Minireview

Epithelial-mesenchymal transition: An emerging target in tissue fibrosis

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Abstract

Epithelial-mesenchymal transition (EMT) is involved in a variety of tissue fibroses. Fibroblasts/myofibroblasts derived from epithelial cells contribute to the excessive accumulation of fibrous connective tissue in damaged tissue, which can lead to permanent scarring or organ malfunction. Therefore, EMT-related fibrosis cannot be neglected. This review highlights the findings that demonstrate the EMT to be a direct contributor to the fibroblast/myofibroblast population in the development of tissue fibrosis and helps to elucidate EMT-related anti-fibrotic strategies, which may enable the development of therapeutic interventions to suppress EMT and potentially reverse organ fibrosis.

Keywords: Epithelial-mesenchymal transition, fibroblast, myofibroblast, tissue fibrosis, anti-fibrosis, TGF-β, dedifferentiation, mesenchymal stem cell

Experimental Biology and Medicine **2016; 241: 1–13. DOI: 10.1177/1535370215597194**

Introduction

Fibrosis is usually the final pathological state of many chronic inflammatory diseases and may lead to organ malfunction.[1,2] Fibroblasts/myofibroblasts are the primary ''effector'' cells in the pathogenesis of fibrosis. When tissues are damaged, the resident fibroblasts increase reactively in response to stimulation of the microenvironment after injury. In addition, a fibroblast that acquires a smooth muscle cell-like phenotype is called a myofibroblast,[3] which exhibits stronger capacities for secretion and contraction than fibroblasts. Active fibroblasts/myofibroblasts secrete excessive extracellular matrix (ECM) components that will deposit into the wound site and replace the provisional matrix. Although the traditional view that the resident fibroblasts and their derived myofibroblasts play a crucial role in fibrosis still holds, an increasing volume of data collected in the last decade suggests that epithelial-mesenchymal transition (EMT) is a direct contributor to the fibroblast/myofibroblast pool during fibrogenesis.[4] Therefore, in addition to targeting fibroblast activation and proliferation, prevention of EMT may be another effective strategy to inhibit tissue fibrosis.

EMT and its roles in tissue fibrosis

EMT has been proved to play vital roles in embryonic development, wound healing, and even cancer progression.[5] Recently, EMT has been classified into three subtypes according to its functional consequences and biomarkers.[6] In an embryo, the primitive epithelial cells give rise to their mesenchymal offspring and migrate to create new organs, which have been classified as type 1 EMT. EMT that occurs in epithelial carcinoma was recently classified as type 3 EMT. The type 2 EMT is characterized by the differentiation of epithelial cells into new fibroblast-like cells in the interstitium that are involved in wound repair, tissue regeneration, or organ fibrosis, which is the major focus of this review. The type 2 EMT involves a change from the apical-basolateral polarity of the epithelial cells to the front-rear polarity of the mesenchymal cells. This change is accompanied by a reduction in the expression of epithelial markers, the acquisition of a spindle-cell shape, and the expression of mesenchymal markers, such as fibroblast-specific protein-1 (FSP1), vimentin, N-cadherin, and α-smooth muscle actin (α-SMA).[4,7,8] The type 2 EMT endows cells with the capacity to synthesize and deposit ECM components.[9–11] Due to the expression of α-SMA in microfilament bundles or stress fibers, these cells also exhibit contractile properties, which play a major role in contraction. Moreover, the mesenchymal state is associated with migratory capacity. All of these characteristics are increasingly recognized as the main pathogenic factors of tissue fibrogenesis after damage.

It is becoming increasingly clear that EMT participates in the organ fibrosis after tissue injury. In tissue repair, the EMT is actually a double-edged sword. Epithelial cells undergoing the EMT gain motility and migrate to the site of injury to fill in defects and preserve the integrity of tissues, similar to the role of EMT in skin wound healing.[12] Moreover, the epithelial cells that gain mesenchymal features secrete ECM components that, when deposited, affect tissue remodeling and can lead to fibrosis and potential organ dysfunction. There is increasing evidence supporting the role of EMT in progressive kidney diseases, lung disease, and possibly liver disease.[13–15]

EMT evidence *in vitro* and *in vivo*

Fibrosis in vital organs, such as kidney, lung, and liver, can cause dysfunction and may be life-threatening, and emerging evidence shows that the EMT process is involved in tissue fibrosis. The role of EMT in tissue fibrogenesis has been suggested through studies on cultured cells *in vitro* and in animal models and human samples *in vivo*.[16–19]

A large body of evidence has shown that the epithelial cells derived from the kidney, lung, and liver could transition into mesenchymal cells *in vitro* in the presence of profibrogenic factors. TGF-β1, a profibrotic factor, is believed to play a key role in the induction of the EMT in various epithelial cells.[17] In addition, it has been noted that a variety of factors can promote the EMT of epithelial cells, including physical factors (hypoxia),[16] chemical factors (high glucose, angiotensin II, and albumin),[20,21] inflammatory mediators,[22] and matricellular proteins.[23,24] These factors induce the loss of the epithelial phenotype, the acquisition of the mesenchymal phenotype, secretion of interstitial matrix components, and the adoption of a spindle-like morphology *in vitro*. The cell culture models are beneficial for us to explore the mechanism of EMT, the related interference factors, and the associated treatment strategies. However, it is worth noting that it is likely to be difficult to show the *in vivo* pathological and physiological conditions in cultured cell models. Moreover, the results from these models must be further confirmed *in vivo*.

