MicroRNAs in TGF- β /Smad-mediated Tissue Fibrosis

Patrick Ming-Kuen Tang · Hui-Yao Lan

Published online: 14 September 2014
© Springer Science+Business Media New York 2014

Abstract Fibrosis is a key process leading to the end-stage organ scarring with loss of functions in a number of human diseases, contributes to about 45 % of deaths in the industrialized world. TGF- β signaling is one of the most important pathways for fibrogenesis in the diseased tissues. Increasing evidence shows that Smad3 is an important mediator of TGF- β /Smad signaling which enhances fibrosis by directly regulating the microRNAs (miRNAs) at transcriptional level. Recent studies clearly show an important role of miRNAs in the pathogenesis of tissue fibrosis in response to TGF- β . It has been reported that Smad3 can regulate a number of TGF- β -dependent miRNAs including miR-21, miR-29, miR-200, miR-192, and miR-433 during tissue fibrosis. Targeting these miRNAs have shown to effectively inhibit fibrosis in the heart, liver, lung, and kidney diseases. A better understanding of the underlying mechanism will provide valuable information for the development of effective therapy for fibrotic diseases.

Keywords microRNAs · Fibrosis · Smad · TGF- β signaling

Introduction

Tissue fibrosis is one of the major causes of organ dysfunction at the end stage of a number of human diseases including idiopathic pulmonary fibrosis (IPF), cirrhosis, cardiovascular disease, chronic kidney disease (CKD), systemic sclerosis, and other fibrotic disorders, contributing to about 45 % of the deaths in the industrialized world [1]. Damage to tissues can initiate the repair process which is beneficial including two major phases: (1) regenerative phase, where injured cells are replaced by new cells of the same type without traceable damage and (2) fibrosis phase, where connective tissues replace the normal parenchymal tissue. However, an inappropriate repair process can be pathogenic. Substantial deposition of extracellular matrix (ECM) components results in permanent fibrotic scar after chronic inflammation and tissue repair [2]. Fibrosis initiates when myofibroblasts synthesize the new collagen is much faster than the rate that it is degraded at the site of the damaged tissue. This process is regulated by a number of metalloproteinases (MMPs) and their inhibitors including the tissue inhibitors of metalloproteinases (TIMPs). Uncontrolled shifts in synthesis versus catabolism of the ECM regulate the equilibrium of collagen within the diseased tissues will lead to fibrogenesis [3]. Fibrosis is the consequence of chronic inflammatory reactions triggered by a variety of factors including tissue injury, allergic responses, persistent infections, chemical insults, autoimmune reactions, radiation, and so on [4]. The signatures of fibrosis including excess accumulation of ECM components, collagens, glycoproteins, as well as myofibroblasts and inflammatory cells within the affected tissues. The aggressive replacement of functional tissues by deposition of ECM leads to the dysfunction and failure of an organ. For example, renal fibrosis is commonly found in CKD

P. M.-K. Tang · H.-Y. Lan (✉)
Li Ka Shing Institute of Health Sciences and Department of
Medicine and Therapeutics, The Chinese University of Hong
Kong, Shatin, New Territory, Hong Kong, Hong Kong SAR,
China
e-mail: hylan@cuhk.edu.hk

P. M.-K. Tang
e-mail: patrick.tang@cuhk.edu.hk

H.-Y. Lan
Shenzhen Research Institute, The Chinese University of Hong
Kong, Shenzhen, China

during the development of end-stage renal failure, and cardiac fibrosis is identified during hypertensive cardiac remodeling [5].

Increasing evidence shows that transforming growth factor- β 1 (TGF- β 1) is an important mediator for fibrogenesis, where TGF- β 1/Smad3 signaling is suggested to be the main pathway leading to tissue fibrosis in a number of diseases including bleomycin-induced pulmonary fibrosis [6], Angiotensin II (Ang II)-induced hypertensive heart disease [7], carbon tetrachloride (CCl₄)-induced hepatic fibrosis [8], unilateral vocal fold injury [9], unilateral ureteral obstructive nephropathy (UUO) [10], and immunologically induced anti-glomerular basement membranous glomerulonephritis (anti-GBM GN) [11]. In the TGF- β /Smad signaling pathway, TGF- β binds to T β RII and stimulates the T β RI-kinase to phosphorylate Smad proteins. The activated Smad2 and Smad3 assemble with Smad4 in the cytosol, and then the complex translocates into the nucleus and regulates the transcription of target genes including their inhibitory feedback protein Smad7. The expressed Smad7 blocks the Smads activation by triggering the degradations of T β RI and Smad proteins which giving out a negative feedback to suppress the TGF- β /Smad signaling [12, 13]. Recently, a number of miRNAs (e.g., miR-21, miR-29, miR-192, miR-200, and miR-433) are demonstrated to be the important regulators of Smad3-mediated TGF- β /Smad signaling during fibrosis [14–18]. As the importance of both miRNA and Smad proteins on TGF β /Smad signaling for fibrogenesis has been recognized, this review will summarize the latest research progresses of miRNAs on the Smad-dependent tissue fibrosis.

