone marrow stem cells and biliary epithelial cells can also be activated into myofibroblasts in the context of chronic liver injury [36]. Liver fibrosis not only affects the amount of ECM, but also leads to an imbalance between MMP and tissue inhibitor of metalloproteinase (TIMP), which bring out changes in the composition and distribution of ECM components. Activated HSCs remodel the ECM into fibril-rich collagen. In normal liver, the basement membrane matrix of the Disse space is composed mainly of collagen IV and VI, which is gradually substituted with collagen I and III and cell fibronectin during fibrogenesis, resulting in so-called sinusoidal capillary vascularization [37,38]. Classical mechanisms for activating HSCs involve two phases. In the initial stage, liver damage leads to oxidative stress, apoptotic

bodies, lipopolysaccharides, paracrine stimulation, resulting in loss of lipid droplets and up-regulation of platelet-derived growth factor receptor (PDGFR) in HSCs. In the continuation phase, HSCs maintain activation. Cytokines secreted through immune cells and autocrine provide signals for maintaining HSCs activation, survival and related ECM deposition [32,39]. Changes occurred in HSCs behaviors include contractility, proliferation, matrix remodeling and inflammatory signals [33]. Proliferation of HSCs ultimately leads to ECM synthesis.

Kupffer cells are resident macrophages in the liver, which produce cytokines such as platelet-derived growth factor (PDGF) to affect the activation and proliferation of HSCs. Activation of Kupffer cells leads to increased activity of the nuclear transcription factor-κB pathway, followed by secretion of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1, so that HSCs are activated and proliferated [40]. Natural killer (NK) cells have anti-fibrotic activity and induce apoptosis of HSCs by producing interferon gamma (IFN-γ). Quiescent HSCs are relatively resistant to apoptotic signals; however, upon activating and downregulating major histocompatibility complex class I expression, they turn into the sensitive state. If the level of NK cells in the liver is reduced, the occurrence of fibrosis will increase [32]. Other cell types that regulate progression and regression of fibrosis include T cells, dendritic cells, macrophages, and endothelial cells [32,41].

In general, the development of liver fibrosis and subsequent cirrhosis are driven by persistent liver damage through a variety of mechanisms. It can be considered as an excessive wound-healing reaction driven by the pathogenic malignant circulation of hepatocyte necrosis, inflammation and excessive ECM deposition.

2.2. *Drug delivery system for liver fibrosis*

2.2.1. *Passive targeting delivery system*

After systemic administration, overcoming several physiological barriers is the premise of the successful delivery and uptake of therapeutic drugs by the liver cells. Firstly, the drug-loaded particles enter the blood circulation, and non-specific interactions with serum proteins occur, which may lead to aggregation of particles. And resident macrophages of the endothelial network in the liver, spleen and bone marrow (such as Kupffer cells in the liver) will specifically remove particles which are larger than 200 nm and negatively charged. The liver sinusoids are characterized by 100–200 nm fenestrations along the endothelial wall and absence of basal lamina, which results in the limitation of the particle size even after reaching the liver cells [42]. Kupffer cells and sinusoidal endothelial cells, which are located in the space of Disse closing to the hepatocytes, are intrinsic parts of the reticuloendothelial system [43]. In terms of liver passive targeting delivery systems, polymeric nanoparticle [44,45], albumin nanoparticle [46,47], liposome [48,49] and solid lipid nanoparticle [50,51] have been developed for the past few years. Consequently, anti-fibrotic drugs can achieve better passive accumulation effect in liver, and passive liver targeting ability contributes to the improved treatment efficacy compared to free drug.

2.2.2. *Active targeting delivery system*

The liver is the main metabolic organ in the body, therefore, the uptake of drugs in the liver is usually high, regardless of whether the drugs are cell-specific. However, drugs ingested by fibrogenic cells in the fibrotic liver is usually low, and off-target effects can be high [52]. However, the efficient therapy may be limited by insufficient concentrations of drugs accumulating around the target cell and low efficiency of accurate therapy.Active targeting delivery system can devote to anti-fibrotic drugs for high delivery efficiency and allow them showing improved therapeutic efficacy and desirable safety profiles. The main advantage of such delivery system is selectively target to active site, which increases the concentration at the target tissue or cell while minimizing the adverse effects (Fig. 2) [53]. Moreover, hepatocyte, HSCs and hepatic macrophages play important roles in the progression of liver fibrosis. Therefore, these three types of cells were selected as targets for the treatment of liver fibrosis.

Hepatocyte targeting delivery system: Hepatocytes, which accounted for 60% of the total liver cells and 80% of the liver volume, is the primary cause of liver fibrosis [54]. Hepatocytes bear the function of sugar, protein and lipid metabolism, liver detoxification and other important functions. Liver fibrosis causes hepatocytes damage and can seriously affect liver function. It is very important to transport liver-protecting drugs selectively into hepatocytes to maintain the function of hepatocytes in treatment of liver fibrosis.

Asialoglycoprotein receptor (ASGP-R) is the extracellular glycoprotein receptor located on the surface of hepatocytes, and the galactose is its specific ligand. Therefore, the delivery system modified by galactose could selectively target hepatocytes and improve delivery efficiency, which became a common hepatocyte-targeting ligand [55,56]. Mandal et al. [57] used galactose-modified liposomes loading quercetin (QC) to combat arsenic-induced hepatic fibrogenesis. The results confirmed that galactosylated liposomal QC could improve drug accumulation in the hepatocytes and protect liver from fibrosis. This research validated the significant role of galactose in liver-targeted delivery, which is conducive to the drug for liver fibrosis therapy. In addition, ASGP-R is also used to judge the level and stage of liver fibrosis for precise treatment. Zhang et al. [58] proved that *in vivo* SPECT/CT imaging and quantification of ASGP-Rs targeting tracer, which can be used for predicting fibrosis response to therapy and selecting more appropriate treatment regimens for patients with chronic liver disease. Therefore, ASGP-R-based delivery systems are necessary for liver diseases and these systems all have the fantastic ability of liver-targeting for liver fibrosis therapy and diagnosis.

HSCs targeting delivery system: HSCs are regarded as the main ECM-producing cells with the injured liver and thus drives the fibrogenic process, which play a critical role in the fibrogenesis of liver. Therefore, HSCs are well accepted to be the target cells of anti-fibrotic therapy. Several kinds of targeted delivery systems that can target the receptors expressed on HSCs have been designed, and shown an attractive targeted and therapeutic potential *in vivo* [59-61]. HSCs contain approximately 80% of the body's vitamin A (VA) with a gradual distribution in the liver lobules [62], and there

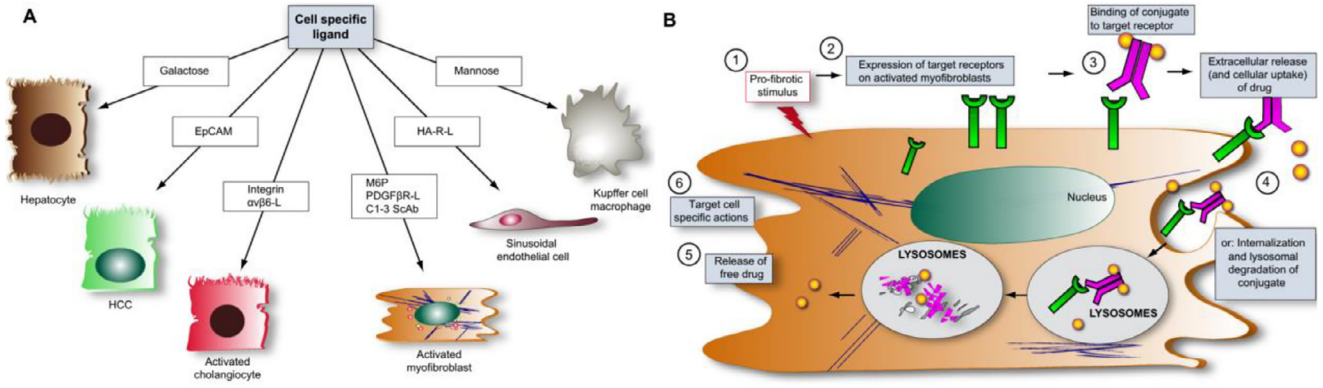

Fig. 2 – Drug targeting in the liver. (A) Cell-specific ligands directed at resident hepatic cells that can be used for targeted therapies of liver fibrosis/cirrhosis. (B) Binding, uptake, internalization and release of compounds targeted at extracellular receptors resulting in target cell-specific actions. Reprinted with permission from [53]. Copyright 2016 Elsevier.

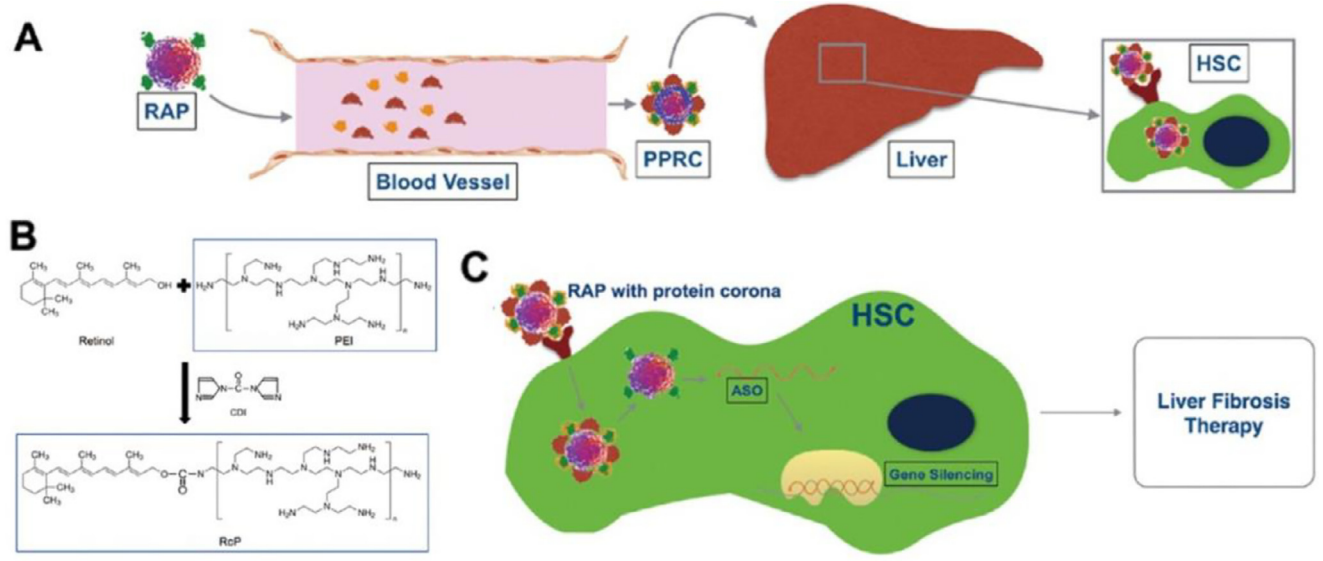

Fig. 3 – (A) Schematic diagram showing the theoretical fate of RAP administered intravenously. (B) Schematic diagram showing the covalent coupling of PEI (1.3 kDa) to retinol via the CDI-activation method. (C) ASO (anti-Col1) was designed to inhibit the expression of collagen I, and the control group (anti-NC) was a scrambled noncoding DNA fragment with the same length and GC contents as anti-Col1. Naked anti-Col1 was set as another control to verify the efficacy of the delivery carriers. Reprinted with permission from [64]. Copyright 2015 American Chemical Society.

are amount of retinol binding protein receptor (RBPR), cell REP (CRBP) and cell retinoic acid binding protein (CRABP) over-expressed on the surface of HSCs. The studies reported that these receptors could selectively transport VA into HSCs [63]. Therefore, VA-based delivery carriers have been utilized for targeting system.