EMT has been proved in animal models. Strutz *et al*.[25] provided the first demonstration of EMT in renal tubular epithelial cells (TECs) in an anti-tubular basement membrane disease model. Subsequent studies showed EMT in nephrectomy and obstructive nephropathy. EMT actively participates in various other animal models of chronic kidney disease, such as diabetic nephropathy, experimental glomerulonephritis, and chronic allograft nephropathy.[18,26,27] It has been shown that a large number of cells express both mesenchymal and epithelial cell marker, suggesting that they are in the transitional state.[28–30] Recently, Chang *et al*.[31] investigated the mouse model of single-walled carbon nanotube-induced pulmonary fibrosis and found an increasing occurrence of N-cadherin-positive epithelial-derived fibroblasts at up to 42 days following exposure. In the bile duct ligation (BDL) model, a portion of the bile duct epithelium co-expressed cytokeratin-19 and α-SMA in the biliary epithelia and periductal region. Meanwhile, these cells actively produced collagen type I,[32] suggesting an epithelial-to-myofibroblast transition in bile duct epithelial cells (BECs) after BDL. In addition, the EMT of cholangiocytes has been detected in a nonalcoholic fatty liver disease animal model.[33]

A lineage tracing technique can be used to more intuitively reflect the cellular changes *in vivo*. The cell fate-tracing study published by Iwano *et al*.[14] provides crucial evidence supporting the existence of EMT in kidney fibrosis and demonstrated that approximately 36% of new fibroblasts originate from a local EMT using a transgenic mouse model. Kim *et al*.[34] reported that lung epithelial cells expressing vimentin in an established pulmonary fibrosis model used a cell fate-reporter mouse with overexpressing active transforming growth factor (TGF)-β1. Wu *et al*.[35] found that some alveolar epithelial cells in the same peribronchial areas and a few bronchial epithelial cells co-expressed E-cadherin and α-SMA in alpha-smooth muscle actin-Cre transgenic mice. Tanjore *et al*.[36] showed that approximately one-third of the FSP1-positive mesenchymal cells were derived from the lung epithelium in a bleomycin-induced pulmonary fibrosis model in cell fate reporter mice. Further evidence obtained with AlbCre.R26RstoplacZ double-transgenic mice *in vivo* has demonstrated that hepatocytes can undergo EMT. Hepatocytes can transdifferentiate into FSP1-positive fibroblasts that have lost albumin and still present an activated Laz gene after carbon tetrachloride (CCl4)-induced liver fibrosis.[14,37]

EMT has been found in human samples. The immunohistological analysis of injured organ has suggested that EMT plays a role in tissue fibrosis in humans. Nadasdy *et al*.[38] reported that a small subset of cells that were positive for epithelial antigens were located in the fibrotic interstitium of the samples derived from human end-stage renal disease. Jinde *et al*.[39] found that the incidence of α-SMA-positive TECs in cortical tubules isolated from fibrotic areas was 0.4% in IgA nephropathy and 3.8% in rapidly progressive glomerulonephritis. The TECs derived from α-SMA-positive cells were also positive for collagen types I and III. Moreover, recently, several research groups clearly detected the EMT of TECs in many samples from patients with various degrees of renal diseases and found that the expression level of these EMT markers correlated well with the extent of renal fibrosis.[40–42] In idiopathic pulmonary fibrosis/usual interstitial pneumonia patients, some of the hyperplastic epithelial cells overlying fibroblastic foci have been found to co-express epithelial markers and α-SMA.[43–45] More recently, studies of the samples from smokers and patients with chronic obstructive pulmonary disease showed that human bronchial epithelial cells upregulated the mesenchymal markers and downregulated the epithelial cell markers.[46] Furthermore, several research groups have found that BECs in human liver disease undergo EMT.[47,48] In addition, two research groups have found that hepatocytes undergo EMT by expressing the mesenchymal markers vimentin and α-SMA in chronic liver disease.[49,50]

However, there are studies arguing that EMT may not be operative in tissue fibrosis. One study failed to find TECs that had migrated outside of the tubular basement membrane and differentiated into mesenchymal-like cells in

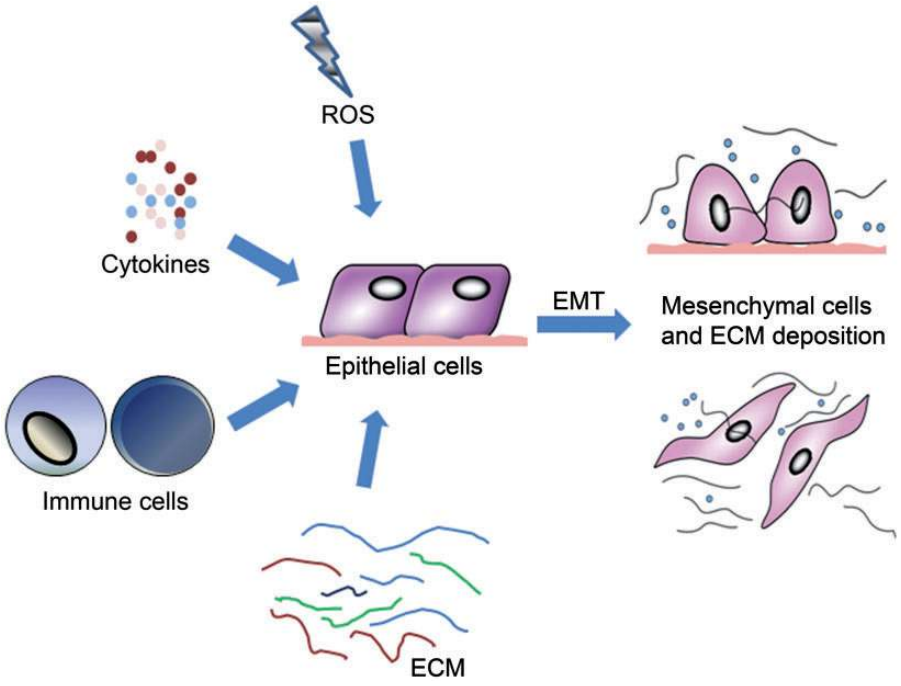

Figure 1 A schematic diagram of critical factors for the induction of the epithelial-mesenchymal transition and the process of EMT-related tissue fibrosis. (A color version of this figure is available in the online journal.)
ROS: reactive oxygen species; EMT: epithelial-mesenchymal transition; ECM: extracellular matrix