Mechanism of miRNA-mediated Gene Regulation

The miRNAs are endogenous, non-coding, and contain only approximately 22 nucleotides; they are well conserved in eukaryotic organisms and can regulate translational repressions by binding to the 3' untranslated region (3'UTR) of its target mRNA in an imperfect complementary manner [19]. Furthermore, miRNAs are predicted to regulate approximately 30 % of protein-coding genes and participate in the post-transcriptional regulation in mammals. In brief, miRNA is transcribed to be a long stem-loop primary miRNA (Pri-miRNA) and cleaved to be a double-stranded shorter miRNA precursor (Pre-miR) in the nucleus, the Pre-miR is then exported to the cytoplasm and further cleaved to be the 20–22 base pairs double-stranded RNAs (mature from) by the RNase III enzyme Dicer. The mature miRNA is unwound and the functional strand is loaded onto the RNA-induced silencing complex (RISC) which guides the complex to bind onto the 3'UTR of its target mRNA. The miRNA-mRNA complex will lead to

mRNA cleavage, translational regulation, as well as transcriptional regulation [20]. The importance of miRNAs has been demonstrated on a number of biological processes including differentiation, development, cell proliferation, immunity, carcinogenesis, glucose homeostasis, as well as fibrosis [21]. The high plasticity of miRNAs facilitates their involvements in the pathogenesis of a number of diseases including tissue fibrosis [16].

Role of miRNAs in TGF- β /Smad-mediated Tissue Fibrosis

miR-21

miR-21 is one of the well study miRNAs in the fibrotic diseases, it is upregulated in a number of disease models including dystrophic muscle [22], thioacetamide-induced hepatic fibrosis [23, 24], bleomycin-induced pulmonary fibrosis (IPF) [25, 26], and renal fibrosis in the unilateral ureteral obstruction (UUO) model [27, 28]. The miR-21-mediated fibrosis is suggested to be Smad-dependent, as the renal fibrosis was prevented in Smad3-deficient mice during UUO induction where miR-21 expression is upregulated [27]. TGF- β stimulation can establish the formation of a microprocessor complex containing Smad3 that triggers the transcription of miR-21, which has been reported on the UUO and liver fibrosis models [24, 27]. The mechanism of Smad3-mediated miR-21 overexpression is further intensified by the discovery of several putative binding sites for Smad3 at the promoter region of miR-21 in a liver fibrosis model [24]. The elevated miR-21 acts as a profibrogenic enhancer by repressing the inhibitory effects of Smad-7 on the TGF- β signaling pathway during liver fibrosis, as well as diabetic nephropathy [24, 29], it is because miR-21 can directly binds to the 3' UTR of Smad7 mRNA in the affected tissue which was confirmed by Abraham's group where high level of miR-21 was found in the lung of mice with bleomycin-induced fibrosis and patients with IPF [26, 30]. Therefore, TGF- β activates Smad3 which directly triggers the transcription of miR-21, and then the evoked miR-21 affects the cellular availability of Smad7 via translational inhibition as a positive feedback to prolong the TGF- β /Smad3 signaling in order to facilitate the development of fibrosis in the affected tissue. Importantly, miR-21 can enhance the phenotypes and progression of the disease: high expression level of miR-21 is positively correlated with inflammation and fibrosis biomarkers (e.g., urine albumin creatine ratio (ACR), collagen IV (ColIV), fibronectin (FN), and TIMP1), but negatively correlated with creatine clearance ratio (Ccr) and MMP-9 expression level in the kk-ay diabetic nephropathy mice [31]. The possibility of miR-21 as

a therapeutic target for tissue fibrosis was demonstrated in a number of studies: inhibiting miR-21 expression by angiotensin-1-7,3,3'-diindolylmethane ameliorates (DIM) or baicalein significantly improved the severity of fibrosis in the animal models of diminished muscle strength, hepatic, and pulmonary fibrosis [22, 32–34]. Administration of antagomir-21 not only improved the Ccr and ACR but also decreased the synthesis of profibrotic proteins (i.e., TIMP1, ColIV, and FN) in the kk-ay diabetic nephropathy mice [31]. Nevertheless, miR-21 suppression significantly reduced the severity of pulmonary fibrosis in the experimental animals even after the lung injury had been already initiated for 5–7 days [30].

miR-29

miR-29 is another well-study miRNAs in the area of fibrosis, it is suggested to have a protective role as an anti-fibrotic mediator in the Smad-dependent tissue fibrosis [15]. Its expression level is significantly reduced in intestinal fibrosis [36], renal fibrosis [28], profibrogenic mediator stimulated hepatic stellate cells [24], diabetic and hypertension nephropathy [37–39], cardiac hypertrophy [40, 41•, 42], and pulmonary fibrosis [43]. Indeed, the involvement of miRNAs in Smad-mediated fibrogenesis was firstly revealed in a study of cardiac remodeling by Mann's group in 2009, where miR-29a were found to be involved in the Smad3-mediated signaling in the heart disease [41•]. The loss of miR-29 expression is suggested to be Smad3-dependent, because it can be prevented in the Smad3 knockout mice and Ang II-induced hypertensive nephropathy mice with Smad7 overexpression [39, 40, 41•, 42, 44]. As an anti-fibrotic factor, miR-29 establishes its protective effect via the suppression of TGF- β /Smad signaling by affecting the expression of TGF- β , Smad3, and connective tissue growth factor (CTGF), down-regulation of these proteins was demonstrated in the miR-29 overexpressed pulmonary fibroblasts in vivo and in vitro [43]. It is interesting that miR-29 as a downstream of Smad3 can establish suppression on its upstream TGF- β and Smad3, a possible working mechanism of this miR-29b-mediated negative feedback on the TGF- β 1/Smad3 signaling was discovered by Lan's group, i.e., miR-29b is able to bind the coding sequence of TGF- β 1 mRNA at exon 3 which blocks the translation of TGF- β 1 so that suppresses the Smad3-dependent fibrosis [45]. Furthermore, the therapeutic potential of miR-29 family on tissue fibrosis has been investigated. The importance of miR-29 on the synthesis of ECM was demonstrated by Mann's group in 2009; it was revealed that miR-29a is able to suppress the expression level of collagen gene in cardiac fibroblasts in vitro [42], and consistent trend was observed on the collagen homeostasis in the tissue samples from the