Here are a few examples of HSC-targeting delivery system. Zhang et al. [64] conjugated VA with low molecular weight polyethylenimine (PEI) to form nanoparticles by further combining with nucleotide (RcP) to allow the antisense oligonucleotide (ASO)-laden RcP carrier (RAP) direct into the HSCs (Fig. 3). This nanoparticle system can actively recruit plasma proteins and in particular, RBP4, to form corona on the surface. Therefore, VA is a promising ligand for activated HSCs-targeting delivery in the treatment of liver fibrosis. In addition, Qiao et al. [65,66] developed two novel vehicles grafting VA as a co-delivery system that synergistically suppressed collagen I accumulation in fibrogenesis (Fig. 4). The result showed that two co-delivery systems can accumulate in fibrotic livers, have specifically HSCs-targeting, efficiently decreased collagen I production and ameliorated liver fibrosis. In addition, Fan et al. [67] reported ECM-penetrating nanodrill micelle as a HSCs-targeting delivery system for liver fibrosis therapy. These targeted HSC delivery systems provide a pioneering approach to targeted therapies for liver fibrosis.

Recently, it was reported that the cyclic Arg-Gly-Asp (RGD) peptide can specifically bind to the collagen VI receptors of HSCs [68]. Yang et al. [69] reported the nanosystem of poly (ethylene glycol)-*b*-poly (epsilon-caprolactone) (PEG-*b*-

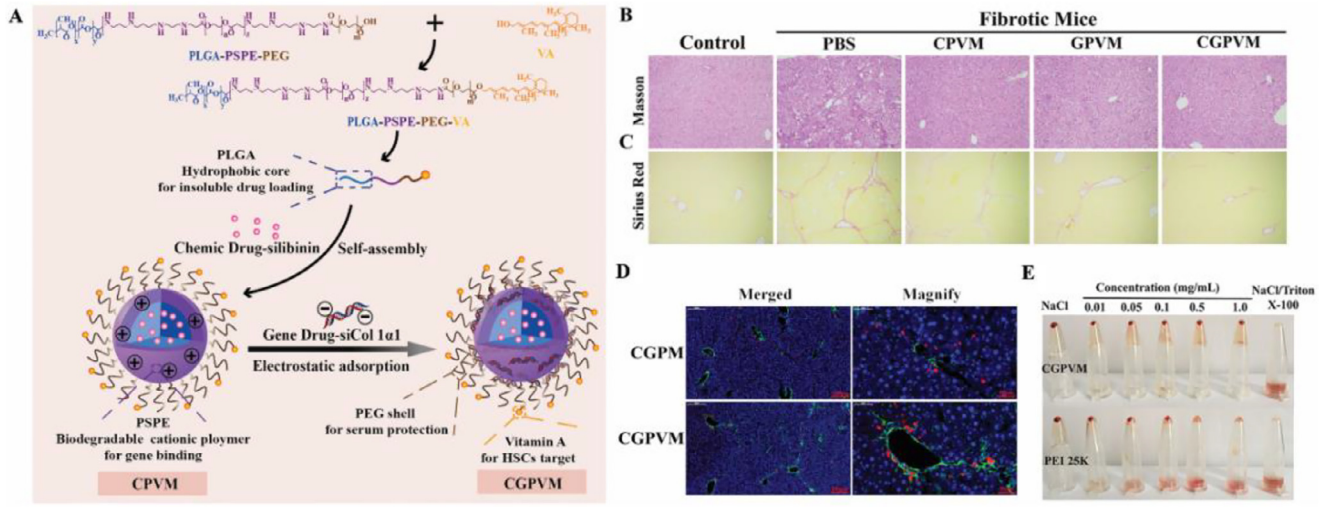

Fig. 4 – (A) The reaction scheme for synthesis of PLGA-PSPE-PEG-VA and illustration on formation of CGPVM. (B) Representative images of H&E (100 ×) and (C) Sirius Red (100 ×) staining of liver tissue sections. (D) Specific delivery of CGPVM to activated HSCs in fibrotic mice. Representative scanning images of liver uptake and cellular distribution of different formulations in fibrotic liver by Pannoramic MIDI Slide scanner. The CGPVM or CGPM was labeled with Cy5 (red), the nuclei were labeled with DAPI (blue) and α-smooth muscle actin (α-SMA) in HSCs was labeled by DyLigh 488 (green). (E) Blood compatibility of CGPVM. Visual observation of hemolysis caused by CGPVM and PEI 25 K in PBS at pH 7.4. Reprinted with permission from [65]. Copyright 2018 Elsevier.

PCL) modified with cyclic RGD peptides to target HSCs. The results demonstrated that the nanosystem could accumulate highly and inhibit the proliferation of HSCs. In addition, HSCs express mannose 6-phosphate/insulin-like growth factor II (M6P/IGFII) receptors, which will be up-regulated when HSCs are activated. Therefore, this is also a potential targeting receptor. Beljaars et al. [70] modified M6P on albumin at different proportions. The results showed that the accumulation of albumin in liver tissue and HSCs increased with the increase of M6P modification. In addition, given CD44 receptors specifically overexpressed on activated HSCs, Gong et al. [71] reported chondroitin sulfate nanomicelles (CS micelles) as a delivery system targeting HSCs for the treatment of liver fibrosis. The results indicated that CS micelles were efficiently delivered to HSCs mediated via CD44 receptors and demonstrated antifibrotic effect. Until now, there are many studies on HSC targeting delivery system. This is because that the activation and proliferation of HSCs are the central event of liver fibrosis. Therefore, HSCs should be the key target cells for the ideal treatment strategy of liver fibrosis.

Hepatic macrophage targeting delivery system: Hepatic macrophages, also known as Kuffer cells, hold a central position in the pathogenesis of chronic liver injury and are the master regulator of dynamic fibrogenesis-fibrosis resolution paradigm. Specifically, macrophages are closely associated with the hepatic scar, directly apposed to the activated HSCs, which can act on the HSCs to induce a pro-fibrotic phenotype because of a rich source of soluble mediators. Macrophages can produce and activate several regulatory factors, such as PDGF, which is a potent stimulator of myofibroblast proliferation; TGF-β, which acts to increase the production of ECM and TIMP-1 by myofibroblast; IL-1β and TNF-α, which are pro-inflammatory cytokines; and a number of chemokines, which can induce further inflammatory cell recruitment to perpetuate the pro-inflammatory pro-fibrotic stimulus [72]. Therefore, hepatic macrophages have been proposed as potential targets in combatting fibrosis.

As researches demonstrated, nanoparticles are easily identified and swallowed by macrophages as a foreign object. Generally speaking, nanoparticles that surface exhibit highly positive to absorb plasma protein are easily recognized and swallowed by macrophages after intravenous injection. However, nanoparticles modified by PEG on the surface will weaken this ability. Besides, macrophages can specifically identify the signal exposed from apoptotic cells, such as phospholipid serine (PS). Therefore, particles modified by PS can be identified as apoptotic cells by macrophages and enhance the macrophages-targeting ability. Wang et al. [73] demonstrated that PS-coated nanoparticles have the ability of efficient macrophages-targeting. Recently, they further designed PS-modified nanostructured lipid carriers prolonged the retention time of drug, enhanced its bioavailability and delivery efficiency to the liver, resulting in reduced liver fibrosis *in vivo* (Fig. 5) [74]. In addition, Kim et al. [75] also developed a glucan-based siRNA carrier system BG34–10-Re-I/siRNA for macrophage-targeted siRNA delivery. Therefore, macrophage targeting delivery systems may have a promising future for the treatment of liver fibrosis.