fate-tracing studies on TECs in various animal models (Habu venom with angiotensin 2, and unilateral ureteral obstruction and ischemia-reperfusion injury).[51] Two parallel studies also showed that none of the resulting fibrotic cells originated from the genetically marked hepatic and BEC cells in CCl4 and 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine models.[52,53]

Although the evidence accumulated to date shows that EMT exists in tissue fibrosis, particularly in the kidney, lung, and liver, contradictory results are constantly emerging. The heated debate of the existence of the type 2 EMT in tissue fibrosis appears irreconcilable. Therefore, great efforts should be made to understand why studies with almost identical experimental settings obtain opposite results. It is possible that the following problems lead to the observed discrepancy. First, there is a lack of specific markers for evaluating EMT. The commonly used mesenchymal markers include Snail, vimentin, FSP1, fibronectin, α-SMA, collagen I, N-cadherin, and matrix metalloproteinase (MMP)-2 and 9. Nevertheless, most of these markers are not exclusively expressed in fibroblasts and are also expressed in other cell types, such as inflammatory cells and endothelial cells. Thus, the combination of a variety of mesenchymal markers is widely used to evaluate EMT. Therefore, fibroblast-specific markers specific for fibroblasts thus need to be found. In addition, epithelial cells derived from different tissues may have specific markers to document EMT, and this is an issue that should be addressed. Second, EMT is an extremely dynamic process. Most of the studies involving EMT primarily depend on identifying the cells at the transitional stage, known as the partial EMT, where cells express both epithelial and mesenchymal markers. It is currently unresolved whether the partial EMT becomes a complete EMT and whether the epithelial cells lose their own phenotypic markers and undergo a phenotypic change to resemble fibroblasts. Another problem that requires further investigation is the relative roles of the partial EMT and the complete EMT in organ fibrosis. Third, the disparate results from the same animal models make it difficult to obtain valuable insights. Animal models cannot fully represent the pathological physiology of human diseases, but the research results from animal models may provide some useful reference information. Thus, it is important to control the reproducibility of a fibrotic model.

At present, supporters and opponents cannot reach a consensus regarding EMT-related tissue fibrosis, and the debate continues. Nevertheless, ample evidence suggests that the transitional proteins expressed in epithelial cells are related to the degree of tissue damage and fibrosis[54] and that the effective control of EMT can significantly improve tissue fibrosis. Therefore, the strategies for anti-EMT are emerging as promising anti-fibrotic treatments. In this review, I summarize the EMT inducers (Figure 1) and propose potential anti-fibrotic treatment strategies that target the EMT (Figure 2).

Contributors to the EMT

The microenvironment can determine the cell phenotype.[55,56] In chronic disease, the microenvironment of the damage site changes, exhibiting characteristics of hypoxia,

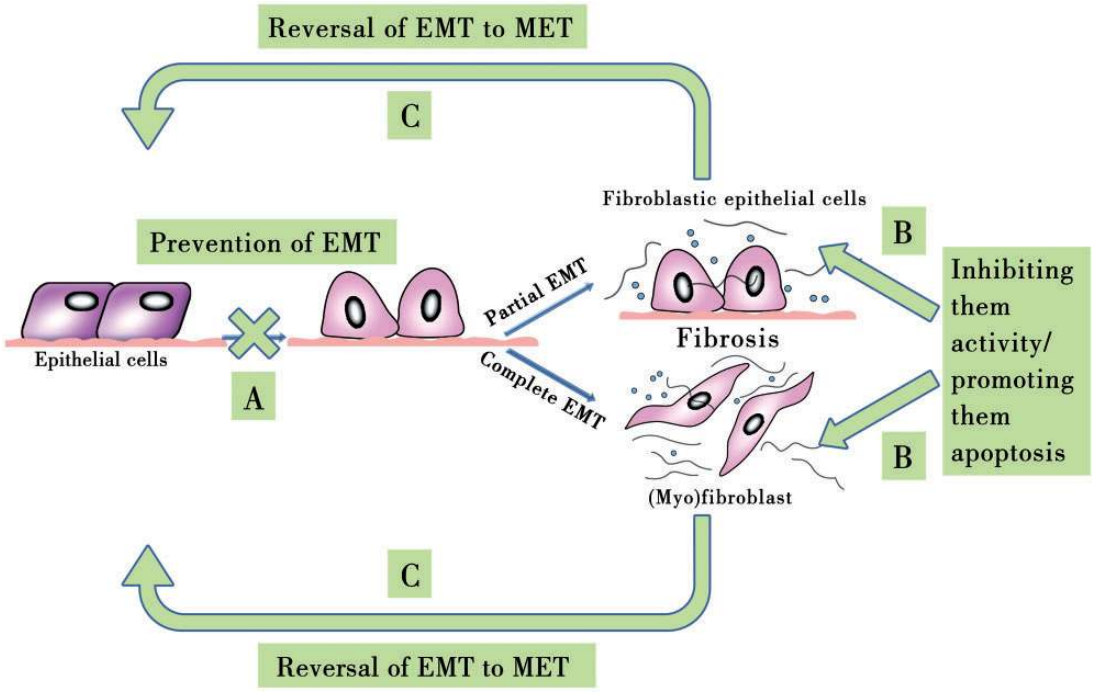

Figure 2 A schematic representation of the EMT in tissue fibrosis and three anti-EMT strategies as potential anti-fibrotic treatments. (a) Prevention of EMT; (b) removal of fibroblastic and/or myofibroblastic epithelial cells; (c) reversal of EMT to MET. (A color version of this figure is available in the online journal.)
EMT: epithelial-mesenchymal transition; MET: mesenchymal-epithelial transition