patients with liver or intestinal fibrosis [24, 36]. In addition, overexpression of miR-29b could inhibit peritoneal fibrosis in a mouse model of peritoneal dialysis by blocking the Sp1-TGF-Smad signaling [46•]. However, the underlying mechanism of miR-29 on fibrosis prevention is still unclear and maybe a tissue or disease-specific response. It is because overexpression of miR-29b was not able to alter the production of TIMP-1, MMP-3, and MMP-12 in the fibroblasts from patient with Crohn's disease, but exogenous overexpression of miR-29 family could block the TGF- β -mediated up-regulation of Adam12 and Adam19 in the UUO and hypertension-induced nephropathy models [36, 38]. Further investigation should be done for evaluating the potential application of miR-29 as a therapeutic target on fibrotic diseases in the clinical settings.

miR-192

The involvement of miR-192 during the development of Smad3-dependent renal fibrosis was firstly demonstrated by Lan's group, a closed association between miR-192 expression and TGF- β /Smad signaling was revealed by a microarray analysis of the fibrotic kidney from a UUO mouse model in vivo. The finding was further confirmed in vitro by the overexpression of Smad7 in NRK52E cells (a rat tubular epithelial cell line) and a rat nephrectomy model, the miR-192 expression was inhibited which lead to an improvement on the experimental-induced renal fibrosis. It is found that miR-192 can be transcriptionally down-regulated by Smad3 via the direct binding of Smad3 protein on its promoter region, so the activated TGF- β /Smad signaling can suppress miR-192 expression and lead to a reduction of collagen matrix expression in order to facilitate the development of renal fibrosis [46•].

miR-200

The involvement of miRNAs in renal fibrosis is the most documented among other Smad-dependent fibrotic diseases. The miR-200a and miR-141 were shown to be down-regulated in the UUO-induced mice at the early phase of the disease, and the mice with early or advanced kidney diseases induced by streptozotocin or adenine [47–49]. Their involvement in TGF- β /Smad3-dependent EMT (epithelial-mesenchymal transition) and fibrosis was also demonstrated on both of the in vivo and in vitro models; where miR-200a suppressed TGF- β 2 expression, Smad3 activity and matrix proteins production by inhibiting TGF- β 2 translation due to its direct binding on the 3'UTR of TGF- β 2 mRNA. The miR-200a also prevented EMT of tubular epithelial cells by down-regulation of E-cadherin transcriptional repressors zinc fingers ZEB1 and ZEB2 [47–49]. Furthermore, not only TGF- β but also high

Fig. 1 An example of miRNA-mediated feedback mechanisms on TGF- β /Smad signaling during tissue fibrosis. TGF- β promotes fibrosis by inducing miR-21, miR-192, and miR-433, but suppressing miR-29 and miR-200 via activated Smad3 during renal fibrosis. Interestingly, the induced miR-21 suppresses the translation of Smad7 mRNA, therefore, reduces the inhibitory effect of Smad7 on Smad3

which prolonging the activation of Smad3 and facilitating fibrosis. In contrast, the suppression on miR-29 reduces inhibition on the translation of TGF- β 1 mRNA that increases the availability of TGF- β 1 and sustains the fibrogenesis in the diseased tissue. (The dashed lines represent pathways that are suppressing by the activated TGF- β /Smad3 signaling during fibrosis)

glucose concentration can trigger the expression of miR-377 in diabetic milieu which contributes to the development of diabetic nephropathy, but the underlying mechanism and the potential role of Smads are still unclear [50].

Other miRNAs

Besides renal diabetic nephropathy, the participation of miRNAs on Smad-dependent fibrogenesis has been reported on systematic fibrotic diseases. For cystic fibrosis, miR-145 and miR-494 are significantly upregulated in the nasal epithelial tissues of the patients [51]. The miR-494 is suggested to be a genetic modifier for modulating the CFTR gene expression and triggering the clinical symptoms of the cystic fibrosis, whereas miR-145 overexpression is correlated with the reduction of Smad3 mRNA level in the nasal airway and epithelial cells of cystic fibrosis patients. It is reported that miR-145 can significantly decrease the activity of Smad3 3'UTR-reporter, which directly silences Smad3 at the transcriptional level. Interestingly, hyper-inflammation is frequently observed at the airways of cystic fibrosis patients. As Smad3 plays an important role in the TGF- β 1-mediated negative regulation of the NF- κ B/IL-8 pathway, but Smad3 expression is

significantly reduced in the airway during cystic fibrosis which it is suggested as a possible mechanism for the improper inflammatory and fibrosis of the airway in cystic fibrosis patients. The miR-145 is suggested as a potential therapeutic target for repairing the abnormality of TGF- β 1/Smad3 signaling in the cystic fibrosis patients [51].