3. Pulmonary fibrosis

3.1. *Mechanism of pulmonary fibrosis*

Pulmonary fibrosis (particularly IPF), is a progressive ILD and its incidence showed a rising upward trend [76], which

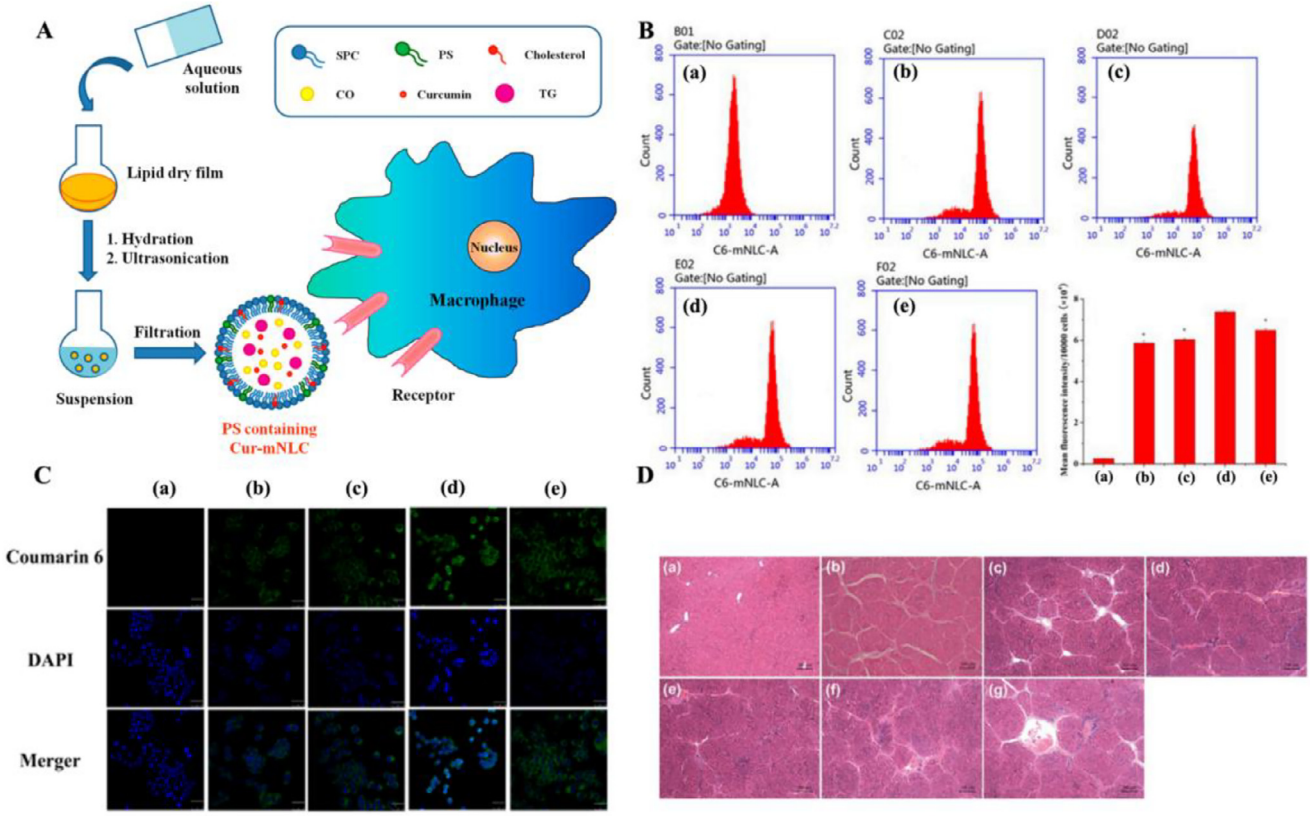

Fig. 5 – (A) A schematic diagram of preparation procedures of PS-based nanoparticles and of endocytotic uptake by RAW 264.7 macrophages. (B) Flow cytometry analysis and mean fluorescence density analysis. (C) CLSM images: (a) negative control group (blank culture medium without NLCs); (b) 0% PS containing C6-mNLCs; (c) 4% PS containing C6-mNLCs; (e) 8% PS containing C6-mNLCs;(e) 12% PS containing C6-mNLCs. (D) Effects of different drug formulations on the histological changes in the liver of CCl4-treated rats as shown by H&E staining: (a) Naive group, (b) Vehicle group (positive control), (c) Free-Cur group, (d) Cur-NLCs group, (e) Cur-mNLCs group, (f) B-mNLCs group, (g) Colchicine group. Data were presented as a typical microscopic view of each group. Reprinted with permission from [73][74]. Copyright 2016, MDPI AG. and Copyright 2017, Informa UK Limited, trading as Taylor & Francis Group.

is an irreversible stage of pathologic development in a variety of lung diseases. According to clinical researches, internal or external factors that may induce or aggravate IPF are as followed: (1) smoke: it induces a self-sustaining lung injury and lower IPF patient survival compared to non-smokers; (2) microorganism: it is a potential risk of pathogenesis. For example, viral infection, such as Epstein-Bar-virus, cytomegalovirus, hepatitis C virus and human herpesvirus-8 were frequently found in the lungs of IPF patients; (3) genetic factor: familial interstitial pneumonia is identified when two or more member of the same biological family are affected. A combination of gene variants and transcriptional changes may induce susceptibility to fibrosis; (4) aging: researches showed that lung fibroblasts of old mice expressed a fibrogenic phenotype which led to resistance to apoptosis and increased susceptibility to the fibrotic response after injury. However, IPF itself is not the main cause of death. A significant proportion of IPF patients will die from another cause: particularly cardiovascular disease or lung cancer [77]. Until now, mortality was significantly higher with increasing age, and males have consistently higher mortality than females. With the progress of disease, the patient mainly presents with long-term, uninterrupted cough with viscous sputum, a scar-like change in the lung parenchyma, a loss of gas exchange in the alveoli and eventually death from respiratory failure. With a progressive course, IPF patients will go through the following stages: the damage to alveolar epithelial cells promotes the proliferation of bone marrow-derived monocytes and their differentiation into macrophages in turn. Myofibroblasts and epithelial and/or endothelial cells can produce MMPs [78], which disrupt the basement membrane and vasodilation recruit a large number of local inflammatory cells, including neutrophils, native macrophages and acidic granulocytes to produce a variety of cytokines (TNF-β, IL-4, IL-13, and IL-1β). These cytokines and CXC chemokines promote the injury and repair process. The release of inflammatory cytokines will promote the differentiation of resident fibroblasts and circulating fibrocytes into myofibroblasts, while myofibroblasts produce excessive collagen at the injury sites [79]. In fibrotic diseases, epithelial cell damage plays an important role in the development of lung disease [80]. Recent studies have shown

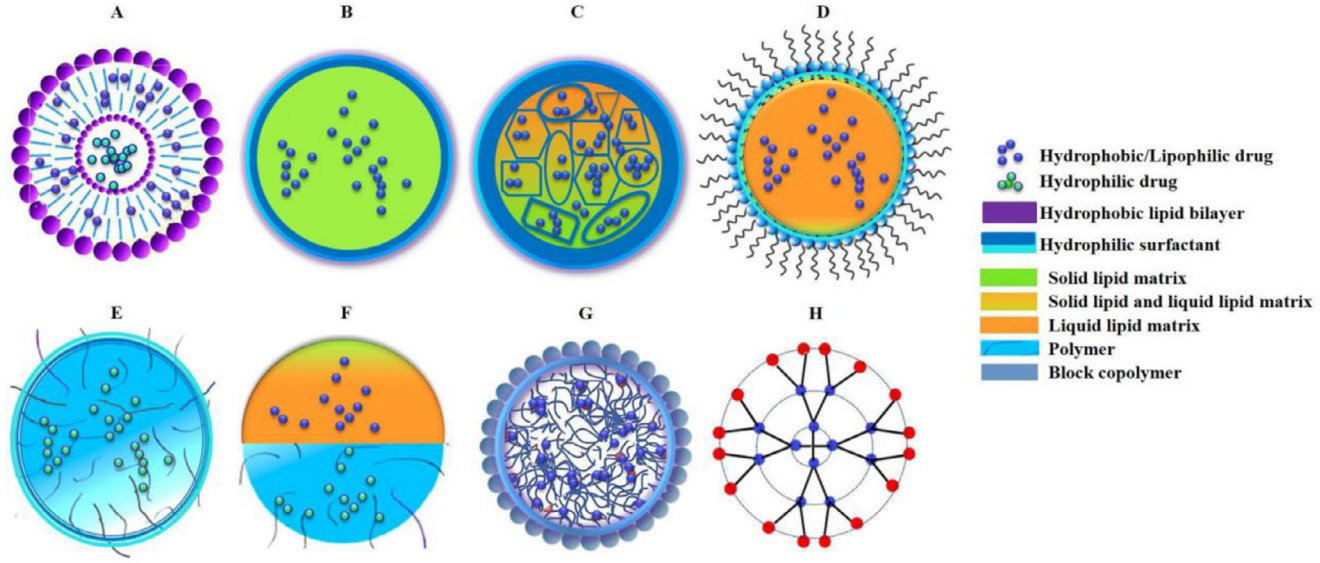

Fig. 6 – The different types of biodegradable nanosized drug delivery systems used in pulmonary nanomedicines (A) Liposomes with hydrophilic drugs in core surrounded by hydrophobic lipid bilayer (B) Solid lipid nanoparticle containing lipophilic drug in solid lipid matrix stabilized by hydrophilic surfactant (C) Nanostructured lipid carrier containing lipophilic drug dispersed in matrix of solid lipid and liquid lipid and stabilized by surfactant (D) Lipid nanocapsule containing lipophilic molecule dissolved in an oily core and stabilized by lecithin and PEGylated surfactant (E) Polymeric nanoparticle containing hydrophilic drug dispersed in matrix and stabilized by surfactant (F) Polymer-lipid hybrid nanoparticle containing hydrophilic and hydrophobic drug dispersed distinctly in polymer and lipid matrix (G) Polymeric micelle containing hydrophobic drug dispersed in hydrophobic core (H) Dendrimer structure. Reprinted with permission from [85]. Copyright 2016 Elsevier.

that damage to the alveolar type II epithelial cells was caused by pulmonary microenvironment disorders and the case of abnormal tissue repair. In the case of normal regulation of the lung tissue, apoptosis of epithelial cells will have a programmed cell or support structure instead [81]. While in an abnormal situation, myofibroblasts continue to secrete excessive proteins called ECM, the overexpressed ECM fills to the alveoli and reduces alveolar space, causing the patient to breathe difficulties and finally even death [82].

3.2. *Inhalation targeting by polymeric carrier*

For the treatment of diseases like IPF in the respiratory tract, drugs need to enter the deep airways. Since the special structure of the lung can communicate with the outside air, the development of non-invasive therapeutic drug has gained increasing interests. The pulmonary administration has the following advantages: the enzyme activity in the lung is lower than that in the intestine, which can avoid the first-pass metabolism. And the large specific surface area of the lung and blood flow of the alveolar membrane, which can promote the rapid systemic absorption of the drug after inhalation administration [83].

At present, there are several treatment strategies, including single drug delivery, dual-drug synergistic treatment and drug-gene synergistic treatment. When different types of biodegradable nanoformulation are delivered by inhalation (Fig. 6) [84,85], the delivery efficiency of nanoparticles to the alveoli is related to the composition, volume and fluid dynamics of the mucus layer [86], which is also related to the active secretion on the alveolar surface. The airway mucus barrier is considered to be the first barrier of lung sections, and the mucus layer is maintaining the hydration and epithelial barrier function of the respiratory tract and regulating immunity. The mucus substances are mainly secreted by goblet cells [87,88], which is composed of glycosylated proteins with the molecular weight of 200 kDa or more. Glycosylated mucin is highly resistant to microbial proteases, and it is beneficial for the clearance of a broad spectrum of bacteria. Mucin having negative charges is interlaced into a porous network with spacing between a few hundred nanometers and micrometers [89]. The mucus network is interacting with foreign substances and generates static electricity or hydrogen bonds, and it can effectively prevent the invasion of foreign substances into alveolar sections [90]. Ivanova et al. reported that the liposomal form of prostaglandin E2 was an attractive drug for the effective inhalation treatment of IPF [91].

