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Review

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Review

Epithelial–mesenchymal transition in organ fibrosis development: current understanding and treatment strategies

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Abstract

Organ fibrosis is a process in which cellular homeostasis is disrupted and extracellular matrix is excessively deposited. Fibrosis can lead to vital organ failure and there are no effective treatments yet. Although epithelial–mesenchymal transition (EMT) may be one of the key cellular mechanisms, the underlying mechanisms of fibrosis remain largely unknown. EMT is a cell phenotypic process in which epithelial cells lose their cell-to-cell adhesion and polarization, after which they acquire mesenchymal features such as infiltration and migration ability. Upon injurious stimulation in different organs, EMT can be triggered by multiple signaling pathways and is also regulated by epigenetic mechanisms. This narrative review summarizes the current understanding of the underlying mechanisms of EMT in fibrogenesis and discusses potential strategies for attenuating EMT to prevent and/or inhibit fibrosis. Despite better understanding the role of EMT in fibrosis development, targeting EMT and beyond in developing therapeutics to tackle fibrosis is challenging but likely feasible.

Key words: EMT, Fibrosis, TGF-β, Smad, HDAC inhibitors, TGF-β antibody, Organ fibrosis, Senescence, Extracellular matrix

Highlights

- Current understanding of the key roles of EMT in triggering fibrosis development in different organs or tissues is updated.
- Cellular signaling pathways that are activated during EMT are extensively discussed.
- Drugs or chemicals that can partially inhibit and reverse EMT, providing potential for preventing and inhibiting fibrosis, are reviewed.

Background

During wound healing, the deposition of extracellular matrix (ECM) produced by myofibroblasts rebuilds the parenchymal tissue architecture. Once ECM production exceeds its degradation, tissue architecture is destructed and subsequently the process of fibrosis is initiated. Fibrosis can occur in vital organs or local tissues, e.g. the kidneys (chronic kidney disease), lungs (idiopathic pulmonary fibrosis (IPF), cystic fibrosis), heart (myocardial fibrosis, atherosclerosis), digestive system (liver cirrhosis, Crohn's disease), lens (cataract), peritoneum (peritoneal fibrosis) and skin (keloid, scleroderma), leading to organ dysfunction or failure (Figure 1). Developing effective strategies to prevent or even slow down fibrotic pathological processes is urgently needed. To date, 6000 new cases of IPF are diagnosed annually [1], 1.2 million people die worldwide from chronic kidney disease [2] and 6.8 million new cases of irritable bowel disease are reported worldwide [3]. In addition, 300 million people suffer from asthma, which was estimated in 2017. It is predicted that there will be over 100 million people by 2025 [4] who have a high risk of lung fibrosis. In the United States, childhood pulmonary fibrosis costs the health service a total of $1.3 billion per year [5]. Due to the high financial burden of fibrosis and the ever-increasing number of patients, advancements in developing strategies and therapies to prevent fibrosis will bring vast benefits to our society.

Fibrosis is characterized by loss of cellular homeostasis and excessive ECM deposition [6]. Myofibroblasts are activated in response to tissue injury and subsequently can synthesize ECM during wound healing and can be differentiated from multiple cell types, such as resident fibroblasts, pericytes, bone marrow, adipocytes, endothelial cells and epithelial cells [7,8]. Epithelial–mesenchymal transition (EMT) is a process in which epithelial cells lose their apical–basal polarity and adhesion with downregulated epithelial markers such as E-cadherin, occludin, claudin-1, and acquire mesenchymal features with upregulated mesenchymal markers such as α-smooth muscle actin (α-SMA), vimentin, fibronectin and fibroblast-specific protein 1 (FSP-1) [9,10] (Figure 2). A recent study demonstrated that during EMT, epithelial cells do not cross the glomerular basement membrane to contribute to myofibroblasts, but just acquire mesenchymal features to release signals with upregulated mesenchymal markers [11]. Therefore, a new concept of partial EMT was proposed (Figure 2). EMT is closely related to fibrogenesis in distinct organs, hence targeting EMT can arguably be considered to be a novel anti-fibrotic therapeutic strategy. This review summarizes current understanding of mechanisms underlying EMT and the possible treatments or strategies under development to slow down organ fibrosis through targeting EMT mechanisms.

Review

Cellular states triggering EMT

Inflammation, hypoxia and senescence are three cellular conditions closely associated with EMT. They interact and converge at transforming growth factor-β (TGF-β) (Figure 3).

Inflammation

Inflammation is widely acknowledged to be closely related to EMT. Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) and IL-1β activate inflammatory cell infiltration in various organs [12]. In turn, these inflammatory cells (macrophages, neutrophils, lymphocytes, etc.) also release pro-inflammatory cytokines [12,13]. Persistent inflammation exacerbates this inflammatory cascade, which stimulates EMT and causes further excessive ECM accumulation. In addition, TNF-α, IL-6 and IL-1β are inducers of the nuclear factor κB (NF-κB) pathway, the activation of which has been recognized to cause inflammation [14]. The inhibition of the toll-like receptor 4 (TLR4)/NF-κB pathway attenuated renal fibrosis by suppressing inflammation and EMT [15]. Furthermore, it has been reported that inflammation mediated by the NF-κB pathway caused cell cycle arrest and subsequently led to renal EMT [16], indicating that the NF-κB signaling pathway plays a significant role during EMT by mediating inflammation. Pro-inflammatory cytokine IL-17 produced by T helper 17 cells (Th17) activates immune cells to generate TGF-β, a well-known EMT inducer, and further causes pulmonary EMT [17]. However, whether this mechanism occurs in other organs remains to be verified. Collectively, inhibition of the inflammatory response can be considered as an anti-EMT treatment.

Hypoxia

Hypoxia is a critical condition which can trigger EMT through hypoxia inducible factor-α (HIF-α) accumulation [18]. Under hypoxic conditions, the inactivation of prolyl hydroxylases (PHD) caused the accumulation and activation of HIF-α, which further induces EMT-related gene expression such as *Snail1*, *Twist1* and *Bmi1* [19]. Upon stimulation of hypoxia, HIF-α activated the TGF-β/Smad and phosphoinositide 3-kinase (PI3K)/Akt pathways to cause renal and pulmonary EMT, respectively [20]. Moreover, in the kidneys, HIF-α induced the expression of *Bmi1*, which is an EMT-related gene. The expression of *Bmi1* activated *Twist1* expression both directly and indirectly, which subsequently stabilized the E-cadherin repressor *Snail1* [18]. This offers a latent mechanism for hypoxia-induced EMT. In addition, the administration of hyperbaric oxygen therapy (HBOT) was shown to ameliorate pulmonary EMT, indicating that relieving hypoxia was a potential treatment for EMT [21].

Senescence

Senescence is a state in which cells undergo cell-cycle arrest and stop dividing [22]. Nevertheless, senescent cells remain metabolically and secretly active, denoted the senescence associated secretory phenotype (SASP) [22]. Cytokines produced by SASP phenotype cells promoted leukocyte infiltration to cause persistent pro-inflammatory cytokines secretion [22]. The p53 protein triggered senescence by upregulating reactive oxygen species (ROS) [23]. Indoxyl sulfate activated

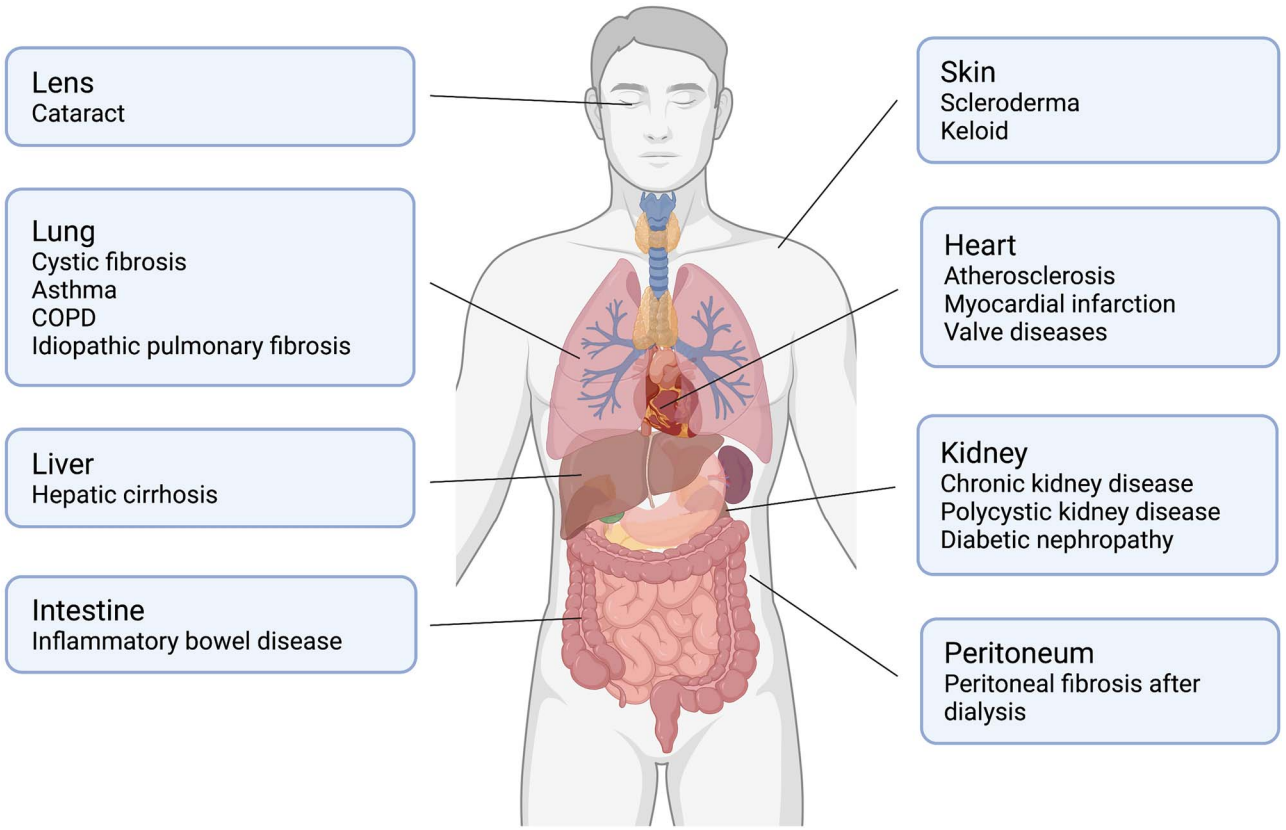

Figure 1. Fibrotic diseases related to multiple organs. Fibrosis is a pathological process that is associated with a variety of diseases. Fibrosis is closely associated with organ dysfunction or failure. *COPD* chronic obstructive pulmoriary disease