The involvement of miR-25 was reported in Smad-mediated fibrogenesis by the first study about Smad-mediated miRNAs in the heart disease [41••]. In addition, miR-133a is one of the highly expressed miRNAs in the heart and found to be suppressed during diabetes-associated cardiac fibrosis in the streptozotocin-induced diabetic animal model by Chakrabarti's group. The reduction of miR-133a was followed by an upregulated expression of major fibrotic markers including TGF- β 1, CTGF, fibronectin, and COL4A1 in vivo, whereas overexpression of miR-133a prevented the phosphorylation of ERK1/2 and Smad-2 following an improvement on the cardiac fibrosis in the diabetic mice [52, 53].

Furthermore, a marked reduction of let-7d, a miRNA belongs to let-7 family which is one of the first discovered and most studied miRNA group on cancer, is found in the tissues of IPF patients [54, 55]. Suppression of let-7d triggers EMT including the up-regulation of mesenchymal

Table 1 Roles of miRNAs in TGF- β /Smad-mediated tissue fibrosis

Disease	miRNA	Expression level	Experiment model	Function	Reference
Renal fibrosis	miR-21	High	UUO-induced kidney disease in mice Rat tubular epithelial cells line NRK52E	Increase TIMP1 expressions Suppress Smad7 and MMP-9 expression	[27–29, 31]
	miR-192	High	5/6 nephrectomy mice and NRK52E cells	Increase collagen I and IV expression	[50, 68]
	miR-377	High	Streptozotocin-induced diabetic nephropathy mice Murine mesangial cells	Increase fibronectin expression	[50, 69]
	miR-29	Low	UUO-induced nephropathy in mice, NRK52E cells AngII-induced hypertensive nephropathy mice	Inhibit collagen I, III, fibronectin, Adam12, and Adam19 expressions	[28, 37, 38, 50, 70]
	miR-141	Low	Maternal protein-restricted mice, NRK52E cells UUO-induced renal fibrosis in mice	EMT and fibrosis development	[47–49, 71]
	miR-200	Low	UUO-induced renal fibrosis in mice	Suppress ZEB1 and ZEB2 expressions	[28, 36, 47]
	miR-200a	Low	NRK52E cells	Inhibit Smad-3 activity, matrix proteins and TGF- β 2 expressions, and prevent EMT	[48, 49]
	miR-433	High	UUO-induced renal fibrosis in mice	Suppress Azin1 expression	[18]
	miR-21	High	Lung tissues of IPF patients Bleomycin-induced fibrosis in mice	Decrease Smad7 expression	[25, 26]
	let-7d	High	Tissue of IPF patients Primary epithelial cell lines from IPF patient	Increase mesenchymal markers expression	[55]
Pulmonary fibrosis	miR-29	Low	Bleomycin-induced lung fibrosis in mice	Inhibit TGF- β and CTGF expression	[42]
	miR-326	Low	Bleomycin-induced lung fibrosis in mice Human cell lines	Decrease profibrotic genes expression Increase Smad7 expressions	[56]
	miR-25, miR-29	Low	Transaortic constricted mice	Increase collagen I and III expression	[41]
	miR-29b	Low	AngII-induced cardiac fibrosis in mice Mouse cardiac fibroblasts	Inhibit collagen I, III, fibronectin expression and TGF- β /Smad3 signaling	[44]
Myocardial fibrosis	miR-133	Low	Streptozotocin-induced diabetic in mice	Inhibit EP300 and major fibrotic markers expression	[53]
Hepatic fibrosis	miR-21	High	Thioacetamide-induced hepatic fibrosis in mice	Increase collagen deposition	[32]
	miR-29	Low	CCl4-induced hepatic fibrosis in mice Human fibrotic liver TGF- β - and PDGF-BB- treated hepatic stellate cells	Regulate collagen homeostasis	[24, 72]
	miR-150	Low	TGF- β 1-induced human hepatic stellate cells	Regulate collagen type I and IV expression	[58]
Cystic fibrosis	miR-145	High	Nasal epithelial tissues of CF patients	Inhibit Smad3 gene transcription	[51]
	miR-494	High	Nasal epithelial tissues of CF patients	Reduce CFTR expression	[51]
Peritoneal fibrosis	miR-29b	Low	Peritoneal dialysis mouse model	Inhibit collagen I, III, fibronectin expression, EMT, and TGF- β /Smad3 signaling	[45]
Dystrophic muscle	miR-21	High	Duchenne muscular dystrophy mice	Increase TCF4-positive fibroblasts	[22]
Intestinal fibrosis	miR-29b	Low	Mucosa of Crohn's disease patients	Regulate collagen type I and III expression	[35]

markers alpha-smooth muscle actin (ACTA2), HMGA2, N-cadherin 2, and vimentin in epithelial cell lines A549, PLE-6TN, and primary bronchial epithelial cells in vitro; and reduction of epithelial marks CDH1 and TJP1 expressions were observed in let-7d knockdown mice by intratracheal administration of let-7d antagomir. In the same study, the regulatory effect of let-7d on EMT in IPF was suggested to be dependent on TGF- β /Smad3 signaling instead of its traditional partner HMGA2 (high-mobility group AT-hook 2) on cancer. Furthermore, it is found that TGF- β signaling can down-regulate the expression of let-7d at transcriptional level by promoting direct binding of Smad3 on the promoter region of let-7d [55]. In addition, loss of miR-326 expression with an associated increase of TGF- β 1 was shown on the bleomycin-induced lung fibrosis mice model and human IPF lung specimens, whereas restoration of miR-326 can inhibit TGF- β 1 expression and attenuate the fibrotic response by down-regulating profibrotic genes (e.g., Smad3, MMP-9) and up-regulating anti-fibrotic genes (e.g., Smad7) in vitro and in vivo [56].