3.3. *Myofibroblast targeting by polymeric delivery system*

In the process of alveolar damage, fibroblast and myofibroblast play a crucial role in the repair of damaged tissue by fibrosis. Myofibroblasts are a major source of ECM secretion at the site of injury. They use ECM as a scaffold for repair and reconstruction of damaged tissue, which can promote tissue regeneration and repair. In the pathology of IPF, a large number of inflammatory factors, including IL-1β, TNF-α,

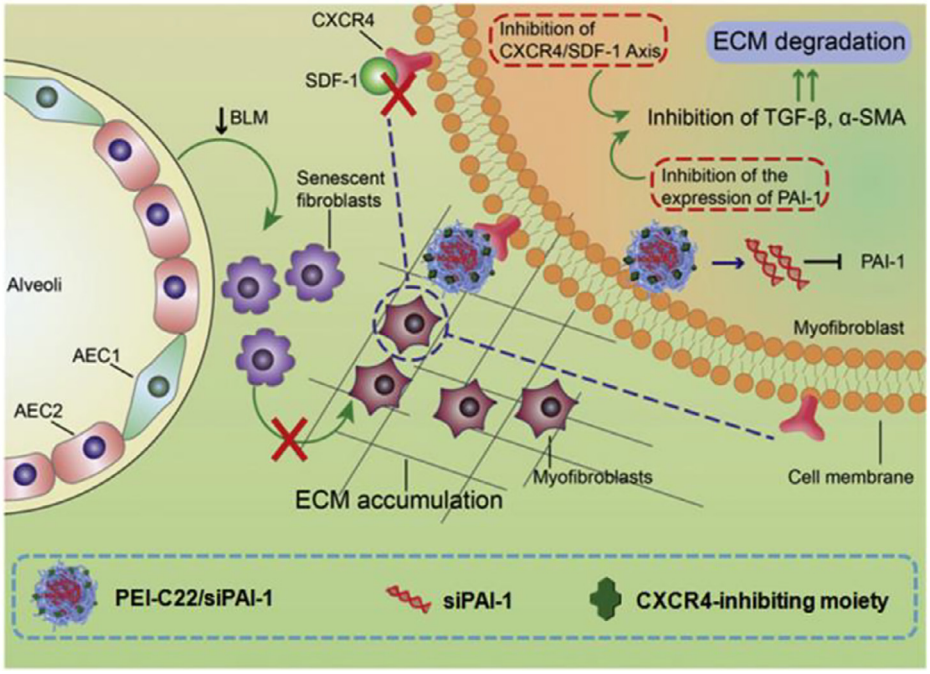

Fig. 7 – Schematic illustration of the mechanism of action of PEI-C22/siPAI-1 polyplexes in the treatment of IPF. Reprinted with permission from [93]. Copyright 2018 Elsevier.

IL-10, and IL-13 and PDGF as a growth factor, can be secreted by mesenchymal stem cell-like resident fibroblasts. PDGF acts as a chemical inducer of interstitial cells which can attract interstitial cells (myofibroblasts) to the damaged sites of alveolar. Once the dysfunction of macrophages occurs, all balances will be destroyed. The massive secretion of inflammatory factors can cause repair-damage of alveolar epithelial cells. And the peripheral fibrocytes expressing chemokine receptor CXCR4 migrate to the injured lung in response to the high levels of its chemokine ligand (stromal cell-derived factor-1) [92], and further activate the conversion of fibroblasts to myofibroblasts. And then the myofibroblasts can secrete large amounts of ECMs again, including collagen, elastin, fibrillin, fibronectin, lamin and proteoglycans. A recent study has focused on the targeted delivery of genes to myofibroblasts by using CXCR4 antagonist. Ding et al. [93] reported the development of polyplexes based on CXCR4-inhibiting PEI derivative (PEI-C) for pulmonary targeted delivery of siRNA to silence plasminogen activator inhibitor-1 (siPAI-1) as a new combination treatment of pulmonary fibrosis (Fig. 7).

4. Renal fibrosis

Renal fibrosis, particularly tubulointerstitial fibrosis, is the common outcome of many CKD independent of the underlying etiology and is characterized by the accumulation of myofibroblasts and excessive ECM deposition [94]. In the past few decades, the number of renal fibrosis patient has significantly increased [95]. Failure to treat renal fibrosis can lead to end-stage renal failure, a devastating disorder which ultimately necessitates dialysis or kidney transplantation. Unfortunately, there is no effective and available strategy to directly treat or reverse renal fibrosis. One of the major obstacles of translating novel anti-fibrotic drug from bench to bedside is due to the intricacies of the drug delivery process.

4.1. *Mechanism of renal fibrosis*

It is now well-accepted that renal fibrosis should not be viewed as a static and uniform ‘scar’, but rather as a dynamic process involving a complexity of cellular events which stimulate the development of fibrotic stages [96]. Many epidemiological studies have shown that the prevalence of patients with end-stage renal disease is increasing all over the world, which has become a major public health problem worldwide [97,98]. However, current therapeutic options for CKD in the clinical setting are scarce and often ineffective. So it’s primary and essential to well understand the cellular and molecular mechanisms of renal fibrosis, not only for obtaining novel insights into the pathogenesis of the process but also for developing rational strategies to treat CKD patients.

4.1.1. *Extracellular matrix*

The production and excessive deposition of a large amount of ECM components are major cellular events in the occurrence and development of renal fibrosis [99]. On one hand, excess ECM which becomes dominated by fibrillar collagen (mainly type I and type III) generates scar tissue that merely maintains organ integrity, in response to sustained inflammation after injury [100,101]. On the other hand, it releases diverse signaling molecules as a consequence of its degradation to cope with physical dysfunction [102,103]. Renal scar formation

involves an excess accumulation of ECM (primarily composed of collagen, fibronectin and proteoglycans) and usually results in loss of function when normal tissue is replaced with scar tissue [104]. With the ongoing deposition of ECM, there is loss of tubules and peritubular capillaries, which can ultimately lead to organ malfunction [105,106]. The main source of ECM deposition in renal fibrosis is the myofibroblast [107,108]. When exposed to pro-fibrotic factors, such as TGF-β1, resident fibroblasts can transform into myofibroblast [109]. Key fibrogenic factors include TGF-β1, PDGF, fibroblast growth factor-2 (FGF-2), connective tissue growth factor (CTGF) and angiotensin II [110,111], whereas hepatocyte growth factor (HGF) and bone morphogenetic protein-7 (BMP-7) inhibit matrix production by antagonizing TGF-β1 action [112,113]. Likewise, signal transduction cascades (between fibrogenic cytokines and specific downstream cells) and expression of matrix genes are also regulated by a variety of micro RNAs [114,115]. ECM is now considered to be a biologically active system that tends to perpetuate inflammation and fibrosis, rather than a passive consequence of chronic kidney injury.

When exposed to internal or external danger stimuli, the body orchestrates an inflammatory response, which is triggered by the damage-associated molecular patterns (DAMPs) [116]. DAMPs consist of a group of heterogenous molecules, such as hyaluronan (HA), biglycan and fibronectin. Recent evidence showed that DAMPs drive not only immune injury but also kidney regeneration and renal scarring [102]. ECM is an important source of DAMPs, whose generation comes from two different processes: either enzymatic degradation of ECM or de novo synthesis from macrophages and renal resident cells in response of various stimuli, such as TGF-β and proinflammatory cytokines [117-119]. DAMPs are involved in fibrogenesis encompass proteoglycans (PGs), HA, heparan sulfate (HS), versican, and so on. For example, two chondroitin/dermatan sulfate with small leucine-rich proteoglycans, biglycan and decorin, are the most characterized ECM-derived DAMP that triggers renal inflammation and fibrogenesis, which act as endogenous ligands of TLR2/4, the adaptor molecules and cluster of differentiation 14 (CD14) and trigger sterile inflammation [120,121]. Furthermore, active HS fragments generated by heparanase-1 serve as TLR4-interacting DAMPs [122]. Thus, it might be a promising approach to reduce ECM-derived DAMPs-driven inflammation to restore impaired kidney function.

4.1.2. *Main signaling targets in renal fibrosis*

TGF-β plays a vital role among the multiple signaling pathways being activated in renal fibrosis, such as ECM production, the proliferation of myofibroblasts and fibroblasts. TGF-β1 is initially secreted in a latent form consisting of a TGF-β1 dimer bound to a latency-associated peptide (laP) [123,124]. There are two mechanisms, mothers against decapentaplegic homologue (SMAD) 3-dependent and SMAD-independent in the dysregulated expression and activation of TGF-β1, which stimulate ECM production and finally lead to the development of glomerulosclerosis and tubulointerstitial fibrosis. Furthermore, TGF-β1 can directly suppress MMPs and induce TIMPs, resulting in a net accumulation of ECM [125]. At the same time, TGF-β1 can also induce the tubular epithelial-to-mesenchymal transition and myofibroblasts-generated interstitial matrix [126]. Thus, the complex regulation of TGF-β might provide specific anti-fibrotic targets, although direct inhibition of TGF-β might have undesired side effects.

On the other hand, BMP-7, a 35-kDa homodimeric protein, is a natural antagonist of TGF-β, which have reversed renal fibrosis in various models. Just like other members of the TGF-β superfamily, BMP-7 signals via heteromeric interactions of BMP receptors type I and type II activate its R-SMADs and SMAD1/5/8, further form complexes with SMAD4, which translocate into the nucleus to regulate transcription of their target genes [127,128]. SMAD6 is an inhibitory SMAD that is induced by BMPs and inhibits both TGF-β and BMP signaling [129]. Numerous researches indicate that BMP-7 antagonizes TGF-β-dependent fibrosis. Thus, BMP-7 signaling agonists such as THR-123 [130] may be an effective therapy for kidney disease.