the phosphorylation of p53 in HK-2 cells and renal failure in mice, which further caused cell senescence [24]. In the kidneys, the inhibition of p53 attenuated the ROS level and led to a decrease in the mesenchymal marker α-SMA [24]. p53 knockdown showed a significant reduction of ROS in olaquindox-induced intestinal cells and liver tissue damage [25]. Thus, it was suggested that injury stimuli led to EMT by triggering the p53/ROS axis to promote cell senescence. ROS augmented TGF-β1 expression during lung fibrosis, and in turn, TGF-β1 stimulated NAPDH oxidase (NOX) generation to synthesize ROS [26]. This positive feedback likely contributed to EMT because the increased senescent markers (including p53, p21 and p16) and NOX and mesenchymal markers were found in multiple-organ fibrosis [22,27]. In addition, the activation of peroxisome proliferator-activated receptor alpha (PPAR-α) inhibited ROS levels and further reduced hepatocyte EMT, accompanied by an increase in glutathione (GSH) [28]. Collectively, senescence caused by p53/ROS may be responsible for EMT in distinct organs.

Signaling pathways promoting EMT induction

Several cellular signaling pathways are involved in the EMT process including TGF-β1, Wnt and Notch pathways.

TGF-β1 TGF-β1 activates TGF-β signaling by binding to TGF-β receptors I and II (TGFβRI and TGFβRII). TGF-β1 is an inducer of cell migration and de-differentiation, and a regulator of ECM components such as fibronectin, elastin and collagen [29,30]. Generally, the TGF-β1 signaling pathway can be categorized into Smad-dependent and Smad-independent pathways.

Smad-dependent pathway TGF-β1/Smad is the most well-studied pathway that induces EMT. The formation of TGFβRI and TGFβRII complexes is activated by TGF-β1, which subsequently leads to Smad2 and/or Smad3 phosphorylation. Phosphorylated Smad2 and/or Smad3 form a complex that heteroligomerizes with Smad4 [30], after which the heterotrimer complex is translocated into the nucleus and then regulates EMT transcription factors such as *Snail1*, *Twist1* and *Slug* [31].

Smad3 phosphorylation was reported to be critical for EMT induction in a variety of organs [32–34], although this is still debatable. For example, a previous study showed that Smad2 reduced ECM deposition by inducing *SnoN* (a negative regulator of TGF-β1) expression [35]. In contrast, another group reported that knockout of Smad2 restored EMT [36]. This implies that Smad2 has both pro- and anti-fibrotic functions.

Several studies revealed that Smad4 acted as an EMT facilitator. In kidney, small interfering RNA (siRNA) suppression of Smad4 blocked TGF-β1-induced EMT [37]. The deacetylation of Smad4 inhibited aristolochic acid-induced EMT by

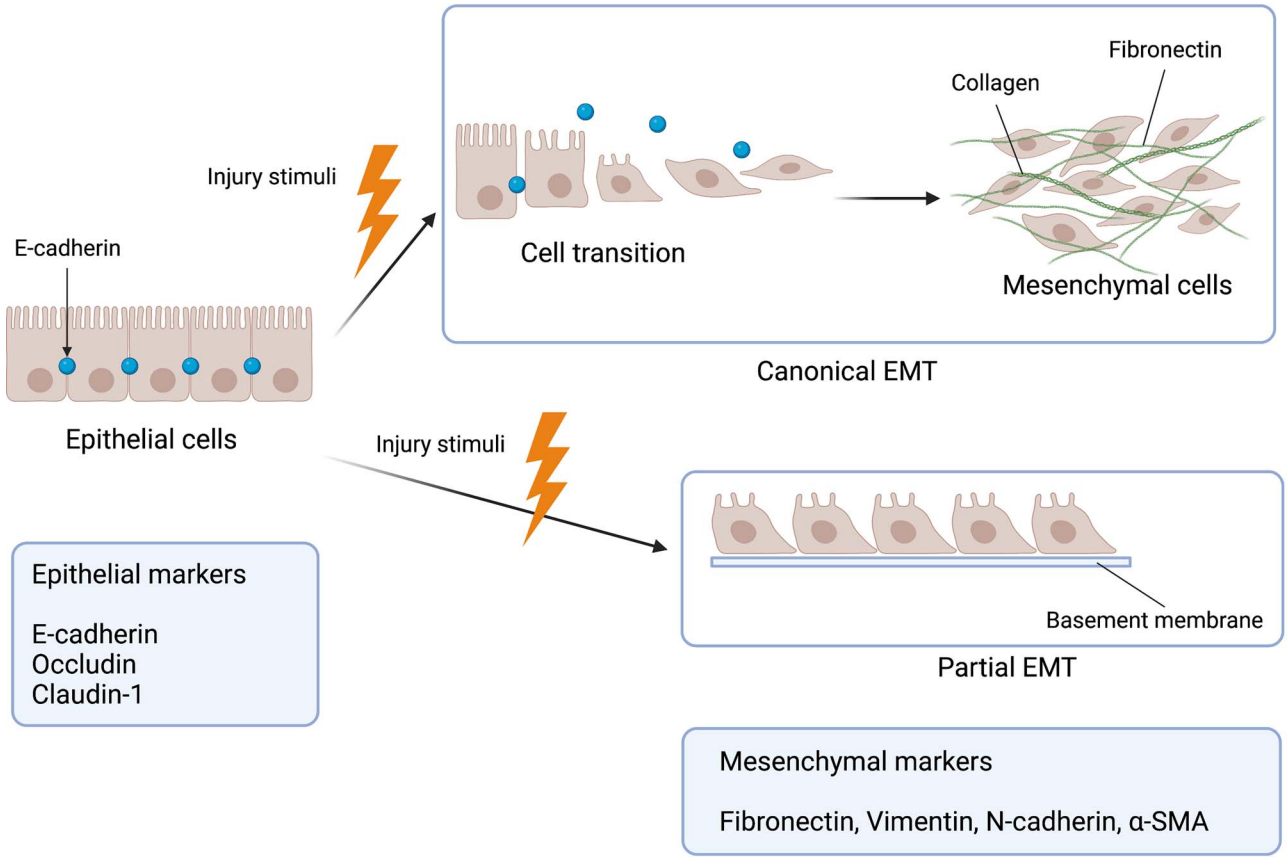

Figure 2. The process of epithelial-to-mesenchymal transition (EMT). Normal epithelial cells connect tightly to each other. During wound healing, EMT is a process in which epithelial cells lose their apical–basal polarity and adhesion with downregulated epithelial markers and transform into mesenchymal cells with upregulated mesenchymal markers. Some studies also discovered a new phenomenon called partial EMT, in which epithelial cells do not cross the glomerular basement membrane to contribute to myofibroblasts, but just acquire mesenchymal features with upregulated mesenchymal markers to release signals. α-SMA α-smooth muscle actin

regulating the TGF-β pathway *in vitro* [38]. Smad7 serves as a negative regulator of TGF-β1 signaling via directly interacting with TGFβRI/TGFβRII and competing with Smad4 for interacting with Smad2/3 [39,40]. This implies that Smad4 and Smad7 are targets for reversing organ fibrosis which is mediated through EMT.

Smad-independent pathways PI3K/Akt and mitogen-activated protein kinase (MAPK) are two Smad-independent pathways induced by TGF-β1 that contribute to EMT.

PI3K/Akt pathway PI3Ks are a family of lipid kinases that participate in regulating a variety of cellular processes, and Akt is a member of the serine/threonine kinases family. The activation of PI3K induces phosphatidylinositol-3,4,5-trisphosphate (PIP3), a phospholipid membrane protein, to bind to Akt [41]. PI3K/Akt pathway activation was widely detected in EMT in different organs. It was associated with hypoxia or the inflammatory response to induce EMT [42,43]. The specific inhibition of PI3K attenuated EMT in different models [44,45]. Under hypoxic conditions, *Bmi1* stabilized *Snail1* by triggering the PI3K/Akt pathway to induce EMT [18]. Overexpression of runt-related transcription factor 1 (RUNX1), triggered by unilateral ureteral obstruction (UUO) and folic acid, promoted the transcription of PI3K subunit P110δ to activate Akt and lead to EMT [46]. The PI3K/Akt pathway was reported to trigger EMT by activating mammalian target of rapamycin (mTOR) and inhibiting glycogen synthase kinase 3β (GSK-3β). mTOR is a downstream substrate of the PI3K/Akt pathway, which is essential for cell-type transformation [19]. mTOR inhibition suppressed EMT in diabetic nephropathy [44]. Curcumin-inhibited EMT exhibited a downregulation of the PI3K/Akt/mTOR pathway [47], indicating that this pathway is involved in EMT development. However, this was just an *in vitro* study and further *in vivo* studies are required.