Up-regulation of miR-150 was firstly reported in the development of non-alcoholic fatty liver disease (NAFLD), and then it has been found to be involved in the pathogenesis of liver fibrosis [57]. Its role of miR-150 in the development of liver fibrosis was demonstrated on LX-2 cells (a hepatic stellate cell line) in vitro, expression of miR-150 was reduced during liver fibrosis by TGF- β 1 stimulation and overexpression of miR-150 would significantly decrease the expression of ECMs and α -SMA. However, miR-150-mediated tissue fibrosis is suggested to be mediated by TGF- β 1 in the hepatic stellate cells without the involvement of Smad2 and Smad3 [58].

Recent Barriers in the Basic Research of miRNA-mediated Fibrosis

The history of miRNAs research in the Smad-mediated fibrosis was relatively short since the related discovery on a cardio disease model by Mann's group in 2009 [41•], but miRNAs have been already discovered more than two decades from *C. elegans* by Ambros's research group [59]. Publications about the discovery and characterization of miRNAs in tissue fibrosis have been rapidly increased, which is properly enhanced by the awareness of the importance of TGF- β /Smad signaling on fibrosis in a number of human diseases. The progress can be further accelerated if the underlying mechanism for controlling the specificity of miRNAs in fibrogenesis can be identified. For example, the research progress of miRNAs in renal diseases has been limited as most of miRNAs have more than one target gene, the low specificity of miRNAs creates difficulty on identifying accurate targets for each miRNA

[15, 60, 61]. Furthermore, the mechanism of miRNA-mediated cellular regulation is still unclear and challenging due to the high flexibility of miRNAs, as the expression of a miRNA can be affected by multiple mediators or pathways and vice versa. These off-target effects of miRNA may cause unexpected side effects on patients if being used as a therapeutic target, which limits the development of specific miRNA therapy for fibrosis diseases [15, 62].

Crosstalk Between Smads and miRNAs During Tissue Fibrosis

According to the available information, Smad3 has been suggested as a key mediator in TGF- β /Smad signaling, and its involvement on miRNA-mediated fibrosis has been demonstrated on a number of disease models. For example, Smad3 can down-regulate anti-fibrotic miRNA miR-200 and up-regulate profibrotic miRNA miR-192 [12, 28, 42]. Interestingly, both miR-21 and miR-29a can be regulated by Smad3 during fibrosis but in an opposite manner [24, 27, 44], where the Smad3-induced miR-21 inhibits Smad7 expression, but the Smad3-suppressed miR-29a prevents TGF- β 1 down-regulation [30, 44]. Therefore, Smad3-mediated miRNA regulation enhances the Smad3 activation and facilitating the TGF- β /Smad-mediated fibrogenesis (Fig. 1). In contract, Smad7 can prevent TGF- β /Smad-mediated fibrosis by up-regulating anti-fibrotic miRNAs miR-29, miR-29b, and miR-192 [7, 38, 47]. Nevertheless, the regulatory roles of miRNAs (Table 1) in the Smad signaling and fibrotic biomarker expression have been reported, for example: miR-155 down-regulates Smad2 in macrophages and myeloid cells [63], miR-145, and miR-200a suppress Smad3 expression and activity in healthy people without CF and renal fibrosis [46•, 51], the protein levels of CFTR, collagen type I and II, and ECMs, as well as induction of EMT can be down-regulated by miR-494, miR-150, and miR-200a [48, 51, 58], whereas miR-143 can up-regulate the collagen type III synthesis in the normal gastric fibroblasts [64]. The interaction between miRNAs and epigenetic is another area need to be elucidated for a better understanding on the regulation of fibrogenesis in specific tissues, i.e., miR-29b inhibits the expression of DNMT1 which further enhances the DNA hypomethylation during the development of systemic lupus erythematosus [65–67].

Conclusion

The importance of miRNAs and TGF- β /Smad signaling on tissue fibrosis has been recognized, and the pathogenic roles and therapeutic potential of miRNAs on fibrosis have

been demonstrated on a number of animal disease models. Further elucidation on the networking and underlying mechanism of miRNAs in TGF- β /Smad signaling will provide useful information for designing a more effective strategy to overcome fibrotic diseases in the future.

Acknowledgments This work was supported by Grants from Major State Basic Research Development Program of China (973 program, No 2012CB517700); the Research Grant Council of Hong Kong (RGC GRF 468711, 469110, CUHK5/CRF/09, and CUHK3/CRF/12R); the Focused Investment Scheme A and B from the Chinese University of Hong Kong.