CTGF is an important modulator of pro-fibrotic TGF-β and anti-fibrotic BMP-7 activities. CTGF is a direct downstream as an early response gene of TGF-β. When binds to TGF-β1 through its CR domain, it leads to myofibroblast activation, de novo expression of α-SMA and extracellular accumulation of fibronectin, which promote tissue fibrosis [131]. CTGF also disrupt the negative feedback loop of the TGF-β signaling pathway via suppression of inhibitory SMAD7 [132]. By contrast, CTGF interacts with BMP-7 to inhibit BMP-7 signaling and ultimately enhances TGF-β signaling [133,134]. Given that CTGF plays a significant role in the pathogenesis of kidney fibrosis, anti-CTGF therapy is a promising strategy to reverse renal fibrosis.

4.2. *Proximal tubule epithelial cell targeting delivery system*

Proximal tubular cells play an important role in the etiology of many renal diseases, such as the formation and development of interstitial inflammation and fibrosis [135]. It is an effective strategy to deliver drugs to the proximal tubules for the treatment of kidney disease. First, Yuan et al. found the low molecular weight chitosan (LMWC) as a potential carrier for the site-specific delivery of prednisolone to kidney. This is mainly because aminoglycoside, a well-known ligand of megalin receptor, shares a similar glucosamine unit level with LMWC [136]. Based on this inspiring results, Qiao et al. [137] designed kidney-specific nanocomplexes from catechol-derived LMWC (HCA-Chi), metal ions and active drug molecules (Fig. 8). The nanocomplexes showed special renal targeting capacity and obvious attenuation of fibrotic progression. Besides, Vimentin is an intermediate filament expressed mainly in cells of mesenchymal origin, which expresses in developing renal tubular epithelial cells and regenerating renal epithelial cells [138]. Kers et al. indicated that vimentin involved in renal fibrosis, which could work as surrogate markers for late interstitial fibrosis and tubular atrophy and renal function [139]. Targeting vimentin receptors can be a vital strategy for efficient and specific delivery of genes to kidney. Kim et al. [140] report that *N*-acetylglucosamine (GlcNAc) with PEI specifically interacted

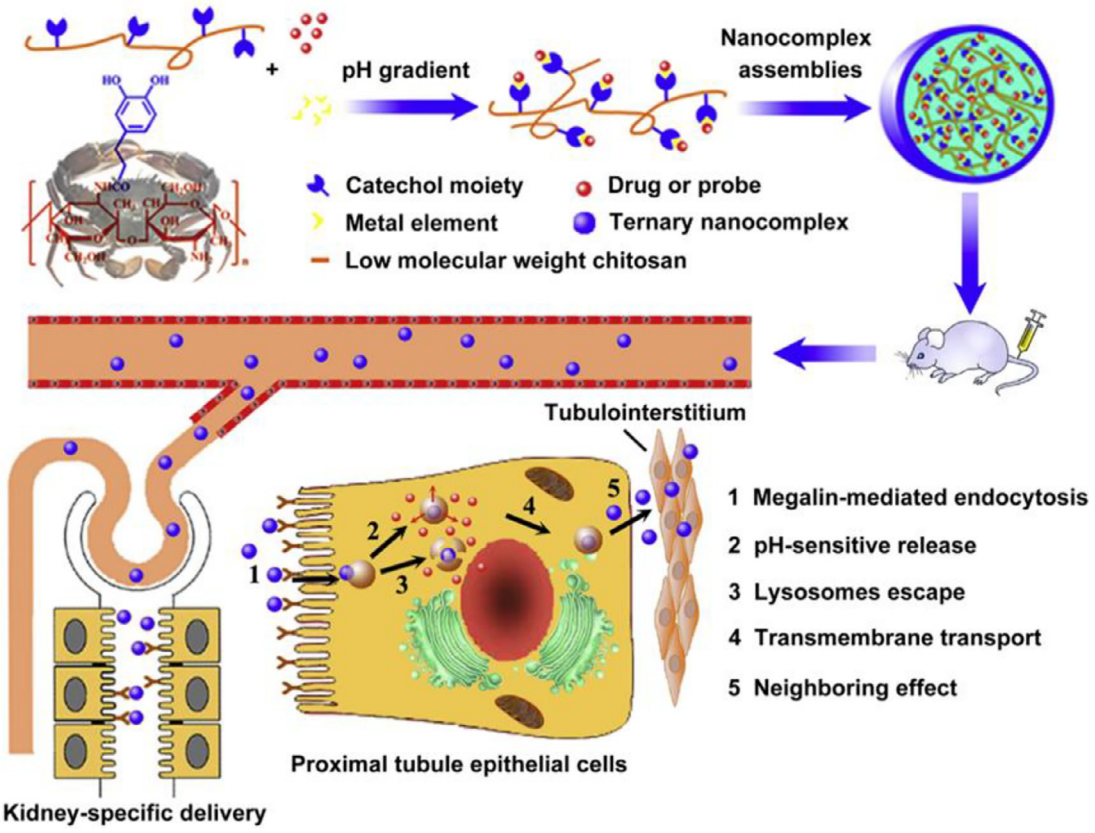

Fig. 8 – pH-response nanodevice based on coordination-driven assembly of HCA-Chi for sequential and kidney-specific drug delivery. Reprinted with permission from [137]. Copyright 2014 Elsevier.

with vimentin-expressing cells such as 293FT and HeLa cells to deliver genes. Conceivably, this gene delivery system could be used to target other vimentin-expressed cell such as renal tubular epithelial cells. Then, based on previous findings, Singh et al. [141] developed other polymeric gene delivery vectors to elucidate the efficiency of GlcNAc ligand for gene delivery. The results showed that high transfection efficiency of PADPG was attributed to the GlcNAc in the polymeric vector, which opens up a new opportunity to combine GlcNAc with another polymer for special vimentin targeting capacity to cure CDK. All of these results make it possible to deliver drugs to proximal tubule epithelial cells for curing CDK.

4.3. *Myofibroblast targeting delivery system*

Kidney fibroblast plays an important role in the fibrotic process, which maintains the homeostasis of interstitial matrix and adjacent tissue. When activated by cytokines, stress and other factors, it tends to myofibroblast phenotype and contributes to matrix deposition [142,143]. Consequently, targeted delivery of drugs to myofibroblasts is effective in suppressing the progress of interstitial renal failure. Poosti et al. [144] conjugated PEGylated IFN-g to PDGFRβ recognizing cyclic peptide C*SRNLIDC* (PPB) [PPB-PEG-IFN-g] to test anti-fibrotic properties *in vitro* and *in vivo* compared with free IFN-g. The results showed special renal myofibroblast targeting capacity and obvious attenuation of fibrotic progression, which could further be studied to reverse fibrosis.

5. Conclusions and perspectives

Fibrosis is a serious disease that threatens human health. The mechanisms underlying the progression of fibrosis are fairly complex (Fig. 9). With the study of molecular mechanisms on fibrosis in recent years, many anti-fibrotic drugs have been continuously discovered and showed strong anti-fibrotic activity *in vitro*. However, most of the therapeutic strategies are still in preclinical phase, and their clinical effects have not been confirmed. And most drugs have low solubility, no specificity and poor accumulation in the action sites, which greatly limits the anti-fibrotic effect *in vivo*. Therefore, it is necessary to develop a safe and effective delivery system for anti-fibrosis drugs. Although much progress in the targeted drug delivery system for fibrosis therapy has been made (Table 1), several challenges still exist, such as delivering single therapeutic agent, low targeting and permeability, and limited therapeutic effect. Given the current research status, the following outlook is proposed: (1) collagenase I can be decorated on nanoparticles to effectively permeate and deliver drugs to action site, which overcomes the difficulty in drug delivery caused by ECM transitional secretion in fibrotic tissue; (2) it is difficult to achieve the desired effect by a single

Table 1 – Summary of representative targeting groups used for drug targeting delivery therapy for liver, lung and renal fibrosis.

Type	Specific cells	Receptors	Targeting groups	Ref.
liver fibrosis	hepatocyte	ASGP-R	galactose	[55-58]
	HSC	RBPR, CRBP andCRABP	vitamin A	[63-65]
	HSC	collagen VI receptor	cyclic RGD	[66,67]
	HSC	M6P/IGFII	M6P	[68]
	HSC	CD44 receptor	chondroitin sulfate	[69]
pulmonary fibrosis	myofibroblast	chemokine receptor CXCR4	AMD3100	[92,93]
renal fibrosis	proximal tubule epithelial cell	glomerular filtration and megalin receptor	LMWC	[136, 137]
	myofibroblast	PDGFRβ	cyclic peptide C*SRNLIDC*	[144]