GSK-3β is at the intersection of distinct pathways. Its inhibition by Akt resulted in EMT occurrence [48].

High-glucose-induced EMT can be attenuated by zinc through inhibiting PI3K/Akt/GSK-3β to inactivate HIF-1α [49]. Nrf2 activator administration partially attenuated high-glucose-induced EMT by dephosphorylating Akt and GSK-3β at serine-473 and serine-9, respectively [48]. The suppressive effects of Nrf2 on the PI3K/Akt/GSK-3β pathway implies its anti-EMT potential.

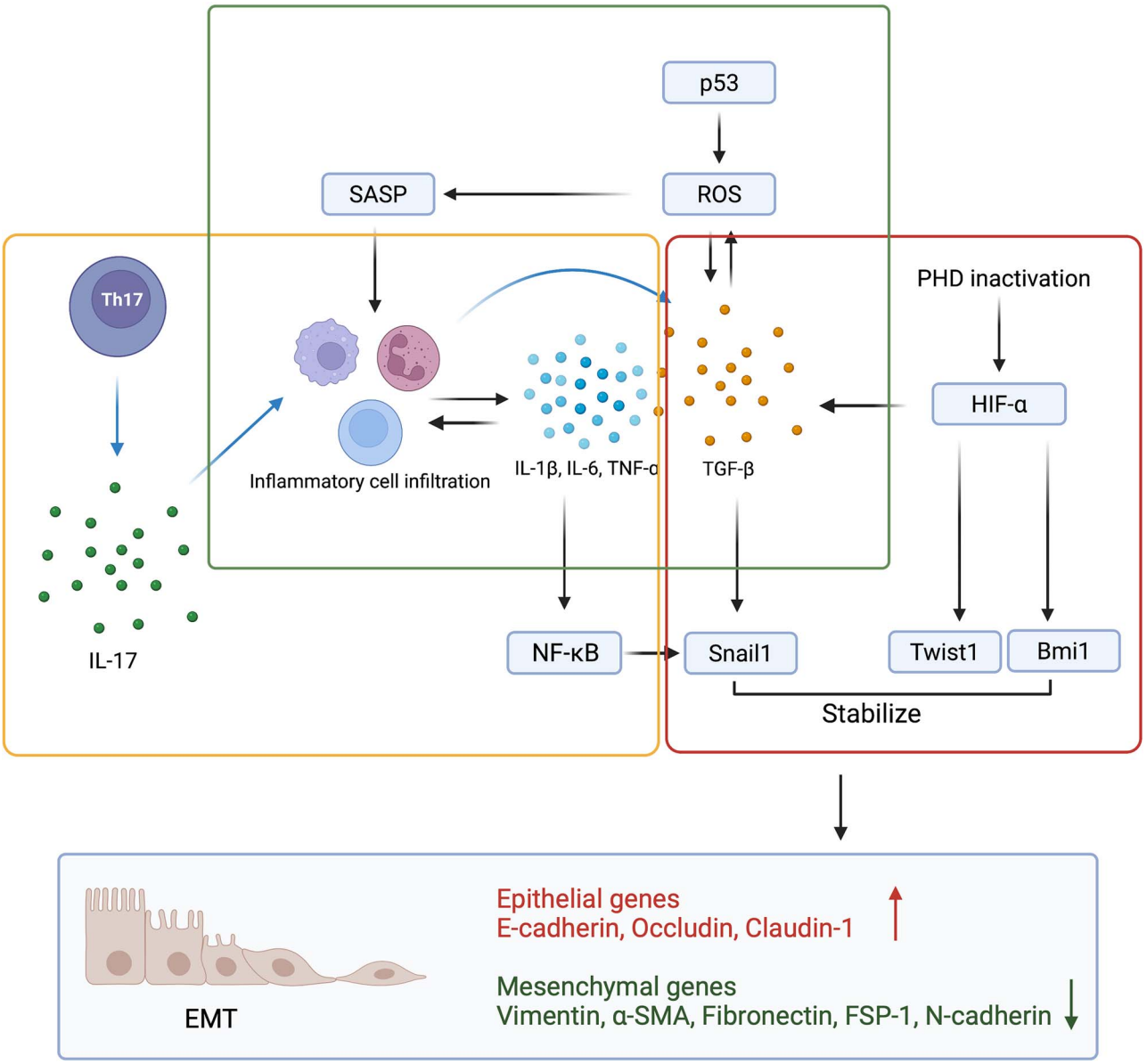

Figure 3. Cellular conditions triggering EMT. Yellow box (inflammation): Th17 cells generate IL-17 to trigger inflammatory cell infiltration. These inflammatory cells produce pro-inflammatory cytokines, and in turn, pro-inflammatory cytokines stimulate inflammatory cell infiltration. Pro-inflammatory cytokines induce EMT by directly activating *Snail1* and indirectly activating the NF-κB pathway to increase *Snail1* expression. Red box (hypoxia): under hypoxic conditions, the inactivation of prolyl hydroxylases (PHD) activates hypoxia inducible factor-α (HIF-α). HIF-α triggers the TGF-β/Smad pathway as well as EMT gene expression such as *Twist1* and *Bm1i* to induce the EMT process. Moreover, *Twist1* binds to the promoter of *Bmi1* to activate the transcription of *Bmi1*, and *Bmi1* further stabilizes *Snail1*. Green box (senescence): *p53* is a well-known gene in senescence. Its activation produces reactive oxygen species (ROS) to trigger cellular senescence. Cells undergoing senescence release cytokines to stimulate inflammatory cell recruitment. Inflammatory cells further generate cytokines to induce EMT. *EMT* epithelial–mesenchymal transition, *SASP* senescence associated secretory phenotype, *TGF-β* transforming growth factor-β, *IL* interleukin, *FSP*-1 fibroblast-specific protein-1

MAPK pathways The MAPK pathway includes p38 MAPK, C-jun N-terminal kinase (JNK) and ERK pathways. The activation of JNK/p38 MAPK/ERK was found in various *in vivo* studies to increase EMT phenotype acquisition and attenuate EMT by blocking these pathways [50].

The blockage of the p38 MAPK pathway attenuated EMT in diabetic nephropathy both *in vivo* and *in vitro* [51,52]. Nevertheless, the mechanism of how p38 MAPK contributes to EMT remains unclear. A recent study reported that inhibition of the MAPK pathway resulted in a decrease in β-catenin [50]. An *in vitro* study reported that p38 MAPK activation inactivates GSK-3β by phosphorylating the C-terminal of GSK-3β to accumulate β-catenin [53]. Although p38 activation is extensively engaged in renal EMT, whether the TGF-β/p38 MAPK/GSK-3β/β-catenin axis leads to EMT *in vivo* remains unknown. Connective tissue growth factor

(CTGF) release was induced under hypoxic conditions, while p38 inhibition-induced EMT suppression was accompanied by CTGF downregulation [54]. This implies a mechanism in which p38 MAPK is associated with hypoxia-related EMT, but the specific mechanism of CTGF regulation remains elusive.

Several inducers contribute to EMT by phosphorylating JNK, which increases the expression of EMT-related transcription factors such as *Snail1* and *Slug* [55,56]. MAPK phosphatase (MKP2) has been revealed to act as a negative upstream regulator of the JNK pathway, not p38 or ERK [57]. JNKs can be separated into three isoforms, JNK1–3. JNK1 mainly phosphorylates c-Jun, while JNK2 decreases the stability of c-Jun [58]. JNK3 is less studied in the existing literature than JNK1/2. Furthermore, currently used JNK inhibitors nonspecifically suppress all JNK proteins. In order to further investigate the features of each JNK protein, specific JNK inhibitors are needed to suppress the expression of each JNK protein separately.

Blockade of the ERK pathway suppressed renal interstitial fibrosis by regulating EMT [59]. Mesenchymal stem cells (MSCs) released extracellular vesicles containing miR-294/miR-133 which reduced renal EMT [60]. In this case, both Smad and ERK pathways were inhibited [60]. Purine receptors of the purinergic receptor family are inflammatory modulators which reduce renal EMT effectively by releasing calcium ions coupled to the MAPK/ERK pathway [61,62]. Purine receptor A2AR reduced EMT by triggering cAMP-related pathways [63], and specific ERK1/2 inhibition decreased the anti-EMT effect of A2AR [63]. This indicates that the activation of purine receptors may mitigate EMT by regulating the ERK pathway.

Wnt activates EMT through the Wnt/GSK-3β/β-catenin axis Wnts contain a family of signaling proteins involved in injury and repair [64]. They interact with frizzled (Fzd) receptors and low-density lipoprotein receptor-related protein (LRP5/6) to transmit signals [65]. Without pathological stimulation, β-catenin forms a complex with Axin and adenomatous polyposis coli (APC), and this heterotrimer interacts with GSK-3β [66]. When the Wnt signaling pathway is stimulated, the cytosolic proteins disheveled (Dvl) are recruited to inhibit GSK-3β phosphorylation [67]. Under such conditions, β-catenin accumulates in the cytoplasm and is then transported into nuclei where it interacts with lymphoid enhancer factor 1 (LEF1) and accelerates the transcription of EMT genes [64,65,68].

Notch signaling pathway is involved in fibrosis in several organs Notch signaling is activated by interaction of Notch receptors (Notch1–4) and multiple ligands including Jagged-1 and Jagged-2 [69]. Notch is a transmembrane receptor which is composed of an extracellular domain (NECD) and an intracellular domain (NICD). γ-Secretase cleaves NICD to promote the translocation of free NICD into the nucleus. Then, the NICD forms a complex with CSL (CBF-1, Suppression of hairless, and Lag), a DNA-binding protein, to stimulate the expression of EMT transcription factors [19,70].