chronic inflammation, cytokine disorder, an imbalance in secreting and degrading ECM, and oxidative stress. Additionally, all of these factors are directly or indirectly involved in the regulation of the EMT program.

Hypoxia and EMT

Hypoxia is one of the important microenvironmental factors in the development of tissue fibrosis. Nevertheless, the potential mechanisms are not well understood. Recent studies investigating the role of EMT in fibrosis in different tissue types have shown that hypoxia decreases the expression of E-cadherin and induces the expression of vimentin and α-SMA in primary epithelial cells, suggesting that hypoxia induces EMT in these cells.[15,57,58] In addition to promoting EMT, hypoxia can directly induce the expression of collagen products to regulate ECM turnover, thereby driving fibrogenesis.[23] It has been demonstrated that hypoxia stimulates EMT in a variety of epithelial cells through a hypoxia-inducible factor-dependent mechanism, which is abasic-helix-loop-helix transcription factor that enables cells to survive in hypoxic-conditions.[15,57,58]

ECM and EMT

The normal ECM microenvironment can direct cellular processes to drive wound repair and regeneration, while an abnormal ECM microenvironment may direct the cells to begin pathological healing. ECM components can induce EMT in multiple cell types,[26] and the target genes within these pathways require further investigation. The MMPs family is one of the classes of ECM and was previously thought to be anti-fibrotic. Nevertheless, recently, MMPs have been found to play a role in the development of tissue fibrosis through EMT and fibroblast activation.[59] The results reported by Zhao *et al*. and Cheng and Lovett demonstrate that MMP-2 and MMP-9 can directly induce the entire course of the renal tubular cell EMT *in vitro*.[60–62] In addition, Rac-1b, an alternatively spliced variant of Rac-1, has been identified as a key factor in the MMP-induced EMT. Induction of the Rac-1b isoform leads to an increased production of cellular reactive oxygen species (ROS), which upregulate Snail, a transcription factor previously implicated in physiological and pathological EMT.[20,36,51] The collagen proteins constitute one class of ECM. It has been reported that collagen type I could further promote the TGF-β1-induced EMT in proximal TECs.[63] Collagen type I starts the EMT process by inducing the destabilization of the E-cadherin adhesion complex *in vitro*.[64] In addition, a recent discovery showed that type VIII collagen may be an important direct inducer of the EMT of TECs in diabetic wild-type mice.[65] Further studies should focus on the mechanobiological mechanisms involved in the EMT and the role of the mechanical forces generated by the cells in their effort to migrate through the stroma.

Cytokines and EMT

There is increasing evidence that cytokines play a critical role in EMT, particularly TGF-β and fibroblast growth factor (FGF)-2. TGF-β1 expression is strongly correlated with tissue fibrosis.[23,66–68] An increasing body of evidence shows that TGF-β1 affects the entire epithelial cell EMT

process. TGF-β1 decreases the expression of epithelial markers by the induction of transcriptional repressors, such as Snail-1, which can downregulate the expression of the TGF-β-receptor-associated protein SARA *in vitro*.[29,69,70] In addition, TGF-β1 induces MMPs, such as MMP-1, MMP-2, and MMP-3, to proteolytically shed E-cadherin *in vitro*.[33,71] Finally, E-cadherin, cytokeratin, the zonula occludens protein, and desmoplakin are repressed, whereas FSP1, fibronectin, and vimentin are upregulated. Furthermore, in response to TGF-β1, the epithelial cells undergo marked reorganization accompanied by the *de novo* expression of α-SMA.

FGF-1 and FGF-2 play different roles in EMT-related tissue fibrosis. FGF-1 is an inhibitory factor of EMT in tissue fibrosis, which will be discussed later in this review. FGF-2 is considered a contributory factor for EMT development by stimulating the critical proteases that are essential for epithelial unit disaggregation, thereby contributing to tissue fibrosis.[72] FGF-2 induces TEC motility across a tubular basement membrane and has been reported to not only reduce epithelial markers (cytokeratin expression) but also induce the expression of mesenchymal markers (vimentin and FSP1) which appear to be affected at the promoter level.[50] In addition, Masola *et al*. found that heparanase is necessary for FGF-2-induced EMT in proximal TECs through mediating the interaction between FGF-2 and its receptor. Therefore, heparanase may be an interesting therapeutic target for intervening in renal fibrosis.[73]