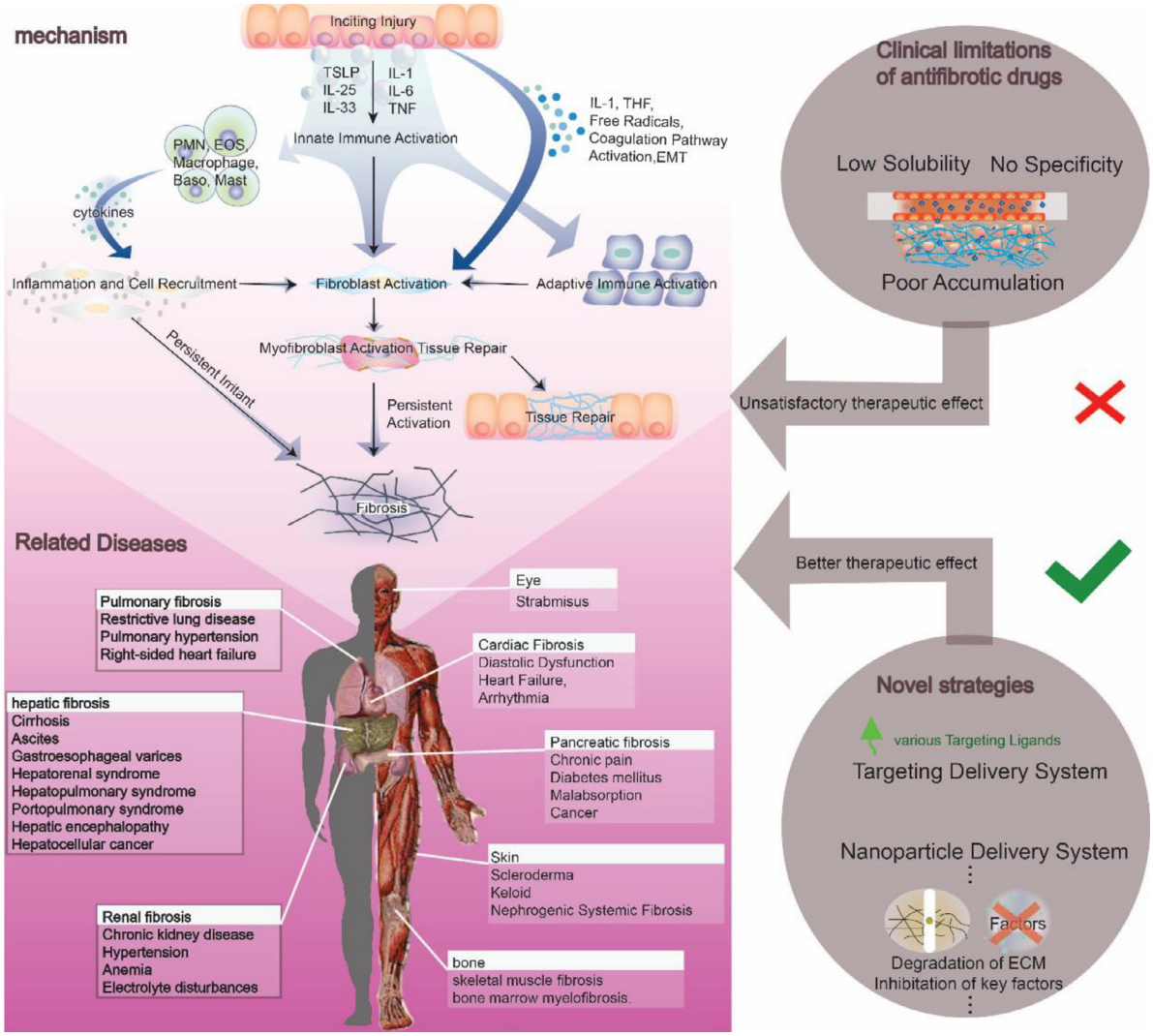

Fig. 9 – The mechanism and outcomes of fibrosis. PMN: polymorphonuclear leukocyte; EOS: eosinophil; Baso: basophil; Mast: mast cell.

target treatment owing to complex mechanisms of fibrosis. Co-delivery strategy can be used to inhibit collagen secretion through multiple pathways; and (3) it is possible to efficiently deliver anti-fibrotic drugs with the help of "cell combination drug delivery" technology based on the pathological features of fibrosis owing to low targeting efficiency of the traditional delivery system.

In addition, through the elaboration of the new pathological mechanisms of fibrosis, more anti-fibrosis drugs and delivery systems will be gradually developed.

And more effective treatment strategies can be adopted for the pathological features of fibrosis. Besides, further development of safe and efficient drug delivery systems will facilitate more precise and more convenient fibrosis therapy strategy compared to the conventional delivery system.

Conflicts of interest

The authors have declared no conflict of interest.

Acknowledgments

This work was financially supported by the National Science and Technology Major Project (2017YFA0205400) and the National Natural Science Foundation of China (81773667, 81573369) and NSFC Projects of International Cooperation and Exchanges (81811540416) and the "111" Project from the Ministry of Education of China and the State Administration of Foreign Experts Affairs of China (D17010).

REFERENCES

[1] Raghu G, Flaherty KR, Lederer DJ, Lynch DA, Colby TV, Myers JL, et al. Use of a molecular classifier to identify usual interstitial pneumonia in conventional transbronchial lung biopsy samples: a prospective validation study. Lancet Respir Med 2019;7(6):487–96.
[2] Borthwick LA, Wynn TA, Fisher AJ. Cytokine mediated tissue fibrosis. Biochim Biophys Acta 2013;1832(7):1049–60.
[3] Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 2012;18(7):1028–40.
[4] Chung W, Jo C, Chung WJ, Kim DJ. Liver cirrhosis and cancer: comparison of mortality. Hepatol Int 2018;12(3):269–76.
[5] Fang C, Jaffer OS, Yusuf GT, Konstantatou E, Quinlan DJ, Agarwal K, et al. Reducing the number of measurements in liver point shear-wave elastography: factors that influence the number and reliability of measurements in assessment of liver fibrosis in clinical practice. Radiology 2018;287(3):844–52.
[6] Schippers M, Beljaars L, Post E, Lotersztajn S, Reker-Smit C, Han B, et al. Upregulation of Epac-1 in hepatic stellate cells by Prostaglandin E2 in liver fibrosis is associated with reduced fibrogenesis. J Pharmacol Exp Ther 2017;363:126–35.
[7] Zhang F, Gu JX, Zou XP, Zhuge YZ. Protective effects of S-adenosylmethionine against CCl_4- and ethanol-induced experimental hepatic fibrosis. Mol Biol Mosk 2016;50:284–90.
[8] Baroni GS, D'Ambrosio L, Curto P, Casini A, Mancini R, Jezequel AM, et al. Interferon gamma decreases hepatic stellate cell activation and extracellular matrix deposition in rat liver fibrosis. Hepatology 1996;23:1189–99.
[9] Gonzalo T, Talman EG, van de Ven A, Temming K, Greupink R, Beljaars L, et al. Selective targeting of pentoxifylline to hepatic stellate cells using a novel platinum-based linker technology. J Control Release 2006;111:193–203.
[10] Toex U, Scheller I, Kern MA, Schirmacher P, Laue O, Jakob B, et al. Effect of the AT1-receptor antagonist candesartancilexetil on liver fibrosis in the rat bile duct occlusion model. Gastroenterology 2003;124:A764.
[11] Traber PG, Zomer E. Therapy of experimental NASH and fibrosis with galectin inhibitors. PLoS ONE 2013;8:e83481.
[12] Kim SG, Kim YM, Choi YH, Lee MG, Choi JY, Han JY, et al. Pharmacokinetics of oltipraz and its major metabolite (RM) in patients with liver fibrosis or cirrhosis: relationship with suppression of circulating TGF-beta1. Clin Pharmacol Ther 2010;88:360–8.
[13] Hsu YC, Chiu YT, Cheng CC, Wu CF, Lin YL, Huang YT. Antifibrotic effects of tetrandrine on hepatic stellate cells and rats with liver fibrosis. J Gastroenterol Hepatol 2007;22:99–111.
[14] Zhang JP, Zhang M, Zhou JP, Liu FT, Zhou B, Xie WF, et al. Antifibrotic effects of matrine on *in vitro* and *in vivo* models of liver fibrosis in rats. Acta Pharmacol Sin 2001;22:183–6.
[15] Ryerson CJ, Corte TJ, Collard HR, Richeldi L. A global registry for idiopathic pulmonary fibrosis: the time is now. Eur Respir J 2014;44(2):273–6.
[16] Karimi-Shah BA, Chowdhury BA. Forced vital capacity in idiopathic pulmonary fibrosis - FDA review of pirfenidone and nintedanib. N Engl J Med 2015;372(13):1189–91.
[17] Wei Q, Su J, Dong G, Zhang M, Huo Y, Dong Z. Glycolysis inhibitors suppress renal interstitial fibrosis via divergent effects on fibroblasts and tubular cells. Am J Physiol Renal Physiol 2019;316(6):F1162–72.
[18] Liu Y. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol 2011;7(12):684–96.
[19] Declèves AE, Sharma K. Novel targets of antifibrotic and anti-inflammatory treatment in CKD. Nat Rev Nephrol 2014;10(5):257–67.
[20] Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet 2013;382(9888):260–72.
[21] Murphy BS, Sundareshan V, Cory TJ, Jr DH, Anstead MI, Feola DJ. Azithromycin alters macrophage phenotype. J Antimicrob Chemother 2008;61:554–60.
[22] Li Z, Liu X, Wang B, Nie Y, Wen J, Wang Q, et al. Pirfenidone suppresses MAPK signalling pathway to reverse epithelial-mesenchymal transition and renal fibrosis. Nephrology 2017;22:589–97.
[23] Wang S, Wilkes MC, Leof EB, Hirschberg R. Imatinib mesylate blocks a non-Smad TGF-beta pathway and reduces renal fibrogenesis *in vivo*. FASEB J 2005;19:1–11.
[24] Shirazi M, Noorafshan A, Farrokhi A. Effects of pentoxifylline on renal structure after urethral obstruction in rat: a stereological study. Cent Eur J Urol 2011;64:30–3.
[25] Liu CF, Liu H, Fang Y, Jiang SH, Zhu JM, Ding XQ. Rapamycin reduces renal hypoxia, interstitial inflammation and fibrosis in a rat model of unilateral ureteral obstruction. Clin Invest Med 2014;37:E142.
[26] Zhou J, Chen X, Wei R. The influence of compound Biejia Ruangan precription on renal interstitial fibroblast proliferation and excreting extracellular matrix induced by TGF-β1. Chin J Integr Med 2014;5:381–4.
[27] Guo Z, Diao Y, Wang Z. Effect of Lianhuang Turbid clearance granules on kidney in spontaneously hypertensive rats. Chin J Integr Med 2008;2:130–3.
[28] Han CY, Koo JH, Kim SH, Gardenghi S, Rivella S, Strnad P, et al. Hepcidin inhibits Smad3 phosphorylation in hepatic stellate cells by impeding ferroportin-mediated regulation of Akt. Nat Commun 2016;7:13817.
[29] Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: concept to treatment. J Hepatol 2015;62:S15–24.
[30] Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev 2017;121:27–42.
[31] Tacke F, Trautwein C. Mechanisms of liver fibrosis resolution. J Hepatol 2015;63:1038–9.