The Notch signaling pathway is engaged in the mechanisms for fibrosis in various organs. Increased expression of Notch1, Jagged-1 and Notch3 was found in pulmonary fibrosis with increased mesenchymal markers and decreased epithelial markers [71]. Hypoxia-induced EMT was promoted in renal tubular epithelial cells by directly targeting Notch1 and Jagged1, and subsequent Notch downstream signaling [72].

A disintegrin and metalloproteinase domain-10 (ADAM10) is a transmembrane protease which was recently found to induce renal fibrosis through EMT by activating the Notch pathway with the complementary help of ADAM17 [73]. However, the specific mechanism of how ADAM10 affects the Notch pathway still remains elusive. A member of TGF-β called Gremlin was found to induce EMT in UUO mice models via interacting with vascular endothelial growth factor receptor 2 and Notch pathway activation was found in this study [74]. Another study found that inhibition of the Notch pathway promoted vascular endothelial growth factor receptor 2 expression [75]. These two findings suggest a potential relationship between the TGF-β pathway and the Notch pathway.

Crosstalk of EMT signaling pathways As stated above, EMT is regulated by various pathways. These pathways crosstalk intricately (Figure 4). GSK-3β serves at the intersection between Wnt and non-Smad pathways. Wnt and Smad-independent pathways both inhibit GSK-3β, which is a β-catenin inhibitor, to induce EMT. Some studies have demonstrated that they can regulate each other in regulating EMT. Inhibition of ERK and JNK prevents the phosphorylation of Smad2 and Smad3, respectively [76,77]. The Wnt pathway requires the regulation of the JNK pathway in pulmonary EMT *in vitro* [78]. Although some studies have found that EMT can be induced by more than one pathway, how these signaling pathways interact and work at different stages requires further investigation.

Epigenetic mechanisms involved in EMT

Epigenetic modifications such as histone deacetylation, lysine methylation, long noncoding RNAs (lncRNAs) and microRNA (miRNA)-induced epigenetic modification have been newly discovered as crucial mechanisms of EMT.

lncRNAs are a group of RNAs with >200 nucleotides that do not code for any proteins [79]. They were demonstrated to affect the EMT process by regulating miRNA. lncRNA nuclear enriched abundant transcript 1 (NEAT1) was shown to promote glucose-induced mouse renal mesangial cell (MMC) EMT and bleomycin-induced pulmonary EMT [79,80]. EMT markers were downregulated by the inhibition of NEAT1 [79,80], but miR-23c depletion partly reversed this effect [79]. This implies that miR-23c may serve as a downstream target of lncRNA NEAT1. Moreover, miR-9-5p was revealed to bind to NEAT1 directly to attenuate

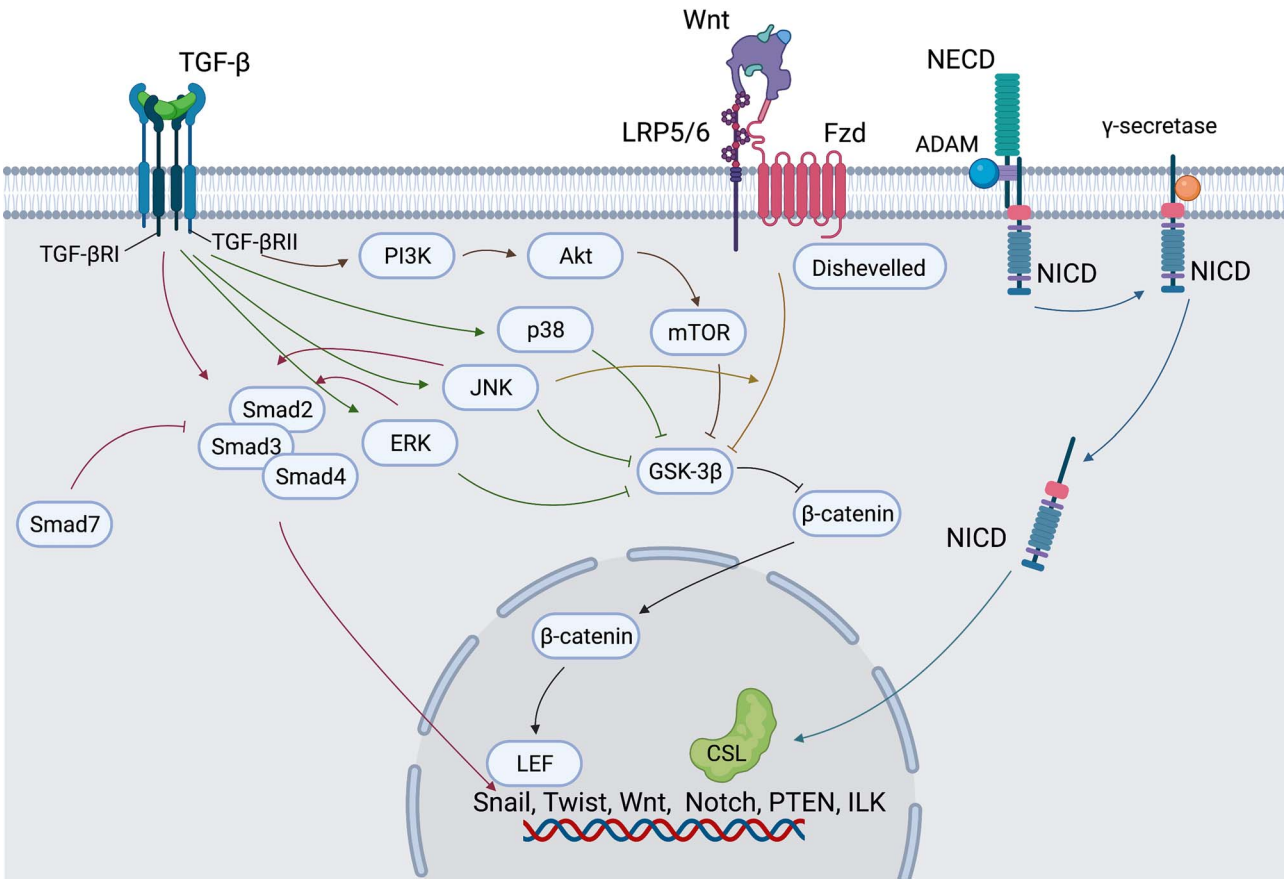

Figure 4. The crosstalk of EMT pathways. EMT is regulated by several pathways and these pathways interplay intricately. TGF-β1 stimulates the activation of Smad and non-Smad pathways. All the non-Smad pathways and the Wnt pathway converge at GSK-3β, which is an inhibitor of β-catenin. β-Catenin is transported into nuclei and interacts with lymphoid enhance factor (LEF) to activate EMT gene transcription. The ERK and JNK pathways regulate the phosphorylation of Smad2 and Smad3 respectively. The Wnt pathway is mediated by the JNK pathway. Notch is composed of two domains, an extracellular domain (NECD) and an intracellular domain (NICD). After ADAM cuts the S2 site of the Notch receptor, γ-secretase cleaves NICD to release it. Then the NICD is translocated into the nucleus and bound to a DNA-binding protein called CSL (CBF-1, suppressors of hairless and Lag) to stimulate the expression of EMT transcription factors. *EMT* epithelial–mesenchymal transition, *TGF-β* transforming growth factor-β, *GSK* glycogen synthase kinase, *JNK* C-jun N-terminal kinase, *ADAM10* a disintegrin and metalloproteinase domain-10

pulmonary EMT [80]. lncRNA plasmacytoma variant translocation 1 (PVT1) triggered liver EMT by binding to miR152 competitively [81].

Apart from lncRNAs and miRNAs, several kinds of histone 3 lysine methylations were found to be involved in EMT. Disruptor of telomeric silencing-1 like (DOT1L) is a methyltransferase which can catalyze histone 3 on lysine 79 (H3K79). DOT1L increased by renal damage was found to cause EMT by dimethylating H3K79 [82]. G9a, a Snail-interacting protein, is a histone methyltransferase. Nagaraja *et al*. demonstrated that upon stimulation by radiation, H3K9 methylation in the E-cadherin promoter region could be modified by ROS to trigger pulmonary EMT via activating G9a [83]. During EMT, decreased H3K9 dimethylation and trimethylation, and increased H3K4 trimethylation on the *snail* promoter region were discovered [83], but the mechanism remains unknown. Under conditions of hypoxia, histone deacetylase 3 (HDAC3) combined with WD repeat domain 5 (WDR5) to recruit histone methyltransferase, leading to acetylated H3K4 decrease and methylated H3K4 increase [84]. Enhancement of IL-6 was shown to regulate paraquat-induced pulmonary EMT [85]. Furthermore, IL-6 expression was reported to be increased by HDAC inhibitors and decreased by histone acetyltransferase (HAT) [85]. Epigenetically, immunoprecipitation data indicated that H3K4 trimethylation and H3K9 acetylation were promoted at IL-6 promoter regions [85].

EMT in fibrosis in different organs

EMT is involved in multiple organs, including lung, liver, kidney, peritoneum, heart, skin and lens. There are multiple cell types that can undergo EMT to acquire myofibroblast features, leading to distinct organ diseases. Targeting these cell types may promote the progress of fibrosis treatment (Figure 5).

