Minireview

Epigenetic Modifications in Fibrotic Diseases: Implications for Pathogenesis and Pharmacological Targets

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Received September 7, 2014; accepted October 30, 2014

ABSTRACT

Organ fibrosis is a complex and chronic disorder that results from a variety of acute injuries and contributes to thirty percent of naturally occurring deaths worldwide. The main feature of organ fibrosis is the excessive accumulation and deposit of extracellular matrix, thereby leading to organ dysfunction, loss of elasticity, and development of a rigid organ. Accumulating evidence shows that epigenetic remodeling, including aberrant DNA methylation and noncoding RNA expression as well as histone post-translational modifications, play important roles in the pathogenesis of fibrosis through the regulation of fibroblast activation, differentiation, and apoptosis, as well as collagen synthesis and profibrotic gene transcription. In this review, we discuss the basic regulation of DNA methylation, noncoding RNA expression, and histone post-translational modification, and their participation in the pathogenesis and development of organ fibrosis. This review also provides the latest insights into the novel biomarkers and therapeutic targets for fibrosis through modulation of epigenetic remodeling.

Introduction

Organ fibrosis is a complex and chronic disorder that results from a variety of acute injuries and accounts for thirty percent of naturally occurring deaths worldwide. The main feature of organ fibrosis is the excessive accumulation and deposit of extracellular matrix, thereby leading to organ dysfunction, loss of elasticity, and development of a rigid organ. In general, the physiologic repair is initiated as a result of an injury of the functional parenchyma and then terminated once the initial injury has been resolved. However, fibrogenesis progresses even when the initial insult has been removed. The molecular mechanisms underlying this excessive fibrogenesis are not fully understood.

Recently, we and other groups have shown that the processes of epigenetic remodeling, including DNA methylation, microRNA (miRNA), and histone post-translational modifications, play important roles in regulating the injury and repair during organ fibrosis (Kim and Shukla, 2005; Kulkarni et al., 2011; Zhang et al., 2011; He et al., 2012; Komatsu et al., 2012; Zhou et al., 2012; Li et al., 2014). Indeed, epigenetic therapies are under development for the treatment of fibrotic diseases (Zhang et al., 2010; Jungel et al., 2011; Pottier et al., 2014; Subramaniam et al., 2014). Here we review the latest insights into epigenetic regulation and its involvement in the fibroblast activation and fibrogenesis.

Cellular and Molecular Pathogenesis of Fibrotic Diseases

Interstitial fibroblasts and myofibroblasts are the principal effector cells for fibrosis of organs, including kidney, lung, liver, and heart. These myofibroblasts have characteristics of both fibroblasts and smooth muscle cells with high expression of α smooth muscle actin and collagens. Accumulating evidence suggests three potential sources of myofibroblasts during fibrogenesis: 1) resident and local fibroblasts, 2) epithelial/endothelial mesenchymal transition (Corvol et al., 2009; Lamouille et al., 2014), and 3) circulating fibrocytes, pericytes, or mesenchymal stem cells (Willis et al., 2006; Lin et al., 2008; Forte et al., 2010) (Fig. 1). Resident fibroblasts are the primary source of myofibroblasts through differentiation, whereas the epithelial/endothelial mesenchymal transition contributes a minor component of the myofibroblast population during the progression of organ fibrosis (Strieter et al., 2009; LeBleu et al., 2013). A recent study shows that pericyte...
Deletion does not improve kidney fibrosis or change the recruitment of myofibroblasts, suggesting the noninvolvement of pericytes in renal fibrosis (LeBleu et al., 2013). However, it remains unclear if this applies to (myo)fibroblast origination in other organs during fibrosis. These findings indicate that the heterogeneous sources of myofibroblasts during fibrogenesis and that targeting these collagen-generating cells would be a potential therapeutic avenue for treating organ fibrosis.

It has been shown that collagen synthesis and profibrotic gene expression can be regulated by a variety of signal pathways. Transforming growth factor β (TGF-β)/Smad signaling is the best studied pathway in regulating profibrotic gene expression and collagen synthesis. Upon binding of TGF-β1 to its receptor II, Smad2/3 form heteromeric complexes with Smad4, which translocate into the nucleus to regulate transcription of targeted genes. Interestingly, Smad2 is able to competitively inhibit Smad3 phosphorylation, thereby preventing Smad3-mediated collagen matrix expression in response to TGF-β1 and other profibrotic mediators, including angiotensin II (Lan, 2011; Duan et al., 2014). Additionally, Smads can interact with other signaling pathways in regulating fibrogenesis, including mitogen-activated protein kinase, nuclear factor κB, and bone morphogenic protein signals (Zeisberg et al., 2003; Meng et al., 2012; Cheng et al., 2013). There are other signal pathways, such as Toll-like receptor 4, Wnt, Ying Yang 1, peroxisome proliferator-activated receptor γ (PPARγ), and purinergic signals, involved in the fibrogenesis (Isayama et al., 2006; Riteau et al., 2010; Lin et al., 2011; Akhmetshina et al., 2012; Lu and Insel, 2014). For example, PPARγ inhibits activation of hepatic stellate cells and fibroblasts in vitro, as well as fibrosis in vivo (Diep et al., 2004; Yang et al., 2006; Kawai et al., 2009; Yu et al., 2010; Kulkarni et al., 2011). The levels of extracellular ATP are increased in bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis (IPF) patients and bleomycin-treated mice. Furthermore, P2X7 receptor-deficient mice show reduced lung inflammation and fibrosis (Riteau et al., 2010). Hence, targeting the components of these signal pathways represents an attractive potential therapeutic approach for fibrosis.