[32] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134:1655–69.
[33] Böttcher K, Pinzani M. Pathophysiology of liver fibrosis and the methodological barriers to the development of anti-fibrogenic agents. Adv Drug Delivery Rev 2017;121:3–8.
[34] Novo E, Cannito S, Paternostro C, Bocca C, Miglietta A, Parola M. Cellular and molecular mechanisms in liver fibrogenesis. Arch Biochem Biophys 2014;548:20–37.
[35] Prakash J, Pinzani M. Fibroblasts and extracellular matrix: targeting and therapeutic tools in fibrosis and cancer. Adv Drug Deliv Rev 2017;121:1–2.
[36] Wells RG. Mechanisms of liver fibrosis: new insights into an old problem. Drug Discov Today 2007;3(4):489–95.
[37] Karsdal MA, Nielsen SH, Leeming DJ, Langholm LL, Nielsen MJ, Manon-Jensen T, et al. The good and the bad collagens of fibrosis-their role in signaling and organ function. Adv Drug Deliv Rev 2017;121:43–56.
[38] Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix Biol 2017;68-69:452–62.
[39] Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev 2017;121:27–42.
[40] Tacke F, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. J Hepatol 2014;60(5):1090–6.
[41] Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: concept to treatment. J Hepatol 2015;62(1 Suppl):S15–24.
[42] Li L, Wang H, Zhan YO, Xu K, Rachel Ee PL, Zheng S, et al. Polymer- and lipid-based nanoparticle therapeutics for the treatment of liver diseases. Nano Today 2010;5(4):296–312.
[43] Poelstra K, Prakash J, Beljaars L. Drug targeting to the diseased liver. J Control Release 2012;161(2):188–97.
[44] Lin TT, Gao DY, Liu YC, Sun YC, Wan D, Liu JY, et al. Development and characterization of sorafenib-loaded PLGA nanoparticles for the systemic treatment of liver fibrosis. J Control Release 2016;221:62–70.
[45] Kumar V, Mondal G, Dutta R, Mahato RI. Co-delivery of small molecule hedgehog inhibitor and miRNA for treating liver fibrosis. Biomaterials 2016;76:144–56.
[46] Ahn KY, Ko HK, Lee BR, Lee EJ, Lee JH, Byun Y, et al. Engineered protein nanoparticles for *in vivo* tumor detection. Biomaterials 2014;35(24):6422–9.
[47] Elzoghby AO, Samy WM, Elgindy NA. Albumin-based nanoparticles as potential controlled release drug delivery systems. J Control Release 2012;157:168–82.
[48] Calvente C, Diken M, Sehgal A, Kim YO, Schuppan D. Specific *in vivo*, delivery of procollagen α1(I) siRNA in lipid-like carriers inhibits the progression of biliary liver fibrosis. J Hepatol 2013;58(Suppl1):S236.
[49] Zhu J, Wang Q, Li H, Zhang H, Zhu Y, Omari-Siaw E, et al. Galangin-loaded, liver targeting liposomes: optimization and hepatoprotective efficacy. J Drug Deliv Sci Technol 2018;46:339–47.
[50] Kong WH, Park K, Lee MY, Lee H, Sung DK, Hahn SK. Cationic solid lipid nanoparticles derived from apolipoprotein-free LDLs for target specific systemic treatment of liver fibrosis. Biomaterials 2013;34(2):542–51.
[51] Rao MP, Manjunath K, Bhagawati ST, Thippeswamy BS. Bixin loaded solid lipid nanoparticles for enhanced hepatoprotection–preparation, characterisation and *in vivo* evaluation. Int J Pharm 2014;473(1–2):485–92.
[52] Bansal R, Nagórniewicz B, Prakash J. Clinical advancements in the targeted therapies against liver fibrosis. Mediat Inflamm 2016;2016:7629724.
[53] Poelstra K, Schuppan D. Targeted therapy of liver fibrosis/cirrhosis and its complications. J Hepatol 2011;55(3):726–8.
[54] Bartneck M, Warzecha KT, Tacke F. Therapeutic targeting of liver inflammation and fibrosis by nanomedicine. Hepatobiliary Surg Nutr 2014;3(6):364–76.
[55] He H. Bolstering cholesteryl ester hydrolysis in liver: a hepatocyte-targeting gene delivery strategy for potential alleviation of atherosclerosis. Biomaterials 2017;130:1–13.
[56] Oh HR, Jo HY, Park JS, Kim DE, Cho JY, Kim PH, et al. Galactosylated liposomes for targeted co-delivery of doxorubicin/vimentin siRNA to hepatocellular carcinoma. Nanomaterials 2016;6(8):141–56.
[57] Mandal AK, Das S, Basu MK, Chakrabarti RN, Das N. Hepatoprotective activity of liposomal flavonoid against arsenite-induced liver fibrosis. J Pharmacol Exp Ther 2007;320(3):994–1001.
[58] Zhang D, Guo Z, Zhang P, Li Y, Su X, You L, et al. Simplified quantification method for *in vivo* SPECT/CT imaging of asialoglycoprotein receptor with (99m)Tc-p(VLA-co-VNI) to assess and stage hepatic fibrosis in mice. Sci Rep 2016;6:25377.
[59] Li X. Placental growth factor silencing ameliorates liver fibrosis and angiogenesis and inhibits activation of hepatic stellate cells in a murine model of chronic liver disease. J Cell Mol Med 2017;21(10):2370–85.
[60] Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: etiology, pathological hallmarks and therapeutic targets. World J Gastroenterol 2016;22(48):10512–22.
[61] Perepelyuk M, Terajima M, Wang AY, Georges PC, Janmey PA, Yamauchi M, et al. Hepatic stellate cells and portal fibroblasts are the major cellular sources of collagens and lysyl oxidases in normal liver and early after injury. Am J Physiol 2013;304(6):G605–14.
[62] Weiskirchen R, Tacke F. Cellular and molecular functions of hepatic stellate cells in inflammatory responses and liver immunology. Hepatobiliary Surg Nutr 2014;3(6):344–63.
[63] Lee YS, Jeong WI. Retinoic acids and hepatic stellate cells in liver disease. J Gastroenterol Hepatol 2012;27(s2):75–9.
[64] Zhang Z, Wang C, Zha Y, Hu W, Gao Z, Zang Y, et al. Corona-directed nucleic acid delivery into hepatic stellate cells for liver fibrosis therapy. ACS Nano 2015;9(3):2405–19.
[65] Qiao JB, Fan QQ, Xing L, Cui PF, He YJ, Zhu JC, et al. Vitamin A-decorated biocompatible micelles for chemogene therapy of liver fibrosis. J Control Release 2018;283:113–25.
[66] Qiao JB, Fan QQ, Zhang CL, Lee J, Byun J, Xing L, et al. Hyperbranched lipoid-based lipid nanoparticles for bidirectional regulation of collagen accumulation in liver fibrosis. J Control Release 2020;321:629–40.
[67] Fan QQ, Zhang CL, Qiao JB, Cui PF, Xing L, Oh YK, et al. Extracellular matrix-penetrating nanodrill micelles for liver fibrosis therapy. Biomaterials 2020;230:119616.
[68] Li F, Sun JY, Wang JY, Du SL, Lu WY, Liu M, et al. Effect of hepatocyte growth factor encapsulated in targeted liposomes on liver cirrhosis. J Control Release 2008;131(1):77–82.
[69] Yang J, Hou Y, Ji G, Song Z, Liu Y, Dai G, et al. Targeted delivery of the RGD-labeled biodegradable polymersomes loaded with the hydrophilic drug oxymatrine on cultured hepatic stellate cells and liver fibrosis in rats. Eur J Pharm Sci 2014;52:180–90.
[70] Beljaars L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis GM, et al. Albumin modified with mannose 6-phosphate: a potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Hepatology 2010;29(5):1486–93.
[71] Luo J, Zhang P, Zhao T, Jia M, Yin P, Li W, et al. Golgi Apparatus-targeted chondroitin-modified nanomicelles