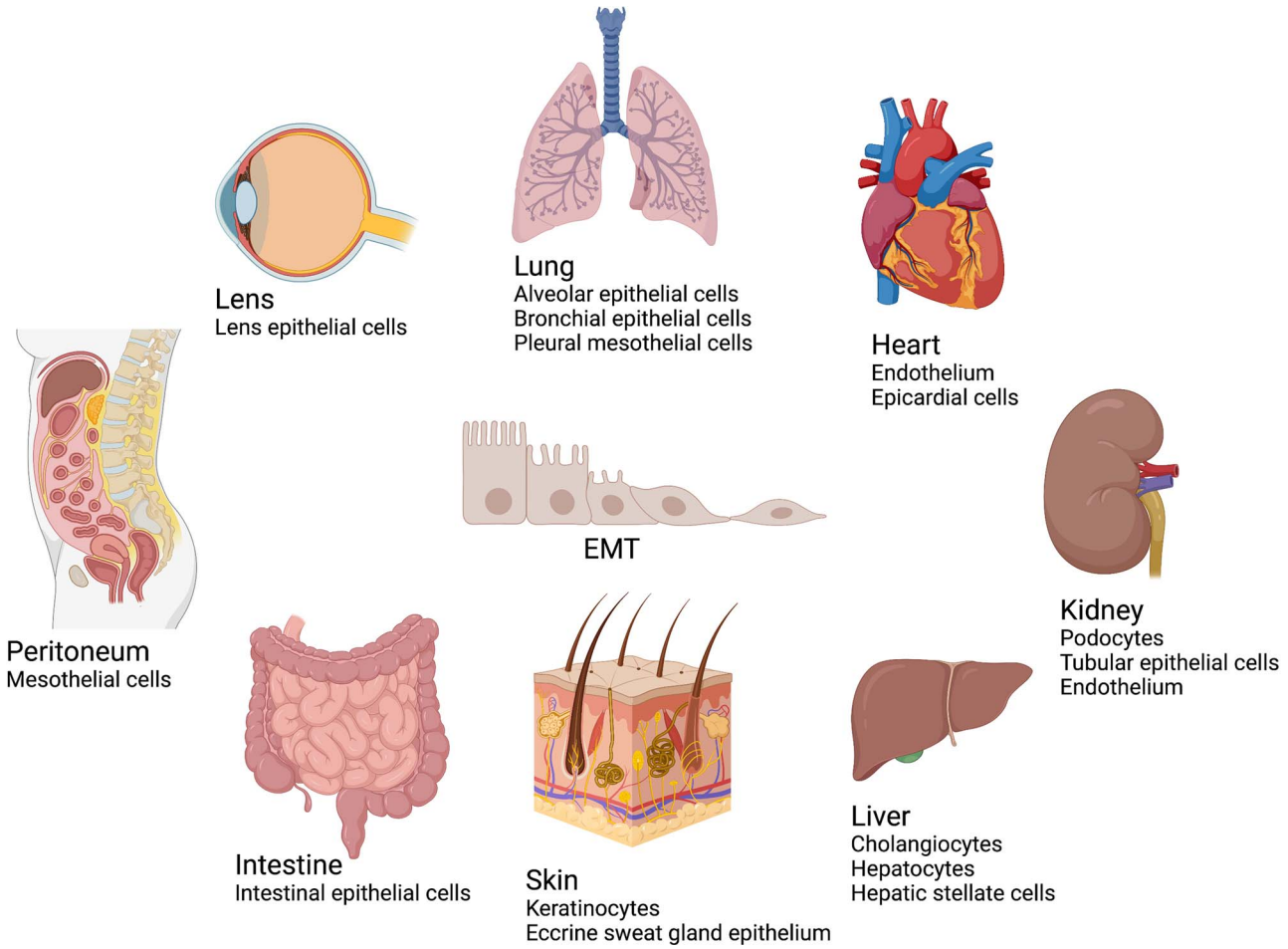

Figure 5. Source of epithelial-to-mesenchymal transition (EMT) in different organ fibrosis. In the lungs, alveolar epithelial cells, bronchial epithelial cells and pleural mesothelial cells are revealed to undergo EMT and cause further airway remodeling. In the liver, after injury, the hepatic stellate cells undergo an EMT-like process with decreased epithelial markers and increased mesenchymal markers. The role of hepatocytes and cholangiocytes in EMT is debatable. In the kidneys, it has been widely accepted that tubular epithelial cells (TECs) are the source of renal EMT. However, some studies demonstrated that TECs undergo partial EMT because they do not cross the renal basement membrane but express mesenchymal and epithelial markers concomitantly. Additionally, studies have shown that podocytes and renal endothelial cells are engaged in renal fibrosis as well. After peritoneal dialysis, peritoneal mesothelial cells tend to undergo EMT and cause peritoneal fibrosis. In the heart, endothelium undergoes endothelial-to-mesenchymal transition (EndMT) in cardiac fibrotic diseases. In the intestines, intestinal epithelial cells undergo EMT to contribute to fibrotic intestinal diseases. In the skin, keratinocytes undergo EMT to cause keloid. Moreover, the eccrine sweat glands epithelium is a potential source of EMT which occurs in scleroderma. In the lens EMT, all the myofibroblasts originate from lens epithelial cells

Lung EMT is involved in a variety of pulmonary fibrosis diseases [86]. A study revealed that bronchial epithelial cells (BECs) stimulated by TGF-β1 are able to transform into myofibroblasts, and lead to peribronchial fibrosis. This process leads to airway remodeling and is significant in the pathogenesis of asthma [87]. In IPF, alveolar epithelial cells (AECs) that undergo EMT lead to the formation of fibroblastic foci and further destroy the blood flow of alveolar septa [88]. Noteworthily, apart from BECs and AECs that contribute to myofibroblasts during EMT, pleural mesothelial cells also undergo mesothelial-to-mesenchymal transition (MMT), a special type of EMT, during IPF pathogenesis. MMT, termed as mesothelial cells that acquire mesenchymal features, is a special form of EMT in all the serosal membranes. A study revealed that pleural mesothelial cells exhibited mesenchymal features and migrated to the parenchyma of the lung after inhaled fibrogenic stimulation *in vivo* [89].

Liver Liver epithelial cells undergo EMT to contribute to myofibroblasts, which has recently attracted more attention. Hepatocyte *Snail1* deletion by using the Cre-loxP technique revealed less EMT markers and inflammatory response compared to the WT group *in vivo*. Rowe *et al*. also found that in the absence of *Snail1*, the liver expressed fewer profibrogenic genes such as Col1a1, Col2a1, Col3a1, vimentin, FSP1, PDGFRB and MMP14 [90]. However, the involvement of hepatocytes and cholangiocytes in liver EMT is controversial. Though hepatocytes treated with TGF-β1 were able to undergo EMT *in vitro* [91], Taura *et al*. demonstrated that

hepatocytes did not express mesenchymal markers *in vivo* in any phase of liver injury [92]. A study revealed that cholangiocytes isolated from bile duct ligation (BDL) mice expressed fewer epithelial markers but more mesenchymal markers (FSP-1) [93]. However, David *et al*. failed to detect the EMT process in cholangiocytes in BDL and carbon tetrachloride (CCl_4) models using a genetic labeling technique [94]. Hence, the authenticity of the roles of hepatocytes and cholangiocytes in liver EMT requires further study. Hepatic stellate cells (HSCs) are currently considered to be the major source of myofibroblasts after liver injury. In a CCL_4 liver injury mouse model, HSCs accounted for 87% of myofibroblasts [95]. During liver development, sub-mesothelial cells underwent EMT to transform into HSCs [96]. Interestingly, in a quiescent state, HSCs expressed more epithelial markers than mesenchymal markers, but they expressed more mesenchymal markers after injury [97]. This implies that although HSCs are not strictly epithelial cells, they can undergo an EMT-like process in the presence of injurious stimulation.

Kidney It has been widely accepted that tubular epithelial cells (TECs) undergoing EMT are responsible for renal fibrosis development [98]. Iwano *et al*. reported that one-third of myofibroblasts in renal fibrosis came from epithelial cells through EMT by using lineage-tracing experiments [99]. However, during EMT, the proportion of epithelial cells that transform into myofibroblasts is still controversial. LeBleu *et al*. later demonstrated that 5 and 10% of myofibroblasts came from tubular epithelial cells and endothelial cells, respectively, by using fate-mapping experiments [7]. Interestingly, Grande *et al*. demonstrated that TECs did not cross the basement membrane to contribute to myofibroblasts but only co-expressed mesenchymal and epithelial markers in *Snail1* reactivation models *in vivo* [11]. Hence, a new conception of partial EMT (pEMT) was proposed. This pEMT phenomenon indicates that EMT likely contributed to fibrosis not because of fibroblast generation but because of inducing loss of functionality, impairing epithelial regeneration and affecting the inflammatory milieu [11,100]. This conception of pEMT offers us a novel perspective on renal fibrosis treatment. Apart from TECs that have been widely recognized to be the source of renal EMT, podocytes and the endothelium have recently been identified as the origins of EMT in the pathogenesis of renal fibrosis as well [101]. A high glucose-induced diabetic nephropathy (DN) model is highly correlated with podocyte EMT [101]. Therefore, targeting of podocyte EMT may offer a new insight for diabetic nephropathy treatment.

In addition, although studies indicated that the endothelium is able to undergo endothelial-to-mesenchymal transition (EndMT) to acquire mesenchymal phenotypes, the proportion of endothelium that contributes to myofibroblasts in renal fibrosis varies from 10 to 50% [7,102]. This discrepancy may be caused by the distinct endothelial markers that researchers used. Hence, a gold standard is required to enhance the specificity of EndMT detection. Genetic deletion of *Twist1* and *Snail1* in renal endothelial cells revealed a decreased renal EndMT and fibrosis level [103]. Moreover, their deletion induced the reduction of $CD8^+$ cell infiltration, indicating that a decrease in the inflammatory response may also contribute to the reduction of fibrosis [103].