Compliance with Ethics Guidelines

Conflict of Interest Patrick Ming-Kuen Tang and Hui-Yao Lan declare they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Recently published papers of particular interest have been highlighted as:

- Of important
- Of major importance

1. Friedman SL, Sheppard D, Duffield JS, Violette S (2013) Therapy for fibrotic diseases: nearing the starting line. *Sci Transl Med* 5(167):167sr1
2. Schuppan D, Ruehl M, Somasundaram R, Hahn EG (2001) Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis* 21(3):351–372
3. Pardo A, Selman M (2006) Matrix metalloproteases in aberrant fibrotic tissue remodelling. *Proc Am Thorac Soc* 3(4):383–388
4. Wynn TA (2011) Integrating mechanisms of pulmonary fibrosis. *J Exp Med* 208(7):1339–1350
5. Wynn TA, Ramalingam TR (2012) Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* 18(7):1028–1040
6. O'Donoghue RJ, Knight DA, Richards CD, Prêle CM, Lau HL, Jarnicki AG, Jones J, Bozinovski S, Vlahos R, Thiem S, McKenzie BS, Wang B, Stumbles P, Laurent GJ, McAnulty RJ, Rose-John S, Zhu HJ, Anderson GP, Ernst MR, Mutsaers SE (2012) Genetic partitioning of interleukin-6 signalling in mice dissociates Stat3 from Smad3-mediated lung fibrosis. *EMBO Mol Med* 4(9):939–951
7. Wei LH, Huang XR, Zhang Y, Li YQ, Chen HY, Yan BP, Yu CM, Lan HY (2013) Smad7 inhibits angiotensin II-induced hypertensive cardiac remodelling. *Cardiovasc Res* 99(4):665–673
8. Wu XL, Zeng WZ, Jiang MD, Qin JP, Xu H (2008) Effect of Oxymatrine on the TGF β -Smad signaling pathway in rats with CCl4-induced hepatic fibrosis. *World J Gastroenterol* 14(13):2100–2105
9. Branski RC, Barbieri SS, Weksler BB, Saltman B, Krishna P, Kraus DH, Broadbelt NV, Chen J, Poppas DP, Felsen D (2009) Effects of transforming growth factor-beta1 on human vocal fold fibroblasts. *Ann Otol Rhinol Laryngol* 118(3):218–226
10. Liu Z, Huang XR, Chen HY, Penninger JM, Lan HY (2012) Loss of angiotensin-converting enzyme 2 enhances TGF- β /Smad-mediated renal fibrosis and NF- κ B-driven renal inflammation in a mouse model of obstructive nephropathy. *Lab Invest* 92(5):650–661
11. Huang XR, Chung AC, Zhou L, Wang XJ, Lan HY (2008) Latent TGF-beta1 protects against crescentic glomerulonephritis. *J Am Soc Nephrol* 19(2):233–242
12. Lan HY, Chung AC (2012) TGF- β /Smad signaling in kidney disease. *Semin Nephrol* 32(3):236–243
13. Lan HY (2008) Smad7 as a therapeutic agent for chronic kidney diseases. *Front Biosci* 1(13):4984–4992
14. Li R, Chung AC, Yu X, Lan HY (2014) MicroRNAs in diabetic kidney disease. *Int J Endocrinol* 2014:593956
15. Chung AC, Yu X, Lan HY (2013) MicroRNA and nephropathy: emerging concepts. *Int J Nephrol Renovasc Dis* 25(6):169–179
16. Vettori S, Gay S, Distler O (2012) Role of microRNAs in fibrosis. *Open Rheumatol J* 6:130–139
17. Blahna MT, Hata A (2012) Smad-mediated regulation of microRNA biosynthesis. *FEBS Lett* 586(14):1906–1912
18. Li R, Chung AC, Dong Y, Yang W, Zhong X, Lan HY (2013) The microRNA miR-433 promotes renal fibrosis by amplifying the TGF- β /Smad3-Azin1 pathway. *Kidney Int* 84:1129–1144
19. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116(2):281–297
20. Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 9(2):102–114
21. Butz H, Rácz K, Hunyady L, Patócs A (2012) Crosstalk between TGF- β signaling and the microRNA machinery. *Trends Pharmacol Sci* 33(7):382–393
22. Acuña MJ, Pessina P, Olguin H, Cabrera D, Vio CP, Bader M, Muñoz-Canoves P, Santos RA, Cabello-Verrugio C, Brandan E (2014) Restoration of muscle strength in dystrophic muscle by angiotensin-1-7 through inhibition of TGF- β signalling. *Hum Mol Genet* 23(5):1237–1249
23. Lv J, Nie ZK, Zhang JL, Liu FY, Wang ZZ, Ma ZL, He H (2013) Corn peptides protect against thioacetamide-induced hepatic fibrosis in rats. *J Med Food* 16(10):912–919
24. Noetel A, Kwiecinski M, Elfimova N, Huang J, Odenthal M (2012) microRNA are central players in anti- and profibrotic gene regulation during liver fibrosis. *Front Physiol* 19(3):49
25. Gao Y, Lu J, Zhang Y, Chen Y, Gu Z, Jiang X (2013) Baicalein attenuates bleomycin-induced pulmonary fibrosis in rats through inhibition of miR-21. *Pulm Pharmacol Ther* 26(6):649–654
26. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E (2010) miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 207(8):1589–1597
27. Zhong X, Chung AC, Chen HY, Meng XM, Lan HY (2011) Smad3-mediated upregulation of miR-21 promotes renal fibrosis. *J Am Soc Nephrol* 22(9):1668–1681
28. Meng XM, Chung AC, Lan HY (2013) Role of the TGF- β /BMP-7/Smad pathways in renal diseases. *Clin Sci (Lond)* 124(4):243–254
29. Zhong X, Chung AC, Chen HY, Dong Y, Meng XM, Li R, Yang W, Hou FF, Lan HY (2013) miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. *Diabetologia* 56(3):663–674
30. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E (2010) miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 207(8):1589–1597
31. Wang J, Gao Y, Ma M, Li M, Zou D, Yang J, Zhu Z, Zhao X (2013) Effect of miR-21 on renal fibrosis by regulating MMP-9 and TIMP1 in kk-ay diabetic nephropathy mice. *Cell Biochem Biophys* 67(2):537–546