suppress hepatic stellate cell activation for the management of liver fibrosis. ACS Nano 2019;13(4):3910–23.
[72] Friedman SL. Mac the knife? Macrophages- the double-edged sword of hepatic fibrosis. J Clin Invest 2005;115(1):29–32.
[73] Wang J., Kang Y.X., Pan W., Lei W., Feng B., Wang X.J. Enhancement of anti-inflammatory activity of curcumin using phosphatidylserine-containing nanoparticles in cultured macrophages. Int J Mol Sci 201617(5):969–87.
[74] Wang J, Pan W, Wang Y, Lei W, Feng B, Du C, et al. Enhanced efficacy of curcumin with phosphatidylserine-decorated nanoparticles in the treatment of hepatic fibrosis. Drug Deliv 2018;25(1):1–11.
[75] Zhang M, Gao Y, Caja K, Zhao B, Kim JA. Non-viral nanoparticle delivers small interfering RNA to macrophages *in vitro* and *in vivo*. PLoS ONE 2015;10(3):e0118472.
[76] Navaratnam V, Fogarty AW, Glendening R, McKeever T, Hubbard RB. The increasing secondary care burden of idiopathic pulmonary fibrosis: hospital admission trends in england from 1998 to 2010. Chest 2013;143(4):1078–84.
[77] Hutchinson JP, Mckeever TM, Fogarty AW, Navaratnam V, Hubbard RB. Increasing global mortality from idiopathic pulmonary fibrosis in the twenty-first century. Ann Am Thorac Soc 2014;11(8):1176–85.
[78] Pardo A, Cabrera S, Maldonado M, Selman M. Role of matrix metalloproteinases in the pathogenesis of idiopathic pulmonary fibrosis. Respir Res 2016;17:23.
[79] Hinz B. Formation and function of the myofibroblast during tissue repair. J Invest Dermatol 2007;127:526–37.
[80] Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. Am J Physiol Lung Cell Mol Physiol 2010;298(6):L715–31.
[81] Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2010;214(2):199–210.
[82] Kolb M, Bonella F, Wollin L. Therapeutic targets in idiopathic pulmonary fibrosis. Respir Med 2017;131:49–57.
[83] Akhmetshina A, Palumbo K, Dees C, Bergmann C, Venalis P, Zerr P, et al. Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis. Nat Commun 2012;3:735.
[84] Carvalho CDS, Daum N, Lehr CM. Carrier interactions with the biological barriers of the lung: advanced *in vitro* models and challenges for pulmonary drug delivery. Adv Drug Delivery Rev 2014;75:129–40.
[85] Haque S, Whittaker MR, Mcintosh MP, Pouton CW, Kaminskas LM. Disposition and safety of inhaled biodegradable nanomedicines: opportunities and challenges. Nanomedicine 2016;12(6):1703–24.
[86] García-Díaz M, Birch D, Wan F, Nielsen HM. The role of mucus as an invisible cloak to transepithelial drug delivery by nanoparticles. Adv Drug Delivery Rev 2017;124:107–24.
[87] Moens E, Veldhoen M. Epithelial barrier biology: good fences make good neighbours. Immunology 2012;135:1–8.
[88] Zanin M, Baviskar P, Webster R, Webby R. The interaction between respiratory pathogens and mucus. Cell Host Microbe 2016;19(2):159–68.
[89] Cone RA. Barrier properties of mucus. Adv Drug Delivery Rev 2009;61:75–85.
[90] Boegh M, Nielsen HM. Mucus as a barrier to drug delivery-understanding and mimicking the barrier properties. Basic Clin Pharmacol Toxicol 2015;116(3):179–86.
[91] Ivanova V, Garbuzenko OB, Reuhl KR, Reimer DC, Pozharov VP, Minko T. Inhalation treatment of pulmonary fibrosis by liposomal prostaglandin E2. Eur J Pharm Biopharm 2013;84(2):335–44.
[92] Wang Y, Hazeldine ST, Li J, Oupický D. Development of functional poly(amido amine) CXCR4 antagonists with the ability to mobilize leukocytes and deliver nucleic acids. Adv Healthc Mater 2015;4(5):729–38.
[93] Ding L, Zhu C, Yu F, Wu P, Chen G, Ullah A, et al. Pulmonary delivery of polyplexes for combined PAI-1 gene silencing and CXCR4 inhibition to treat lung fibrosis. Nanomedicine 2018;14(6):1765–76.
[94] Klinkhammer BM, Goldschmeding R, Floege J, Boor P. Treatment of renal fibrosis-turning challenges into opportunities. Adv Chronic Kidney Dis 2017;24(2):117–29.
[95] Barreira AL, Takiya CM, Castiglione RC, Maron-Gutierrez T, Barbosa CML, Ornellas DS, et al. Bone marrow mononuclear cells attenuate interstitial fibrosis and stimulate the repair of tubular epithelial cells after unilateral ureteral obstruction. Cell Physiol Biochem 2009;24:585–94.
[96] Efstratiadis G, Divani M, Katsioulis E, Vergoulas G. Renal fibrosis. Hippokratia 2009;13(4):224–49.
[97] Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, et al. Prevalence of chronic kidney disease in the United States. JAMA 2007;298(17):2038–47.
[98] Zhang L, Zhang P, Wang F, Zuo L, Zhou Y, Shi Y, et al. Prevalence and factors associated with CKD: a population study from Beijing. Am J Kidney Dis 2008;51(3):353–7.
[99] Du Y, Liu P, Chen Z, He Y, Zhang B, Dai G, et al. PTEN improve renal fibrosis *in vitro* and *in vivo* through inhibiting FAK/AKT signaling pathway. J Cell Biochem 2019;120(10):17887–97.
[100] Hewitson TD. Renal tubulointerstitial fibrosis: common but never simple. Am J Physiol Renal Physiol 2009;296:F1239–44.
[101] Karsdal MA, Manon-Jensen T, Genovese F, Kristensen JH, Nielsen MJ, Sand JMB, et al. Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2015;308(10):G807–30.
[102] Anders HJ, Schaefer L. Beyond tissue injury-damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis. J Am Soc Nephrol 2014;25(7):1387–400.
[103] Liu Y. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol 2011;7:684–96.
[104] Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 2007;117:524–9.
[105] Hewitson TD. Fibrosis in the kidney: is a problem shared a problem halved? Fibrogenesis Tissue Repair 2012;5(S1):S14.
[106] Jiao J, Friedman SL, Aloman C. Hepatic fibrosis. Curr Opin Gastroenterol 2009;25(3):223–9.
[107] Meran S, Steadman R. Fibroblasts and myofibroblasts in renal fibrosis. Int J Exp Pathol 2011;92(3):158–67.
[108] Hinz B. It has to be the αv: myofibroblast integrins activate latent TGF-β1. Nat Med 2013;19(12):1567–8.
[109] Lebleu VS, Taduri G, O'Connell J, Teng Y, Cooke VG, Woda C, et al. Origin and function of myofibroblasts in kidney fibrosis. Nat Med 2013;19:1047–53.
[110] Boor P, Floege J. Chronic kidney disease growth factors in renal fibrosis. Clin Exp Pharmacol Physiol 2011;38(7):391–400.
[111] To WS, Midwood KS. Plasma and cellular fibronectin: distinct and independent functions during tissue repair. Fibrogenesis Tissue Repair 2011;4:21.
[112] Yang J, Dai C, Liu Y. Hepatocyte growth factor suppresses renal interstitial myofibroblast activation and intercepts Smad signal transduction. Am J Pathol 2003;163(2):621–32.
[113] Li RX, Yiu WH, Tang SC. Role of bone morphogenetic protein-7 in renal fibrosis. Front Physiol 2015;6(1):114.
[114] Trionfini P, Benigni A, Remuzzi G. MicroRNAs in kidney physiology and disease. Nat Rev Nephrol 2015;11(1):23–33.
[115] Chung AC, Lan HY. MicroRNAs in renal fibrosis. Front Physiol 2015;6:50.

[116] Schaefer L. Complexity of danger: the diverse nature of damage-associated molecular Patterns. J Biol Chem 2014;289(51):35237–45.
[117] Wight TN, Potterperigo S. The extracellular matrix: an active or passive player in fibrosis? Am J Physiol Gastrointest Liver Physiol 2011;301(6):G950–5.
[118] Frey H, Schroeder N, Manon-Jensen T, Iozzo RV, Schaefer L. Biological interplay between proteoglycans and their innate immune receptors in inflammation. FEBS J 2013;280(10):2165–79.
[119] Ebid R, Lichtnekert J, Anders HJ. Hyaluronan is not a ligand but a regulator of Toll-like receptor signaling in mesangial cells: role of extracellular matrix in innate immunity. ISRN Nephrol 2014;2014:714081.
[120] Hsieh LT, Nastase MV, Roedig H, Zeng-Brouwers J, Poluzzi C, Schwalm S, et al. Biglycan- and sphingosine kinase-1 signaling crosstalk regulates the synthesis of macrophage chemoattractants. Int J Mol Sci 2017;18(3):595.
[121] Merline R, Moreth K, Beckmann J, Nastase MV, Zeng-Brouwers J, Tralhão JG, et al. Signaling by the matrix proteoglycan decorin controls inflammation and cancer through PDCD4 and MicroRNA-21. Sci Signal 2011;4(199):ra75.
[122] Goodall KJ, Poon IK, Phipps S, Hulett MD. Soluble heparan sulfate fragments generated by heparanase trigger the release of pro-inflammatory cytokines through TLR-4. PLoS ONE 2014;9:e109596.
[123] Huang XR, Chung AC, Wang XJ, Lai NK, Lan HY. Mice overexpressing latent TGF-beta1 are protected against renal fibrosis in obstructive kidney disease. Am J Physiol Renal Physiol 2008;295(1):F118–27.
[124] Choi ME, Ding Y, Kim SI. TGF-β signaling via TAK1 pathway: role in kidney fibrosis. Semin Nephrol 2012;32(3):244–52.
[125] Lee SY, Kim SI, Choi ME. Therapeutic targets for treating fibrotic kidney diseases. Transl Res 2015;165(4):512–30.
[126] Kriz W, Kaissling B, Le HM. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? J Clin Invest 2011;121(2):468–74.
[127] Tampe D, Zeisberg M. Potential approaches to reverse or repair renal fibrosis. Nat Rev Nephrol 2014;10:226–37.
[128] Zeisberg M. Bone morphogenic protein-7 and the kidney: current concepts and open questions. Nephrol Dial Transplant 2006;21(3):568–73.
[129] Wang S, Hirschberg R. Bone morphogenetic protein-7 signals opposing transforming growth factor in mesangial cells. J Biol Chem 2004;279:23200–6.
[130] Motazed R, Colvillenash P, Kwan JT, Dockrell ME. BMP-7 and proximal tubule epithelial cells: activation of multiple signaling pathways reveals a novel anti-fibrotic mechanism. Pharm Res 2008;25(10):2440–6.
[131] Lipson KE, Carol W, Teng Y, Suzanne S. CTGF is a central mediator of tissue remodeling and fibrosis and its inhibition can reverse the process of fibrosis. Fibrogenesis Tissue Repair 2012;5(S1):S24.
[132] Wahab NA, Weston BS, Mason RM. Connective tissue growth factor CCN2 interacts with and activates the tyrosine kinase receptor TrkA. J Am Soc Nephrol 2005;16(2):340–51.
[133] Nguyen TQ, Roestenberg P, van Nieuwenhoven FA, Bovenschen N, Li Z, Xu L, et al. CTGF inhibits BMP-7 signaling in diabetic nephropathy. J Am Soc Nephrol 2008;19(11):2098–107.
[134] Ito Y, Aten J, Bende RJ, Oemar BS, Rabelink TJ, Weening JJ, et al. Expression of connective tissue growth factor in human renal fibrosis. Kidney Int 1998;53(4):853–61.
[135] Lin Y, Li Y, Wang X, Gong T, Zhang L, Sun X. Targeted drug delivery to renal proximal tubule epithelial cells mediated by 2-glucosamine. J Control Release 2013;167(2):148–56.
[136] Yuan ZX, Zhang ZR, Zhu D, Sun X, Gong T, Liu J, et al. Specific renal uptake of randomly 50% N-acetylated low molecular weight chitosan. Mol Pharm 2009;6:305–14.
[137] Qiao H, Sun M, Su Z, Xie Y, Chen M, Zong L, et al. Kidney-specific drug delivery system for renal fibrosis based on coordination-driven assembly of catechol-derived chitosan. Biomaterials 2014;35(25):7157–71.
[138] Holthöfer H, Miettinen A, Lehto VP, Lehtonen E, Virtanen I. Expression of vimentin and cytokeratin types of intermediate filament proteins in developing and adult human kidneys. Lab Invest 1984;50(5):552–9.
[139] Kers J, Xu-Dubois YC, Rondeau E, Claessen N, Idu MM, et al. Intragraft tubular vimentin and CD44 expression correlate with long-term renal allograft function and interstitial fibrosis and tubular atrophy. Transplantation 2010;90:502–9.
[140] Kim SJ, Ise H, Goto M, Komura K, Cho CS, Akaike T. Gene delivery system based on highly specific recognition of surface-vimentin with N-acetylglucosamine immobilized polyethylenimine. Biomaterials 2011;32:3471–80.
[141] Singh B, Maharjan S, Kim YK, Jiang T, Islam MA, Kang SK, et al. Targeted gene delivery via N-acetylglucosamine receptor mediated endocytosis. J Nanosci Nanotechnol 2014;14(11):8356–64.
[142] Boor P, Ostendorf T, Floege J. Renal fibrosis: novel insights into mechanisms and therapeutic targets. Nat Rev Nephrol 2010;6:643–56.
[143] Hinz B. The myofibroblast: paradigm for a mechanically active cell. J Biomech 2010;43(1):146–55.
[144] Poosti F, Bansal R, Yazdani S, Prakash J, Post E, Klok P, et al. Selective delivery of IFN-γ to renal interstitial myofibroblasts: a novel strategy for the treatment of renal fibrosis. FASEB J 2015;29(3):1029–42.