General Epigenetic Modifications

In general, epigenetics refers to change of gene function, which is inheritable, but not the result of an alteration in DNA sequence. The epigenetic modifications include DNA methylation, noncoding RNAs (e.g., miRNA), and histone modifications (e.g., acetylation/deacetylation and methylation/demethylation), which create an epigenetic landscape for regulating gene expression (Keller, 2014; Shenderov and Midtvedt, 2014). These epigenetic modifications can also influence each other so as to regulate gene expression.

**DNA Methylation.** DNA methylation is an addition of a methyl group at the C5 position of the cytosine ring. This leads to the generation of 5-methyl cytosine that precedes a guanosine in the DNA sequence, which is called the CpG dinucleotide (Patil et al., 2014; Subramaniam et al., 2014) (Fig. 2). These CpG clusters are in short stretches of DNA with 300–3000 base pairs (CpG islands) (Han and Zhao, 2009; Liu et al., 2014). The functional consequence of DNA methylation is highly dependent on the site of the corresponding CpG islands. The best studied DNA methylation of CpG islands has been within proximal promoter regions (Lokk et al., 2014). The CpG island promoters are typically unmethylated. The transcriptional activity of genes is suppressed when it is methylated in CpG island promoters (Ramirez-Carrozzi et al., 2009; Deaton and Bird, 2011; Lokk et al., 2014). It should be noted that DNA hypomethylation provides a permissive state for transcriptional activity but does not correlate with high expression. DNA methylation is an addition of a methyl group at the C5 position of the cytosine ring (A). In most conditions, DNA methylation induced by DNMTs causes the recruitment of chromatin inactive proteins, leading to chromatin gene repression (B).

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**Fig. 1.** Postulated sources of myofibroblasts during fibrogenesis. There are three sources of myofibroblasts for fibrogenesis during chronic injury: resident fibroblasts, epithelial/endothelial mesenchymal transition, and circulating fibrocytes, pericytes, or mesenchymal stem cells. Excessive collagen secreted by activated myofibroblasts is deposited and leads to fibrogenesis in organs, including liver, lung, kidney, and heart. Col 1, collagen 1; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; α-SMA, α smooth muscle actin.

**Fig. 2.** Regulation of DNA methylation of gene expression. DNA methylation is an addition of a methyl group at the C5 position of the cytosine ring (A). In most conditions, DNA methylation induced by DNMTs causes the recruitment of chromatin inactive proteins, leading to chromatin gene repression (B).
gene expression (Wong et al., 2006). However, recent studies have shown promoter hypomethylation as a mechanism of activation of oncoproteins and metastatic genes (Stefanska et al., 2011, 2013; Mayol et al., 2012). Further studies are required to dissect these differences in the regulation of promoter hypomethylation of gene expression. It has been shown that almost half of all human gene promoters do not localize within CpG islands (Takai and Jones, 2002). The non-CpG methylation occurs on CpA, CpT, and CpC sites with the most frequent at CpA dinucleotides in a tissue-specific manner (e.g., stem cells and brain tissues), or at particular regions of the genome. Non-CpG methylation cannot be maintained through cell division owing to asymmetry of sequence, which is different from CpG methylation. The link of methylation of non-CpG island promoters to gene expression is controversial (Han et al., 2011; Patil et al., 2014). Most studies have shown that aberrant methylation of non-CpG island promoters is associated with gene silencing (Inoue and Oishi, 2005; Barres et al., 2009, 2013). In addition, DNA can be methylated in different genomic contexts, including transcriptional start sites with or without CpG islands, in gene bodies, at regulatory elements, and at repeat sequences (Jones, 2012). Indeed, DNA methylation is more prevalent within gene-bodies with poor CpG dinucleotides than seen in promoters, and this is associated with gene transcription (Jingo et al., 2012; Nerl et al., 2013). A recent study has shown how gene-body methylation interferes with transcriptional elongation (Jingo et al., 2012). In somatic cells, gene body methylation is one of the major causes for gene mutations in tumor suppressor genes, including tumor protein 53. However, it is not clear whether or how DNA methylation in these different regions communicates and thereby controls gene expression.