32. Zhang Z, Gao Z, Hu W, Yin S, Wang C, Zang Y, Chen J, Zhang J, Dong L (2013) 3,3'-Diindolylmethane ameliorates experimental hepatic fibrosis via inhibiting miR-21 expression. *Br J Pharmacol* 170(3):649–660
33. Gao Y, Lu J, Zhang Y, Chen Y, Gu Z, Jiang X (2013) Baicalein attenuates bleomycin-induced pulmonary fibrosis in rats through inhibition of miR-21. *Pulm Pharmacol Ther* 26(6):649–654
34. Zhang Z, Gao Z, Hu W, Yin S, Wang C, Zang Y, Chen J, Zhang J, Dong L (2013) 3,3'-Diindolylmethane ameliorates experimental hepatic fibrosis via inhibiting miR-21 expression. *Br J Pharmacol* 170(3):649–660
35. Nijhuis A, Biancheri P, Lewis A, Bishop CL, Giuffrida P, Chan C, Feakins R, Poulsom R, Di Sabatino A, Corazza GR, MacDonald TT, Lindsay JO, Silver AR (2014) In Crohn's disease fibrosis-reduced expression of the miR-29 family enhances collagen expression in intestinal fibroblasts. *Clin Sci (Lond)* 127(5):341–350
36. Lan HY (2012) Transforming growth factor- β /Smad signalling in diabetic nephropathy. *Clin Exp Pharmacol Physiol* 39(8):731–738
37. Ramdas V, McBride M, Denby L, Baker AH (2013) Canonical transforming growth factor- β signaling regulates disintegrin metalloprotease expression in experimental renal fibrosis via miR-29. *Am J Pathol* 183(6):1885–1896
38. Liu GX, Li YQ, Huang XR, Wei LH, Zhang Y, Feng M, Meng XM, Chen HY, Shi YJ, Lan HY (2014) Smad7 inhibits AngII-mediated hypertensive nephropathy in a mouse model of hypertension. *Clin Sci (Lond)* 127(3):195–208
39. Wei LH, Huang XR, Zhang Y, Li YQ, Chen HY, Heuchel R, Yan BP, Yu CM, Lan HY (2013) Deficiency of Smad7 enhances cardiac remodeling induced by angiotensin II infusion in a mouse model of hypertension. *PLoS ONE* 8(7):e70195
40. Wei LH, Huang XR, Zhang Y, Li YQ, Chen HY, Yan BP, Yu CM, Lan HY (2013) Smad7 inhibits angiotensin II-induced hypertensive cardiac remodelling. *Cardiovasc Res* 99(4):665–673
41. Divakaran V, Adroque J, Ishiyama M, Entman ML, Haudek S, Sivasubramanian N, Mann DL (2009) Adaptive and maladaptive effects of SMAD3 signaling in the adult heart after hemodynamic pressure overloading. *Circ Heart Fail* 2(6):633–642. *This is the first study revealing the involvement of miRNA in tissue fibrosis*
42. Xiao J, Meng XM, Huang XR, Chung AC, Feng YL, Hui DS, Yu CM, Sung JJ, Lan HY (2012) miR-29 inhibits bleomycin-induced pulmonary fibrosis in mice. *Mol Ther* 20(6):1251–1260
43. Liu GX, Li YQ, Huang XR, Wei LH, Zhang Y, Feng M, Meng XM, Chen HY, Shi YJ, Lan HY (2014) Smad7 inhibits AngII-mediated hypertensive nephropathy in a mouse model of hypertension. *Clin Sci (Lond)* 127(3):195–208
44. Zhang Y, Huang XR, Wei LH, Chung AC, Yu CM, Lan HY (2014) miR-29b as a therapeutic agent for angiotensin II-induced cardiac fibrosis by targeting TGF- β /Smad3 signaling. *Mol Ther* 22(5):974–985
45. Yu JW, Duan WJ, Huang XR, Meng XM, Yu XQ, Lan HY (2014) MicroRNA-29b inhibits peritoneal fibrosis in a mouse model of peritoneal dialysis. *Lab Invest* 94(9):978–990
46. Chung AC, Huang XR, Meng X, Lan HY (2010) miR-192 mediates TGF-beta/Smad3-driven renal fibrosis. *J Am Soc Nephrol* 21(8):1317–1325. *This study demonstrates the inhibitory effect of Smad3 on the expression of miR-192 at transcriptional level during fibrosis*
47. Xiong M, Jiang L, Zhou Y, Qiu W, Fang L, Tan R, Wen P, Yang J (2012) The miR-200 family regulates TGF- β 1-induced renal tubular epithelial to mesenchymal transition through Smad pathway by targeting ZEB1 and ZEB2 expression. *Am J Physiol Renal Physiol* 302(3):F369–F379
48. Wang B, Koh P, Winbanks C, Coughlan MT, McClelland A, Watson A, Jandeleit-Dahm K, Burns WC, Thomas MC, Cooper ME, Kantharidis P (2011) miR-200a Prevents renal fibrogenesis through repression of TGF- β 2 expression. *Diabetes* 60(1):280–287
49. Lan HY, Chung AC (2011) Transforming growth factor- β and Smads. *Contrib Nephrol* 170:75–82
50. Megiorni F, Cialfi S, Cimino G, De Biase RV, Dominici C, Quattrucci S, Pizzuti A (2013) Elevated levels of miR-145 correlate with SMAD3 down-regulation in cystic fibrosis patients. *J Cyst Fibros* 12(6):797–802
51. Ge Q, Moir LM, Black JL, Oliver BG, Burgess JK (2010) TGF β 1 induces IL-6 and inhibits IL-8 release in human bronchial epithelial cells: the role of Smad2/3. *J Cell Physiol* 225(3):846–854
52. Chen S, Puthanveetil P, Feng B, Matkovich SJ, Dorn GW 2nd, Chakrabarti S (2014) Cardiac miR-133a overexpression prevents early cardiac fibrosis in diabetes. *J Cell Mol Med* 18(3):415–421
53. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 403(6772):901–906
54. Pandit KV, Corcoran D, Yousef H, Yarlagadda M, Tzouvelekis A, Gibson KF, Konishi K, Yousem SA, Singh M, Handley D, Richards T, Selman M, Watkins SC, Pardo A, Ben-Yehudah A, Bouros D, Eickelberg O, Ray P, Benos PV, Kaminski N (2010) Inhibition and role of let-7d in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 182(2):220–229
55. Das S, Kumar M, Negi V, Pattnaik B, Prakash YS, Agrawal A, Ghosh B (2014) MicroRNA-326 regulates profibrotic functions of transforming growth factor- β in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 50(5):882–892
56. Estep M, Armistead D, Hossain N, Elarainy H, Goodman Z, Baranova A, Chandhoke V, Younossi ZM (2010) Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 32(3):487–497
57. Zheng J, Lin Z, Dong P, Lu Z, Gao S, Chen X, Wu C, Yu F (2013) Activation of hepatic stellate cells is suppressed by microRNA-150. *Int J Mol Med* 32(1):17–24
58. Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75(5):843–854
59. Singh S, Narang AS, Mahato RI (2011) Subcellular fate and off-target effects of siRNA, shRNA, and miRNA. *Pharm Res* 28(12):2996–3015
60. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, Linsley PS (2003) Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol* 21(6):635–637
61. Yousef M, Trinh HV, Allmer J (2014) Intersection of MicroRNA and Gene Regulatory Networks and their Implication in Cancer. *Curr Pharm Biotechnol* 15(5):445–454
62. Naito Y, Sakamoto N, Oue N, Yashiro M, Sentani K, Yanagihara K, Hirakawa K, Yasui W (2014) MicroRNA-143 regulates collagen type III expression in stromal fibroblasts of scirrhous type gastric cancer. *Cancer Sci* 105(2):228–235
63. Louafi F, Martinez-Nunez RT, Sanchez-Elsner T (2010) MicroRNA-155 targets SMAD2 and modulates the response of macrophages to transforming growth factor- β . *J Biol Chem* 285(53):41328–41336
64. Guo L, Sun B, Wu Q, Yang S, Chen F (2012) miRNA-miRNA interaction implicates for potential mutual regulatory pattern. *Gene* 511(2):187–194
65. Bian EB, Zhao B, Huang C, Wang H, Meng XM, Wu BM, Ma TT, Zhang L, Lv XW, Li J (2013) New advances of DNA methylation in liver fibrosis, with special emphasis on the crosstalk between microRNAs and DNA methylation machinery. *Cell Signal* 25(9):1837–1844