Aryl hydrocarbon receptor (AhR) activation is associated with chronic kidney disease (CKD) progression. 1-Aminopyrene (AP) was found to be a novel mediator of EMT by upregulating AhR mRNA and the expression of its three target genes [104]. This implied a critical role of aryl-containing metabolites (ACM) in EMT. Recent studies also showed that CKD patients and mice had gut microbes dysbiosis, which resulted in the accumulation of uremic toxins. A UUO and 5/6 nephrectomized rat models revealed the proportion of altered gut microbiota and plasma metabolites, with upregulation of EMT markers such as α-SMA, collagen I and fibronectin [105,106]. Moreover, using plant-derived drugs to restore the dysbiosis of gut microbiota could downregulate the expression of EMT markers. Collectively, these studies imply the potential mechanism by which gut microbes regulates serum metabolites to induce EMT in CKD patients.

Chen *et al*. discovered that five metabolites including 5-methoxytryptophan (5-MTP) can distinguish all clinical CKD stages. Among them, 5-MTP administration could inhibit the activation of EMT markers, and knockdown of tryptophan hydroxylase-1 (TPH-1) increased the expression of these markers [107]. TPH-1 is an enzyme that is involved in 5-MTP synthesis, and it was downregulated in ischemia reperfusion injury (IRI) and UUO mice. TPH-1 may serve as a critical target of EMT by controlling 5-MTP synthesis. Furthermore, the administration of 5-MTP not only attenuated the expression of pro-inflammatory factor NF-kB and its downstream gene products monocyte chemoattractant protein-1 (MCP-1) and cyclooxygenase-2 (COX-2), but also increased the presence of the anti-inflammatory transcription factor Nrf2 and its target genes Heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase-1 (NQO-1) [107]. Taken together, this may suggest that TPH-1 may attenuate EMT by targeting NF-kB and Nrf2 pathways.

Intestine An increasing number of studies showed that EMT was involved in inflammatory bowel disease (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) [108]. In IBD patients, recurrent intestinal inflammatory factors triggered mucosal healing reactions, leading to ECM deposition to form fibrosis [108]. Transgenic mice expressing high levels of tumor necrosis factor-like ligand 1 A (TL1A) were sensitive to dextran sodium sulfate (DSS) and expressed more EMT markers with upregulation of the TGF-b/Smad3 pathway [108]. This indicates the critical role of TL1A in intestinal EMT. EMT also plays a critical role in the formation of fistulas in patients with CD [109]. Recent research revealed that CD patients had increased levels of succinate and its receptor SUCNR1 in fistula tract, accompanied by high levels of EMT markers, and Wnt signaling pathway activation was

found to induce intestinal epithelial EMT [110]. Therefore, the Wnt pathway was suggested to contribute to fistula formation. These reports imply that SUCNR1 is a potential therapeutic target for preventing fistula formation in CD.

Peritoneum Peritoneal dialysis is an alternative to hemodialysis in patients who have suffered from end-stage renal failure. However, during peritoneal dialysis, the persistent exposure to bio-incompatible dialysate and plastic circuits as well as hemoperitoneum can all trigger EMT to induce peritoneal fibrosis [111,112]. MMT is considered an early mechanism underlying peritoneal fibrosis, suggesting early intervention of mesothelial cells (MC). Therefore, EMT may maintain mesothelial barrier integrity [113].

Heart EMT and its special type, EndMT, are involved in cardiac healing during pathological states such as myocardial infarction, atherosclerosis and valve dysfunction [114,115]. Epicardial EMT was activated after injury to initiate angiogenesis and healing [114]. One-quarter of epicardial cells treated by Wnt1 were found to have mesenchymal phenotypes [116]. Although previous studies revealed that EndMT was involved in cardiac fibrosis, the proportion of endothelial cells that contribute to myofibroblasts is under debate and warrants further study [117]. More *in vivo* lineage-tracing experiments are required to clarify the proportion of myofibroblasts with endothelial origin. Moreover, since the conception of pEMT in renal fibrosis was proposed [118], the discrepancy of endothelium-derived myofibroblasts measured in different organs may be caused by partial EndMT.

Compared to other organs such as lung, kidney and liver, the understanding of signaling pathways that are involved in cardiac EMT is limited. The activation of PPAR-γ alleviates cardiac EMT by inhibiting the ERK pathway, with the promotion of MMP2 and MMP9 [119]. In addition, a previous study showed that epicardial-specific β-catenin-deletion mice had an ameliorated epicardial EMT after IRI [116], implying the pivotal function of β-catenin-related pathways such as Wnt, MAPK and PI3K/Akt.

However, although EMT plays a role in cardiac healing, it is not sufficient to repair injury completely. The therapeutic strategy for cardiac fibrosis requires the targeting of other mechanisms in addition to EMT [114].

Skin Evidence suggested that EMT was engaged in cutaneous fibrosis such as keloid and scleroderma. In keloid tissue, EMT markers were found in keratinocytes, and this process was activated by proinflammatory factors [120]. Scleroderma is an autoimmune disease which primarily affects dermis and subcutaneous regions of the skin, with a thick and tight appearance [121]. A study demonstrated that scleroderma was associated with the EMT process in eccrine sweat glands with fibronectin and also affects E-cadherin expression [122]. This study may indicate that the myoepithelium in eccrine glands is a potential source of EMT.

Lens EMT of lens epithelial cells is vital in the pathogenesis of fibrotic cataract formation after cataract surgery and under high-glucose conditions [123]. It is worth noting that since there are no resident fibroblasts in lens, all the myofibroblasts in lens are derived from lens epithelial cells. This is different from other organs which have diverse potential origins of myofibroblasts [124].

Preventive strategies

Although some drugs have been proven to suppress or partially reverse EMT in laboratory experiments, further clinical trials are needed for their clinical application in the treatment of fibrosis. Unfortunately, in clinical settings, anti-fibrosis drugs are only used to relieve symptoms but not to reverse fibrosis completely.

Naturally derived therapies

Botanical compounds Multiple herbal extractions were reported to attenuate EMT. Resveratrol (RSV) and its analog pterostilbene are extracted from the red grape. They partially inhibited EMT by regulating Akt and Smad pathways [125,126]. Curcumin extracted from *Curcuma longa* suppressed EMT by regulating both Smad and non-Smad pathways [43,127]. Inhibition of the PI3K/Akt/mTOR pathway by curcumin was found to attenuate renal and liver EMT *in vivo* [28,43]. During peritoneal EMT, curcumin inhibited TAK1, and further downregulated the expression of p38 and JNK, indicating its regulatory role in MAPK pathways [127]. Curcumin also regulated the tetratricopeptide repeat domain 3/Smad ubiquitination regulatory factor 2 (SMURF2)/Smad axis [28]. It blunted tetratricopeptide repeat domain 3 which subsequently enhanced SMURF2 expression to prevent Smad2/Smad3 formation in liver EMT [28]. Some RAS inhibitors, such as members from poricoic acid groups extracted from *Poria cocos* and 25-O-methylalisol F (MAF) extracted from *Alismatis rhizoma*, were found to suppress EMT [128]. MAF combined with TGF-β receptor I competitively prevented the interaction of Smad3 with TGF-β receptor I [128]. The poricoic acid group contains poricoic acid ZA (PZA), ZC (PZC), ZD (PZD), ZE (PZE), ZF (PZF), ZG (PZG) and ZH (PZH). Of those, PZC, PZD, PZG and PZG belong to the secolanostane tetracyclic triterpenoid group while PZE and PZH belong to lanostane tetracyclic triterpenoid group [129,130]. These poricoic acids, except PZF, were demonstrated to exert anti-EMT function by inhibiting the Wnt/β-catenin and Smad pathways [129,130]. They were found to suppress the interaction of Smad3, but not Smad2, with TGF-β receptor I [129,130], indicating that Smad3, rather than Smad2, was a critical therapeutic target of poricoic acid groups. However, some herbal extractions have been observed to attenuate EMT only *in vitro*, such as ferulic acid, PZG and PZH [131]. Therefore, more animal experiments should be carried out to test their efficacy. Furthermore, PZF had no effect

on anti-EMT, and PZE had a weaker anti-EMT function compared to other secolanostane tetracyclic triterpenoid groups [129,130]. This may be caused by the discrepancy in their chemical structure and the increased number of hydroxyl groups on side chains, whereas carboxyl groups may be more renoprotective [130]. Triptolide (TPL) is a diterpenoid epoxide which is extracted from *Tripterygium wilfordii*. TPL reduced *Snail1* and Twist to inhibit EMT in phosphatase and tensin homolog-induced diabetic kidney disease by interacting with the RING domain of mex-3 RNA binding family number C (MEX3C) to prevent its combination with ubiquitin-conjugating enzyme E2S (UBE2S) [132]. This provides a structural mechanism of how TPL inhibits ubiquitin ligase.

In addition, some plant extractions were shown to mitigate EMT by altering microbial composition. In chronic kidney disease, poricoic acid A and *P. cocos* were found to decrease the level of glycine-conjugated compounds and polyamine metabolites, which are derived from microbes [105]. *Polyporus umbellatus* and its extraction, ergone, were revealed to increase plasma tryptophan levels and decrease some tryptophan metabolites to mitigate EMT [106]. Collectively, improving the levels of plasma metabolites that help the recovery of intestinal barrier function, may provide a novel solution to treat EMT.