DNA methylation is catalyzed by DNA (cytosine-5)-methyltransferases (DNMTs). Currently, three DNA methyltransferases have functional enzymatic activity in mammals, namely, DNMT1, DNMT3A, and DNMT3B (Bestor, 2000; Subramaniam et al., 2014). DNMT3A and DNMT3B belong to de novo methyltransferases that are capable of methylating unmethylated CpG sequences, whereas the most abundant DNMT1 has a higher affinity for hemi-methylated DNA leading to the propagation and maintenance of established DNA methylation (Baylin and Herman, 2000; Eng et al., 2000). These DNMTs are responsible for both CpG and non-CpG methylation that are often coincident. Unfortunately, it remains elusive how to target and extrapolate the biologic functions of non-CpG methylation without changing CpG methylation and vice versa. The mechanisms for DNA demethylation are complicated and involve the removal of 5-mC or further modification of cytosine bases. Histone deacetylases (HDACs) can be recruited on methylated DNA via the methyl-DNA binding motifs coregulating gene expression (Nan et al., 1998). Interestingly, a recent study has shown that DNMT1, DNMT3A, and DNMT3B could act as active DNA methylases to remove a methyl group from methylated DNA in a Ca$^{2+}$ and reduct state–dependent manner (Chen et al., 2012). Further study to identify novel DNA demethylases and to dissect the underlying mechanisms for DNMTs/DNA demethylases imbalance, and their communications with HDACs, would enhance the understanding of the pathogenesis of diseases where aberrant DNA methylation occurs.

**miRNAs.** miRNAs are noncoding single-stranded RNAs with 18–24 nucleotides. In addition to miRNAs, noncoding RNAs also include tRNA, rRNA, snRNAs, snoRNAs, and long ncRNAs (Esteller, 2011; Fatica and Bozzoni, 2014; Sela et al., 2014). There are several steps for miRNA biogenesis (Fig. 3). First, miRNAs are transcribed into long primary transcripts (pri-miRNAs) by RNA polymerase II. These pri-miRNAs are further processed into pre-miRNAs with 70–100 nucleotides by Drosha (an RNA polymerase III) along with its cofactor DGCR8 (Sohn et al., 2007; Bartel, 2009). Pre-miRNAs are transported into the cytosol by expotin-5, and then undergo final cleavage into a ~22-nucleotide DNA duplex by the RNase III nuclease Dicer. Finally, the mature single-stranded miRNA is incorporated into the RNA-induced silencing complex (RISC), including Dicer and Argonaute proteins, leading to translational repression and mRNA cleavage.

![miRNA biogenesis](image-url)

**Fig. 3.** Biogenesis of miRNAs. Several steps are involved in miRNA biogenesis. This includes the transcription by RNA polymerase II and further processing by the RNA polymerase III Drosha and its cofactor DGCR8 into pre-miRNAs. Then pre-miRNAs are transported into cytoplasm where they are cleaved into a DNA duplex by the RNase III nuclease Dicer. Finally, the mature single-stranded miRNA is incorporated into the RNA-induced silencing complex (RISC), including Dicer and Argonaute proteins. miRNAs have been shown to regulate the translation of more than 60% of protein-coding genes. miRNAs control gene expression through the seed sequence–selective targeting of mRNAs, resulting in translational repression or mRNA degradation, depending on the degree of complementarity between miRNAs and target mRNA sequences (Kozomara and Griffiths-Jones, 2011). There are more than 2000 miRNAs identified so far in all known mammalian genomes, and each can target a variety of different mRNAs. Each mRNA can also be controlled by several miRNAs. The interaction between miRNA and targeted mRNA is regulated by many factors, such as the position and accessibility of the miRNA-binding sites, the presence of other the miRNA-binding sites, and RNA-binding proteins. Bioinformatics-based algorithms provide a model of prediction for miRNA-targeting mRNAs (Lewis et al., 2005). This implicates that miRNAs consist of a complex regulatory network that regulates various cellular process, including cell proliferation, apoptosis, differentiation, development, and tumorigenesis (Macfarlane and Murphy, 2010). Therefore, regulation of miRNAs provides the potential...
and promising therapeutic targets for diseases such as cancer in which miRNAs are aberrant.