66. Cheng C, Huang C, Ma TT, Xu T, Wang YR, Zhang L, Jun L (2014) New surprises of suppressor of cytokine signalling in liver fibrosis. *Expert Opin Ther Targets* 18(4):415–426
67. Sun L, Zhang D, Liu F, Xiang X, Ling G, Xiao L, Liu Y, Zhu X, Zhan M, Yang Y, Kondeti VK, Kanwar YS (2011) Low-dose paclitaxel ameliorates fibrosis in the remnant kidney model by down-regulating miR-192. *J Pathol* 225(3):364–377
68. Wang Q, Wang Y, Minto AW, Wang J, Shi Q, Li X, Quigg RJ (2008) MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB J* 22(12):4126–4135
69. Qin W, Chung AC, Huang XR, Meng XM, Hui DS, Yu CM, Sung JJ, Lan HY (2011) TGF- β /Smad3 signaling promotes renal fibrosis by inhibiting miR-29. *J Am Soc Nephrol* 22(8):1462–1474
70. Sene Lde B, Mesquita FF, de Moraes LN, Santos DC, Carvalho R, Gontijo JA, Boer PA (2013) Involvement of renal corpuscle microRNA expression on epithelial-to-mesenchymal transition in maternal low protein diet in adult programmed rats. *PLoS One* 8(8):e71310
71. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, Janssen J, Koppe C, Knolle P, Castoldi M, Tacke F, Trautwein C, Luedde T (2011) Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology* 53(1):209–218