Inflammation and EMT

There is increasing evidence that inflammation at the site of tissue damage is involved in triggering the gene expression changes associated with EMT. In addition, inflammation likely induces EMT through the following mechanisms. First, the inflammatory cells directly induce EMT. Chronic inflammation with the feature of inflammatory cell infiltration has been proved to be a common characteristic of many chronic diseases and is also implicated in the progression of tissue fibrosis. Li *et al*.[74] showed that monocytes possessed the ability to induce tubular EMT via an NF-kB-dependent pathway only when they directly contacted with TECs. Second, tissue injury is followed by inflammatory cell infiltration, which then produces a variety of proinflammatory cytokines to provide a directional signal for EMT induction. Several studies have demonstrated an accentuated effect of inflammatory cytokines on TGF-β1-driven EMT. It has been proved that inflammatory factors, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1)β, enhance the effect of TGF-β1 on EMT induction in bronchial epithelial cells.[75–77] The study conducted by Ji *et al*.[78] showed that Th2-derived IL-4 and Th17-derived IL-17A provide an inflammatory microenvironment that favors the TGF-β1-dependent EMT induction in the bronchia and which is related to ERK1/2 activity. Furthermore, IL-22, another Th17 cytokine, coordinates the TGF-β1-induced promotion of the expression of EMT-related transcription factors (Snail-1 and Zeb1) and downregulates the expression of epithelial markers (E-cadherin and MUC5AC) in asthmatic bronchial epithelial cells.[79] In addition, it has been shown that TNF-α is an important proinflammatory cytokine that could suppresses vitamin D receptor (VDR) expression and which results in the sensitization of TECs to TGF-β1-triggered EMT in renal fibrosis.[80] Third, inflammatory cells produce profibrotic mediators that act in a paracrine manner to induce EMT.[74] Previous studies have shown that macrophage-derived TGF-β participates in tissue fibrosis via the paracrine activation of the myofibroblasts and the upregulation of TEC-derived myofibroblasts. Moreover, macrophages can synthesize and secrete collagens to affect the ECM milieu.[81] Although the relationship between inflammation and EMT has been proposed, the underlying mechanisms between inflammation and EMT, as well as the subsequent tissue fibrosis, have yet to be studied.

ROS and EMT

A number of recent studies have indicated that ROS also function as second messengers to mediate the EMT process. The relationship between EMT and oxidative stress in the kidney has been studied in TGF-β-induced renal proximal TECs,[82] chronic allograft nephropathy,[83] diabetic kidney disease,[84,85] and other kidney diseases. ROS generation can be augmented through the increased formation of advanced glycation end-products,[86] high glucose,[87] TGF-β,[88] and other proteins that drive the activation of the EMT process. For example, the study conducted by Rhyu *et al*.[82] demonstrated that ROS coordinate TGF-β1 to induce the TEC-EMT by activation of the MAPK and Smad pathways. Moreover, it has been demonstrated that ROS are associated with the EMT in a chronic allograft tubular atrophy/interstitial fibrosis model due to the high inducible nitric oxide synthase levels and the co-staining of α-SMA and Gp91 (the NADPH oxidase enzyme).[89] Recent reports provide evidence suggesting that the production of free radicals induces the EMT in the lung epithelium.[90] The ROS-mediated role in EMT may be due not only to their critical impact on signaling pathways but also to their oxidative modifications of structural proteins.[91]

The existing data suggest that multiple factors at the injured site constitute a microenvironment that induces or promotes EMT. However, it is not clear whether there are specific factors that are essential for the process. These factors are believed to interact with and complement each other to form a large regulatory network that controls EMT, and their effect varies depending on the tissue. In addition, there may be other as yet undiscovered factors involved in the process. Recent progress in gene expression profiling may lead researchers to define additional factors that are associated with EMT and which may help clarify its underlying mechanisms. Despite the recent findings highlighting the influence of multiple factors on EMT, additional studies are required to elucidate how these cues act synergistically to control the transition between the epithelial and mesenchymal phenotypes. Furthermore, it is important to note that only a portion of the epithelial cells transition into mesenchymal cells at the injury site, and further exploration of the biological characteristics of these cells is warranted.

What did we learn from EMT-related anti-fibrotic therapy and where should we go?

A variety of strategies have been developed to counter EMT-related fibrosis that are primarily involved in the following three aspects: prevention of EMT, removal of fibroblastic and/or myofibroblastic epithelial cells, and the re-transdifferentiation of myofibroblasts to epithelial cells.[92] These methods are summarized in this section, and promising strategies are proposed.

Prevention of EMT

To date, studies on EMT-related anti-fibrosis have primarily focused on blocking the occurrence of EMT, particularly through the use of an antagonist to the EMT-promoting factors. The main EMT inducer is TGF-β. During the pathogenesis of tissue fibrosis, TGF-β signaling, a pivotal inducer for fibroblast activation and EMT program is thought to interact with other inducers. Thus, interfering with TGF-β signaling may be an effective intervention to reverse established fibrosis.[93] In addition, many research studies have shown that interference with these signaling pathways almost completely inhibits the EMT process.

Many strategies have been investigated to inhibit TGF-β signaling and, therefore, ameliorate tissue fibrosis. Based on the characteristics of the TGF-β1 signaling pathway, there are various methods that can specifically disrupt its signaling at various levels, including the use of: (1) TGF-β1 antisense oligodeoxynucleotides to inhibit TGF-β mRNA expression[94,95]; (2) peptide ligands that occupy the same binding sites on TGF-β receptor I (TβRI) and TβRII to compete for receptor binding,[96] soluble TβRII fragments, or synthetic peptides to interfere with ligand-receptor interactions[97]; (3) small molecule that antagonize TGF-β kinase activity[98,99]; (4) a small molecular agent to decrease phosphorylated Smad2 levels and the nuclear translocation of Smad2[98]; and (5) overexpression of the natural TGF-β signaling inhibitor Smad7 to induce Smad7 expression.[100]