Compounds derived from living species Eicosapentaenoic acid (EPA), derived from fish oil has been reported to inhibit inflammatory responses and exert anti-fibrotic activity through regulating Smad3 signaling and activating miR-541 [133,134]. EPA's function of attenuating renal EMT has only been tested *in vitro* and further experiments are needed to confirm its *in vivo* functions. Melittin is the major component of bee venom, which can be applied in anticancer and anti-inflammation therapies. One study indicates that melittin suppressed hepatic EMT via regulating JNK and Smad-dependent pathways both *in vitro* and *in vivo* [56]. These reports suggest that EPA and melittin have the potential to attenuate EMT in clinical use.

Epigenetic modification

HDAC modulators Mammalian HDAC contains 18 subtypes that are classified into four classes. Class I contains HDAC 1–3 and 8; class II contains class IIa (HDAC 4, 5, 7 and 9) and class IIb (HDAC 6, 8 and 10); class IV only contains HDAC 11, and class III contains sirtuins (SIRT) 1–7 [135]. HDACs act as pro-inflammation molecules that are involved in fibrogenesis. HDAC mediators have an effect on EMT by regulating histones epigenetically.

HDAC inhibitors The inhibition of HDACs restrained E-cadherin reduction and decreased mesenchymal markers as well as ECM components [136], indicating that HDACs inhibitors are potential treatments for renal fibrosis by targeting EMT.

MS-275 is an HDAC 1–3 inhibitor that was proved to attenuate peritoneal and renal EMT [136,137]. The anti-EMT function of MS-275 on the peritoneum depends on H3 acetylation, *Snail* impairment and rescue acetylation of E-cadherin promoter, which is in accordance with HDAC I gene silencing results [137]. Tubastatin A is an HDAC 6 inhibitor that attenuates peritoneal and renal EMT by inhibiting Smad3 phosphorylation and acetylating histone H4 [138]. However, a study demonstrated that although tubastatin A ameliorated bleomycin-induced pulmonary EMT through targeting the PI3K/Akt pathway, knockdown of HDAC6 did not have this function [139]. Hence, whether the anti-EMT function of tubastatin A depends on inhibiting HDAC6 remains obscure. Valproic acid (VPA) is a selective class I HDAC inhibitor. Pretreatment with VPA attenuated pulmonary EMT and restored lung structure *in vivo* by downregulating p-Smad2/Smad3 and p-Akt [140]. VPA ameliorated renal EMT by increasing Smad7 and by acetylating histone H3 in mice with diabetic nephropathy, and the promoter enrichment of *Fn1* and *Col1a1* in H3 was decreased by VPA [141]. Different from tubastatin A and VPA, an HDAC8 specific inhibitor called PCI34051 attenuated renal EMT *in vivo* with no histone H3 and H4 acetylation [142]. Whether H1 and/or H2 or histone-related protein are mediated by PCI34051 remains unknown and requires further study. Trichostatin A (TSA) is a class HDAC I and II inhibitor, and it was found to attenuate EMT in UUO-induced renal fibrosis by promoting M1-to-M2 macrophage transition and increasing the number of anti-inflammatory M2c instead of proinflammatory M2a macrophages [143]. However, the specific mechanism of how TSA affects macrophages is unclear. A recent study indicated that only the JNK signaling pathway was involved in the anti-EMT function of TSA, which led to the inactivation of Notch-2 [144]. Suberoylanilide hydroxamic acid (SAHA) is an HDAC inhibitor approved by the food and drug administration (FDA) to treat cutaneous T-cell lymphoma. SAHA was shown to inhibit the CKD process in *col4a3*$^{-/-}$ mice with decreased α-SMA and fibronectin [145]. The administration of SAHA decreased mesenchymal markers and increased epithelial markers in HSCs [146]. Interestingly, like TSA, only JNK signaling was found to be inhibited by SAHA [145], indicating that the JNK pathway may be promising for reversing EMT. RSV is a well-studied plant-derived drug which can relieve fibrotic EMT in the lungs and kidneys by inhibiting the ROS and Smad pathways [126,147]. However, research on how RSV affects HDAC epigenetically is limited. Its metabolite, piceatannol, was revealed to suppress EMT protein expression by inhibiting HDAC4 and 5 [148]. The inhibition of the p38 MAPK pathway, rather than the Smad pathway, was also detected under the administration of piceatannol [148], indicating the potential relationship between the p38 pathway and class IIa HDAC. A specific HDAC class IIa inhibitor, MC1568, attenuated renal EMT through blocking the TGF-β/Smad pathway [149]. Collectively, HDAC 7 and/or 9 may have an important impact on EMT through activating TGF-β-related pathways.

On this basis, epigenetic regulation plays a potential role in ameliorating EMT. However, whether these HDAC inhibitors affect histone acetylation by modulating histone chaperones or binding to histones directly is unclear. No HDAC inhibitors are currently approved for clinical use in fibrosis treatment.

SIRT activators SIRT are a series of Class III HDAC which require adenine dinucleotide (NAD^+). They play significant roles in physiology and pathology [150]. Based on their roles in fibrosis, several studies found SIRT activators have an anti-EMT effect in different organs.

RSV activated SIRT1 to inhibit EMT dose-dependently. The underlying mechanism of RSV is to decrease Smad3 phosphorylation and further impede Smad3 and Smad4 complex formation [151]. Astragaloside IV (AS-IV) and salvianolic acid B (Sal B) impair EMT by activating SIRT1 to inhibit NF-κB p65 acetylation and phosphorylation [101,152]. Honokiol (HKL) is a kind of SIRT3 activator which can reduce the level of α-SMA and ECM components in renal fibrosis induced by UUO. Since SIRT3 suppression is associated with EMT emergence, HKL was suspected of preventing fibrosis by inhibiting the EMT process. Therefore, epithelial markers need to be studied to confirm HKL's role in inhibiting EMT. HKL also increased NF-κB p65 phosphorylation, indicating that it exerts anti-EMT function probably by anti-inflammatory processes [153]. Decreased Smad2 and Smad3 phosphorylation were also found in kidneys treated with HKL, but the specific mechanism is unknown.

SIRT6 interacts with the promoters of β-catenin target genes to induce histone H3K56 deacetylation to prevent the transcription of fibrosis-related genes. Hence, SIRT6 reduced the transcription of genes associated with β-catenin through epigenetic regulation [154]. However, to date no SIRT6 activator is currently being investigated for the attenuation of EMT. Since several EMT-related signaling pathways converge at β-catenin, finding a SIRT6 activator may be promising for EMT inhibition.

miRNA and lncRNA Some lncRNAs and miRNAs were reported to regulate EMT in some models. lncRNA zinc finger E-box binding homeobox 1 antisense (ZEB1-AS1) was downregulated upon high-glucose treatment. Overexpression of lncRNA ZEB1-AS1 was shown to attenuate renal EMT by targeting miR-216a-5p directly assessed with a luciferase reporter assay [155]. In CCl_4-treated hepatic EMT models, Salvianolic acid B upregulated miR-152, which further contributed to DNA methyltransferase 1 (DNMT1) inhibition and DNA demethylation [156].

Histone methylation EPZ5676 is a DOT1L inhibitor. DOT1L was shown to methylate H3K79 during renal EMT [82]. Inhibition of methyltransferase DOT1L reduced Snail, Twist and Notch1 expression and reversed the reduction of renoprotective factors such as Klotho, Smad2 and Smad7 [82]. Hence, using EPZ5676 to block H3K79 methylation may have therapeutic potential. KDM6A is a histone lysine demethylase which can regulate E-cadherin expression. Its inhibitor, GSK-J4, was found to restore EMT in streptozotocin (STZ)-induced renal injury mice [157]. In addition, miR-199b-3p overexpression targeted KDM6A to prevent EMT [157]. However, which lysine of histone is targeted by KDM6A to regulate EMT remains unclear. As a large number of EMT epigenetic mechanisms have been found, blocking histone methylation is a prospective therapeutic strategy.

Therapies related to TGF-β The activation of TGF-β signaling contributes to EMT in various scenarios. Hence, targeting TGF-β signaling to reverse or stop EMT may be worth developing for clinical use.

Bone morphogenetic protein-7 Bone morphogenetic protein-7 (BMP-7) is a member of the TGF-β superfamily and its administration alleviates EMT in pulmonary, intestine and renal cells [108,158]. The binding of BMP-7 and BMP receptors (BMPRs) stimulated Smad1/5/8 phosphorylation. Subsequently, phosphorylated Smad1/5/8 can compete with Smad2/Smad3 to form a heteromeric complex with Smad4 [159]. BMP-7 was found to suppress EMT in renal HK-2 cells by inhibiting Wnt3a/b-catenin and TGF-β1/Smad2/3 pathways [160]. In RLE-6TN cells, BMP-7 induced mesenchymal-to-epithelial transition (MET) and blunted the EMT process by inhibiting the p38 MAPK pathway [161]. A recent study used protein transduction domain (PTD)-fused BMP-7 to treat A549 cells and UUO mice [158]. It has been demonstrated that the protein transduction domain fused BMP-7 in micelle (mPTD-BMP-7) was transduced into cells via an endosomal pathway and secreted to exosomes containing BMP-7. EMT was successfully ameliorated by mPTD-BMP-7 through activating the Smad1/5/8 pathway [158]. Furthermore, the protein transduction domain fused BMP-7 in micelle (mPTD-BMP-7) was found in Bowman's space after its intra-arterial administration to porcine renal artery, and the fibrosis caused by UUO was alleviated [158]. However, whether this effect was related to EMT has not yet been identified. Interestingly, one study showed that BMP-7 treatment in diabetic nephropathy almost completely reverses the expression of E-cadherin and α-SMA [162].