The purpose of these strategies is primarily to block the TGF-β signaling pathway. It has been shown that TGF-β has a wide range of biological functions, affecting all of the cells that are involved in wound repair.[101,102] Therefore, blocking the TGF-β signaling pathway is bound to affect the process of healing. In chronic disease, TGF-β is usually overexpressed at the wound site. Thus, it is important to develop some strategies for adjusting the level of TGF-β to a physiological level. Unfortunately, this has been difficult to implement so far. A method that uses cytokines to directly act on the biological effects of TGF-β may be more suitable. It has been proved that hepatocyte growth factor (HGF) has an anti-fibrotic effect in part due to the reduction of TGF-β and the modulation of the EMT,[103] and its anti-fibrotic properties have been demonstrated in experimental models of lung fibrosis, kidney fibrosis, heart fibrosis, skin fibrosis, and liver fibrosis. Other cytokines, such as bone morphogenetic protein-7 (BMP-7),[104] BMP-2, and vascular endothelial growth factor,[73,105] block TGF-β1-induced mesenchymal marker expression and restore E-cadherin expression in a dose-dependent manner. In addition, zinc plays a vital role in preventing EMT-driven fibrosis *in vivo* by significantly inhibiting TGF-β1 and ROS production, likely through the inhibition of the TGF-β/Smad, MAPK and NF-κB pathways.[106] miRNAs not only have profibrotic properties but also exhibit anti-fibrotic properties. For example, the let-7d,[107] miR-141,[108] miR-382,[109] and miR-200[110] families inhibit the EMT through TGF-β-dependent or TGF-β-independent mechanisms. More importantly, most of these cytokines and mediators have positive roles in wound repair and regeneration.[111–113] Therefore, they not only inhibit the TGF-β pathway to inhibit fibrosis but also adjust the microenvironment in the damaged area to promote healing. Therefore, the method of using cytokines to directly act on TGF-β seems to be a feasible solution.

In addition, based on the direct role of immune cells in the induction of EMT, we hypothesize that eliminating the local immune cells or blocking the effects of the inflammatory cytokines secreted from these immune cells may effectively inhibit the EMT.

Two methods can be used to deplete macrophages in a wound site. For example, a series of studies have reported that usage of anti-macrophage serum, liposomal clodronate, or sublethal irradiation, which nonselectively depletes macrophages, can abrogate persistent inflammation in experimental acute kidney damage to block the development of fibrosis.[114–117] However, macrophages have many subtypes with different functions. For example, in hepatic fibrosis, hepatic macrophages not only regulate the proliferation of stellate cells[118] but also play a critical role in ECM regression during the remodeling phase after hepatic injury.[119] Moreover, Chazaud *et al*.[120] found that macrophages activated muscle progenitor cells by releasing pleiotropic cytokines and promoted myogenic growth after damage. Furthermore, Arnold *et al*.[121] reported that CX3CR1hiLy-6C+ macrophages rapidly switched to an M2 phenotype with anti-inflammation functions after skeletal muscle damage. Therefore, exploring the subpopulations of macrophages will help us to selectively deplete the macrophages that are involved in tissue fibrosis.

Furthermore, various lines of evidence show that neutralizing inflammatory factors inhibits the accentuation of EMT. The results reported by Borthwick *et al*.[75] show that blocking TNF-α using a TNF-α neutralizing antibody relieves EMT occurrence in bronchiolitis obliterans syndrome. Recently, Mi *et al*.[122] found that blocking IL-17A, a glycoprotein secreted from IL-17-producing cells, can inhibit reverse pulmonary fibrosis through both TGF-β1-dependent and -independent mechanisms. In addition, nuclear factor-κB (NF-κB) is a central regulator in inflammation. Both the inactivation of NF-κB and the upregulation of an endogenous inhibitor of NF-κB improve inflammatory injury or fibrosis. For example, Miyajima *et al*.[123] found that NF-κB inhibition through the administration of dehydroxymethylepoxyquinomicin decreased the mean interstitial fibrosis in the obstructed kidney. In contrast, enhancing the expression of the endogenous inhibitor of the NF-κB pathway, I-κB, or inhibiting the pathway through the use of curcumin or berberine[124] alleviates lung or renal fibrosis and macrophage infiltration.[125,126] These findings demonstrate that the inhibition of inflammatory factors may delay

the progression of tissue fibrosis by preventing the EMT representing a novel strategy requiring further study.

Removal of fibroblastic and/or myofibroblastic epithelial cells

A number of previously developed anti-fibrotic strategies are primarily aimed at preventing myofibroblast formation by interfering with critical factors in the process of differentiation.[127] However, once fibrosis has already progressed, inhibiting myofibroblast formation may not be successful in relieving fibrosis. The established myofibroblasts in wounds are likely a potential target for the development of effective anti-fibrotic therapies.

Promoting the apoptosis of fibroblast/myofibroblast in the damaged area may be a feasible way to alleviate local fibrosis. A large number of pathways are available that promote the apoptosis of fibroblasts/myofibroblasts. Nerve growth factor and basic FGF are able to trigger myofibroblast apoptosis through the Rho/Rho-kinase signaling pathway and the phosphatidylinositol-3-kinase/Akt pathway.[128,129] Another promising approach to promote apoptosis is interfering with myofibroblast mechanoreception. Stress is important to sustain the MF phenotype, and a condition with stress release often drives MFs toward suicide.[130] It has been demonstrated that both the pharmacological disruption of this mechanotransduction pathway, such as the ROCK inhibitor fasudil, and the remodeling of the ECM composition can induce fibroblast/myofibroblast apoptosis.[131,132] Cell transplantation has been documented as another effective method for promoting fibroblast/myofibroblasts apoptosis. Nunes de Carvalho *et al.*[133] showed that bone marrow mononuclear cell transplantation can stimulate myofibroblast apoptosis. Moreover, a number of targeted strategies are emerging to delete the myofibroblasts. These strategies include modifying drug-carrying peptides to recognize type VI collagen or platelet-derived growth factor receptors. In addition, Douglass *et al*. developed a single-chain antibody (C1-3) that specifically targets α-SMA-positive liver myofibroblasts. The C1-3-targeted gliotoxin was found to deplete a twofold increase in liver myofibroblasts compared with the free gliotoxin group.[134] These data demonstrate that specifically inducing myofibroblast apoptosis is one promising strategy for anti-fibrogenic therapy. However, the fate of the fibroblastic/myofibroblastic epithelial cells in the fibrotic tissue and the distinction between the biological characteristics of the fibroblasts/myofibroblasts originating from different epithelial cells, which may have different sensitivities to apoptosis-inducing factors, have not been addressed.

Re-transdifferentiation of myofibroblasts to epithelial cells or dedifferentiation of myofibroblasts to fibroblasts

Studies have shown that myofibroblasts are not the terminally differentiated cells. In fact, these cells can revert into their original cells, which include epithelial cells and fibroblasts.

There are mediators for myofibroblast re-transdifferentiation into epithelial cells. Brown *et al*.[135] reported that TGF-β promotes the EMT program in epithelial cells, but these cells restore an epithelial phenotype in the absence of continuous signaling. Shukla *et al*.[136] recently demonstrated that HGF reverses the TGF-β-induced EMT in human and rat alveolar epithelial-like cell lines by inducing Smad7, the inhibitor of the TGF-β signaling pathway. In addition, FGF-1 has the ability to reverse the TGF-β-induced EMT by affecting the mitogen-activated protein kinase/ERK pathway, and subsequently inhibiting Smad2 phosphorylation. Moreover, a number of polyphenols, such as procyanidins and proanthocyanidins, have anti-inflammatory and antioxidant properties which can reverse the EMT process.[137,138]

Furthermore, there are mediators for myofibroblast dedifferentiation. Although this review mainly focuses on EMT in tissue fibrosis, the reversal of myofibroblasts may be common in cells from different origins. Thus, future studies should investigate methods to reverse the fibroblast-derived myofibroblasts. Recent evidence has suggested that several types of anti-fibrotic factors or drugs possess the ability to reverse the myofibroblast phenotype into the original cells. Thus, we will briefly review several mediators that reverse myofibroblast differentiation.

As discussed in this review, mechanical stress participates in myofibroblast formation. Consistent with this hypothesis, Li *et al*. found that the human amniotic membrane stromal extract not only helps maintain the primary amniotic membrane stromal cells fibroblastic phenotype *in vitro* but can also induce the dedifferentiation of myofibroblasts into a fibroblast phenotype without affecting their proliferation. Moreover, these effects may be due to the loss of mechanical stress.[139] Prostaglandin E2 (PGE2) was recently shown to have the potential to dedifferentiate myofibroblasts. PGE2 treatment effects a dose-dependent decrease in a-SMA and collagen I expression in TGF-β- or endothelin-induced fibroblast differentiation, which is associated with the inhibition of focal adhesion kinase signaling. A focal adhesion kinase inhibitor was also capable of reversing the myofibroblast phenotype.[140] In addition, two previous studies have reported that capsaicin, the pungentphenolic constituent of various peppers, can induce the dedifferentiation of myofibroblasts to hepatic stellate cells by anti-fibrotic and anti-inflammatory actions to enhance peroxisome proliferator-activated receptor-γ expression or decreasing the expression of the proinflammatory mediator COX-2.[141,142] Although several studies have focused on the anti-inflammatory properties of liposomal Cu/Zn superoxide dismutase (SOD), Vozenin-Brotons *et al*.[143] recently identified that SOD can downregulate TGF-β expression *in vitro* and reduce the levels of myofibroblast markers to yield anti-fibrosis effects. Furthermore, several transcription factors contribute to the dedifferentiation of myofibroblasts. The expression of MyoD has also been associated with the presence of myofibroblasts in tissue repair/fibrosis.[144,145] Hecker *et al.* found that the suppression of MyoD in myofibroblasts increases cellular proliferation and dedifferentiation. Moreover, the dedifferentiation process in myofibroblast was found to be mediated by

the mitogen-ERK1/2MAPK-CDK pathway, which leads to the downregulation of MyoD and α-SMA expression.[146] This result was further confirmed by Yang *et al*.[147] In addition, it has been reported that NF-E2-related factor 2 activation by Keap1 siRNA or sulforaphane could induce the dedifferentiation of myofibroblasts into a control-like phenotype by downregulation of collagen Iα1 and a-SMA and decreasing the proliferation, the migration, and contraction of the cells.[148] These data suggest that myofibroblasts are not terminally differentiated cells and are more plastic than previously appreciated.

It appears that inhibiting the formation and activation of the myofibroblasts is an effective method for preventing and treating fibrosis. However, we should note that the appropriate activation of fibroblasts and myofibroblasts is beneficial for tissue repair, because these cells can adjust to the local microenvironment and provide a supporting structure for other cells by secreting ECM components. Therefore, an effective anti-fibrosis treatment should focus on controlling/maintaining the appropriate amount of fibroblasts/myofibroblasts.