TGF-β monoclonal antibody TGF-β is a well-known fibrosis inducer that has been found in multiple organs. TGF-β activation can induce EMT by stimulating its downstream signaling pathways, such as Smad-dependent and Smad-nondependent pathways, as previously described. Antagonizing TGF-β by using its monoclonal antibody (mAb) was reported to reduce ECM deposition and attenuate fibrosis in various scenarios, suggesting its role in anti-EMT. Hence, several studies investigated whether TGF-β mAb can ameliorate EMT after tissue damage. 1D11 is a murine antibody which targets all three TGF-β isoforms and reversed pre-existing thioacetamide-induced liver fibrosis by decreasing ECM production and increasing ECM degradation [163]. In addition, the administration of TGF-β mAb reduced PAI-1 expression [163], a plasminogen activator inhibitor whose interaction with the urokinase-type plasminogen activator receptor (uPAR)

caused ECM accumulation by triggering TGF-β-related signaling pathways [164]. This indicates that TGF-β antibody has a therapeutic impact on hepatic fibrosis. Notably, the expression of TGF-β2 and TGF-β3 was not changed by 1D11 [163]. It is unclear whether this was due to 1D11 affinity or the intracellular distribution of TGF-β2 and TGF-β3.

TGF-β neutralizing antibody injection reduced collagen deposition and expression of fibronectin extra domain A (FnEDA) in UUO mice [165], indicating the anti-EMT role of TGF-β neutralization antibody during renal injury. A bispecific antibody containing both FnEDA and TGF-β domains was also constructed to reverse EMT during renal injury. Nevertheless, injecting TGF-β mAb alone demonstrated a diffused distribution in contrast to the bispecific antibody in the kidney [165]. This indicates that TGF-β mAb therapeutic efficiency can be improved by bispecific antibody construction. Further experiments are required to investigate the effectiveness of TGF-β mAb in pre-existing renal fibrosis.

Collectively, the use of TGF-β mAb provides a novel approach for anti-EMT and subsequent fibrosis development in different organs. However, TGF-β is a critical cytokine with multiple functions such as regulating the adaptive immune system and T cell differentiation [166]. Therefore, the risk/benefit ratio of TGF-β mAb therapy needs to be fully assessed before clinical use can be implemented.

Stem cells Stem cell transplantation is emerging as a novel approach for EMT inhibition. The administration of adipose-derived stem cells (ADSCs) attenuated cigarette smoke extract-induced pulmonary EMT *in vitro* [167], and renal EMT *in vivo* [167]. This process was suggested to be associated with the TGF-β pathway, but which specific downstream signaling pathway is involved remains unknown. Human umbilical mesenchymal stem cells (usMSCs) inhibited aristolochic acid-induced renal EMT by regulating the TGF-β/Smad pathway [168]. Vesicles containing miRNA derived from MSCs were proved to be essential in preventing EMT in renal fibrosis. Vesicles which carried miR-294 and miR-133 inhibited EMT and renal fibrosis by regulating the Smad and/or ERK pathway [60]. Anti-miR-let-7 carried by MSC vesicles increased tuberous sclerosis complex 1 (TSC1) expression level and reduced the phosphorylation of mTOR/p70S6K [169]. However, the exact mechanism is unclear. Let-7i-5p, a regulator of fibrogenesis EMT, was found to bind to TSC1 [169]. In normal physiological conditions, TSC1 and TSC2 form a complex. Akt phosphorylation can inactivate TSC2 and impede its interaction with TSC1 [170]. Hence, MSC vesicles may carry anti-miR-let-7 through targeting the Akt/TSC2/mTOR axis.

Erythropoietin (EPO) is a glycoprotein secreted by kidney which attenuates renal fibrosis in different models by inhibiting EMT [171]. However, it is unfeasible to inject low-dose EPO continuously in clinical events. Therefore, MSCs transfected with EPO genes (named hEPO-MSCs) were produced to tackle this concern. Interestingly, microparticles released by hEPO-MSCs were proved to attenuate renal fibrosis by targeting Smad and p38 MAPK EMT pathways [171].

Extracellular vesicles released by bone marrow stem cells (BMSC-EVs) were demonstrated to attenuate EMT *in vivo* by releasing milk fat globule-epidermal growth factor 8 (MFG-E8) [172]. Klotho is an anti-aging protein mainly expressed in renal tubular cells [173] and it has been shown to be essential for anti-EMT in renal fibrosis in a variety of studies. Using adenovirus to build Klotho-overexpressing BMSC had an anti-EMT effect and inhibited renal fibrosis by targeting the Wnt pathway *in vivo* [173]. However, stem cells and/or their derivative therapies are still not ready to be applied clinically.

Clinical medication Unfortunately, no medication can cure or completely reverse fibrosis currently; all drugs in clinical settings focus on relieving symptoms.

Pirfenidone and nintedanib are anti-fibrotic drugs used for IPF clinically [174,175]. However, their severe gastrointestinal and skin adverse effects require mitigation [175]. Both nintedanib- and pirfenidone-treated patients have decreased liver function [175]. Further investigations such as dosage use should be carried out to prevent these side effects.

Losartan is an angiotensin II receptor antagonist that has been widely used in hypertension and end-stage renal disease treatment [176]. Previous studies have demonstrated that losartan reduced EMT in mitral valve remodeling after myocadiac infarction and renal fibrosis through regulating the TGF-β1 pathway [176,177]. Nevertheless, the administration of losartan is not sufficient to reverse the fibrotic clinical manifestations [178]. Noteworthily, other renin-angiotensin-aldosterone system (RAAS) intervention drugs such as eplerenone, telmisartan and spironolactone are proved to prevent EMT *in vitro*. Hence, this implies that RAAS intervention has the potential to be administrated in clinical events.

Chinese medicine recipes were reported to be used clinically for patients who suffered from fibrosis. [179–181]. Although these herbal recipes are capable of ameliorating EMT in experiments and are used for treating fibrosis in some cases, the side effects of herbal remedies such as nephrotoxicity and hepatotoxicity make their clinical use debatable.

Conclusions

EMT is a key mechanism during fibrogenesis in various organs. It is regulated by several pathways which crosstalk intricately. Recent studies have also explored the significance of epigenetic mechanisms during EMT. Several treatments were shown to inhibit or reverse EMT partially but not completely. Despite better understanding of the role of EMT in fibrosis development, targeting EMT and beyond in developing therapeutics to tackle fibrosis is challenging but feasible.

Abbreviations

α-SMA: α-smooth muscle actin; ADAM: A disintegrin and metalloproteinase domain; AEC: Alveolar epithelial

cell; BDL: Bile duct ligation; BEC: Bronchial epithelial cells; BMP-7: Bone morphogenetic protein-7; CCl4: carbon tetrachloride ; CD: Crohn's disease; CKD: Chronic kidney disease; COX-2: Cyclooxygenase-2; CSL: CBF, Hairless, and Lag; CTGF: Connective tissue growth factor; DOT1L: Disruptor of telomeric silencing-1 like; ECM: Extracellular matrix; EndMT: Endothelial-to-mesenchymal transition; EMT: Epithelial-to-mesenchymal transition; EPA: Eicosapentaenoic acid; EPO: Erythropoietin; FDA: Food and drug administration; FnEDA: Fibronectin extra domain A; FSP: Fibroblast-specific protein 1; GSH: Glutathione; GSK-3: Glycogen synthase kinase 3; H3K79: histone 3 on lysine 79; HDAC3: Histone deacetylase 3;HKL:Honokiol;HSC:Hepatic stellate cells; HIF-α: Hypoxia inducible factor-α; IBD: Inflammatory bowel disease; IL: Interleukin; IPF: Idiopathic pulmonary fibrosis; JNK: C-jun N-terminal kinase; HO-1: Heme oxygenase-1; lncRNA: Long noncoding RNA; mAb: Monoclonal antibody; MAPK: Mitogen-activated protein kinase; MC: mesothelial cells; MCP-1: Monocyte chemoattractant protein-1; MET: Mesenchymal-to-epithelial transition; miRNA: Micro-RNA; MMT: Mesothelial-to-mesenchymal transition; 5-MPT: 5-Methoxytryptophan; mTOR: Mammalian target of rapamycin; MEX3C: mex-3 RNA binding family number C; mPTD-BMP7: protein transduction domain fused BMP-7 in micelle; NEAT1: Nuclear enriched abundant transcript 1; NF-κB: Nuclear factor-κB; NICD: Notch intracellular domain; NQO-1: NADPH quinone oxidoredctase-1; pEMT: partial epithelial–mesenchymal transition; PIP3: Phosphatidylinositol-3,4,5-trisphosphate; PPAR-α Peroxisome proliferator-activated receptor alpha; RAAS: Renin-angiotensin-aldosterone system; ROS: reactive oxygen species; RSV: Resveratrol; SAHA: Suberoylanilide hydroxamic acid; siRNA: Small interfering RNA; SMURF2: Smad ubiquitination regulatory factor 2; TEC: Tubular epithelial cells; TGF-β: Transforming growth factor-β; TL1A: Tumor necrosis factor-like ligand 1 A; TLR4: Toll-like receptor 4; TNF-α: Tumor necrosis factor-α; TPH-1: Tryptophan hydroxylase-1; TPL: Triptolide; TSA: Trichostatin A; TSC1: Tuberous sclerosis complex 1; UBE2S: Ubiquitin-conjugating enzyme E2S; UUO: Unilateral ureteral obstruction; VPA: Valproic acid; WDR5: WD repeat domain 5.

Authors' contributions

DM conceived the project. LL wrote the first draft. All authors read, revised, edited and approved the final manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Competing interests

None declared.

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