A promising strategy for anti-EMT-related fibrosis: Mesenchymal stem cells

The methods that have been developed for anti-EMT-related fibrosis to date are associated with the TGF-β signaling pathway directly or indirectly, even though they can improve tissue fibrosis. However, this is not sufficient. Furthermore, the analysis of the cytokines, inflammation, and ROS that induce the EMT has demonstrated that the microenvironment of the epithelial cells changes as a result of the joint actions of many factors. Therefore, a strategy to inhibit only a certain factor or inflammation is not reliable. Cell therapy is likely to be required to help restore the original cellular milieu due to the paracrine effects of stem cells. Stem cells can not only physically and functionally replace cells lost to tissue damage but also secrete a large number of cytokines to adjust the local microenvironment. In addition to the above-mentioned characteristics of mesenchymal stem cells (MSCs), these cells also have the ability of immune regulation, which give MSCs more advantages over other stem cells in the context of tissue repair and regeneration. A few studies on the use of MSCs for the treatment of fibrosis are emerging.

MSC-based therapy is becoming an attractive treatment for tissue fibrosis. Studies on animal tissue fibrosis models have demonstrated that the intravenous and local administration of MSCs attenuates injury and fibrosis, suggesting a potential clinical application for MSCs in the treatment of tissue fibrosis.[149,150] In addition, MSCs with low immunogenicity can be isolated from various tissues. More importantly, when the cells are implanted *in vivo,* they do not transdifferentiate into a malignant phenotype.[151] Therefore, MSC-based therapy for tissue fibrosis is promising. At present, the paracrine signaling theory is preferred as a plausible explanation for the therapeutic effects of MSCs. Studies have shown that MSCs secrete numerous mediators that play a role in anti-EMT-related fibrosis through the following two mechanisms.

MSCs secrete a myriad of proteins to antagonize and regulate factors that contribute to the EMT, thereby balancing the cytokines in wounds. HGF and BMP-7 which we mentioned above play a role in preventing EMT. They are essential growth factors secreted by the MSCs, and as potent mediators in preventing tissue fibrosis.[137,152,153] In a renal injury animal model, Du *et al*.[154] demonstrated that WJ-MSCs can delay the occurrence of tubular EMT and rescue of renal fibrosis by enhancing native and foreign HGF synthesis, thereby modulating the balance of HGF/TGF-β1. In addition, HGF derived from MSCs was found to inhibit the TGF-β1-induced EMT by blocking TGF-β1 signaling in human peritoneal mesothelial cells in a high-glucose environment.[155] Lv *et al.* recently found that bone marrow-derived MSCs secrete a large amount of BMP-7 and that the expression of BMP-7 was markedly increased, leading to decreased TGF-β and phosphorylated Smad2 and Smad3 expression in the diabetic kidney after MSC treatment. In addition, MSC transplantation was found to effectively prevent α-SMA protein upregulation and E-cadherin protein downregulation in TECs of the diabetic kidney.[156]

MSCs secrete a number of cytokines to regulate the inflammation microenvironment in wounds. Among the growing list of molecules secreted by MSCs are those which have been shown to possess an inflammation suppression function, such as keratinocyte growth factor, IL-1 receptor antagonist, and TNF-α-inducible gene-6, by antagonizing cytokines, including TNF-α and IL-1.[157–159] In addition, MSCs from bone marrow and adipose tissue have been shown to reduce inflammation in injured lung tissue by effectively decreasing the numbers of inflammatory cells likely through the suppression of chemokines, such as monocyte chemotactic protein-1 and macrophage inflammatory protein-1a.[160] As discussed above, inflammation plays a vital role in the occurrence of EMT. Thus, the anti-inflammatory effect of MSCs may prevent the EMT through immunoregulation.

In addition, MSCs can significantly promote myofibroblast apoptosis to reduce the local deposition of ECM, thereby improving fibrosis.[133] Therefore, the mechanism by which MSCs alleviate fibrosis is multi-faceted, requiring the orchestration of various endogenous and exogenous factors to conduct the EMT process, which may make MSC-based therapy superior to the traditional strategies.

Conclusion and perspectives

Many studies have demonstrated that a growing list of epithelial cells derived from different tissues after stress/injury transition into fibroblasts or myofibroblasts which contribute to the pathogenesis of tissue fibrosis. Many extracellular signaling factors that control EMT have been identified and can be explored in the future development of anti-fibrotic therapeutics. This review highlights the research evidence of EMT in the kidney, lung and liver and enumerates the problems that need to be resolved. Future research efforts with the goal of developing an anti-fibrosis strategy should address the challenges and problems highlighted in this review, including the identification of reliable biomarkers and stable and reproducible

animal models, the elucidation of the control center of various signaling pathways for the EMT transcriptional program, and the design of effective clinical trials to evaluate the safety and effectiveness of different strategies. Nevertheless, novel anti-fibrosis strategies are continuously emerging, and the mechanisms mediating anti-fibrosis effects *in vitro* and *vivo* in different fibrosis-related diseases need to be further researched. In this review, we summarize the existing anti-fibrosis strategies and postulate that stem cell-based therapy will be a promising strategy for the inhibition of EMT-related fibrosis. It is expected that a more in-depth understanding of the process of EMT and the molecular mechanisms underlying fibrosis will aid the development of effective strategies for the treatment of fibrotic disease disorders in the near future.

Author contributions: All authors contributed to the writing of this review article and have read and approved the final manuscript. ML, FL and YZ contributed equally to this work.

ACKNOWLEDGEMENTS

This research was supported in part by the National Basic Science and Development Program (2012CB518103, 2012CB518105), the 863 Projects of Ministry of Science and Technology of China (2013AA020105 and 2012AA020502), National Natural Science Foundation of China (81201479, 81121004, and 81230041), Military Medical Foundation (AWS11J008), and Key Sciences and Technology Project in Hainan Province (ZDZX2013003).

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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(Received December 8, 2014, Accepted June 19, 2015)