miRNA can be inhibited by antisense oligonucleotides through base-pair complementarity. There are three types of antisense oligonucleotides: locked nucleic acids, anti-miRNA oligonucleotides, and antagonirs developed for inhibiting miRNAs (Krutzfeldt et al., 2005; Elmen et al., 2008; Essau, 2008). An antagonist is a small synthetic RNA used to silence endogenous miRNAs by preventing miRNAs from binding to a desired site on an mRNA. Owing to the complexity of disease pathogenesis, silencing a single miRNA to target a specific gene expression may not be sufficient to control disease. Thus, an ideal approach is to target multiple miRNAs that are aberrantly expressed in disease, such as the multiple-target anti-miRNA antisense oligodeoxyribonucleotides or the competitive inhibitors of miRNAs preventing their association with targeting genes (Cohen, 2009; Lu et al., 2009; Esteller, 2011). Accumulating evidence shows that the production of miRNAs is reduced in certain diseases, including cancer. Therefore, the restoration of miRNA function through miRNA replacement therapy or miRNAaome-based strategy provides a potential therapeutic avenue in diseases in which global miRNA is reduced (Kumar et al., 2007; Esteller, 2011). Nevertheless, future effort is needed to screen and identify all functional miRNAs using emerging genomic, epigenomic, and bioinformatic technologies, as well as to determine their alteration and roles in the pathogenesis of diseases.

Histone Post-Translational Modifications. The core histones consist of two molecules of each of the histones H2A, H2B, H3, and H4, which are wrapped by DNA in chromatin. The histone post-translational modifications, including phosphorylation, acetylation, and methylation, alter and influence the accessibility for transcription factor binding, thereby regulating gene transcription (Imhof and Wolffe, 1998). Generally, acetylation of N-terminal tails of the core histones augments the accessibility of transcription factors and subsequent transcription activation (Fig. 4). Specifically, acetylation of lysine (K) residues on histone H4 (lysine residues K5, K8, K12, K16) and H3 (K9 and K14) is directly linked to gene transcription (Imhof and Wolffe, 1998; Rahman et al., 2004; Guillemette et al., 2011; Karmodiya et al., 2012). Histone acetylation and deacetylation are regulated by the opposing activities of histone acetyltransferases (HATs) and HDACs, respectively. Histone phosphorylation is known as an early step in chromatin remodeling and has been shown to be important during chromosomal replication. This modification is associated with inducible proinflammatory gene expression (Rossetto et al., 2012).

Histones can also be methylated on either lysine (K) or arginine (R) residues through histone methyltransferases, including the protein arginine methyltransferase 1 family, the SET-DOMAIN–containing protein family, and the non–SET-domains DOT1/DOT1L (Zhang and Reinberg, 2001; Bannister and Kouzarides, 2005). Histone methylation modulates gene activation or repression in a site-dependent manner (Zhang and Reinberg, 2001). For example, the methylation of H3K9 and H3K27 is linked to the transcriptional repression, whereas H3K4, H3K36, and H3K79 methylation are associated with transcriptional activation (Martin and Zhang, 2005). It is interesting to note that there is cross-talk among different histone modifications and thereby control of gene transcription (Cheung and Lau, 2005; Wang et al., 2008b). For instance, histone H3 Ser10 phosphorylation has been shown inhibit H3K9 methylation, which in turn blocks H3K9 acetylation. Histone H4R3 methylation promotes the acetylation of H4K5, H4K8, H4K12, and H4K16, as well as the methylation of H3R2, H3R17, and H3R26. Therefore, this cross-talk among histone post-translational modifications have combinatorial effects on the transcription repression or activation. Moreover, direct functional connections between histone and DNA methylation have been discovered, as a result of the finding that H3K9 methylation is a prerequisite for DNA methylation in Neurospora (Tamaru and Selker, 2001). Additionally, DNMT1 knockdown reduces histone H3K9 methylation in human colon cancer cells (Espada et al., 2004). Further study of the cross-talk among epigenetic modifications will reveal their co-operative roles in the regulation of the gene expression involved in different cellular processes, such as cell differentiation, inflammation, apoptosis, autophagy, and senescence.

Epigenetic Modifications in Fibrotic Diseases

DNA Methylation in Fibrotic Diseases. Organ fibrosis is associated with aberrant promoter methylation. Accumulating evidence demonstrates that DNA methylation occurs in genes involved in fibroblast activation and fibrogenesis (Yu et al., 2010) (Table 1). We and others demonstrated that RASAL1, a tumor suppressor, is hypermethylated, leading to hyperactive Ras signals as well as fibroblast activation and proliferation (Bechtel et al., 2010; Tao et al., 2011). Loss of RASAL1 protects kidney against fibrosis (Bechtel et al., 2010). Nuclear receptor PPARγ exhibits antifibrotic properties, and its DNA hypermethylation is associated with liver fibrosis (Zhao et al., 2013a,b). Interestingly, ligand activation of PPARγ inhibits the expression of DNMT1 gene and protein, suggesting the autoregulation of PPARγ DNA hypermethylation during fibrogenesis. Phosphatase and tensin homolog...
(Pten) is known as a tumor suppressor, and its downregulation promotes the proliferation of fibroblasts and hepatic stellate cells, leading to the development of liver fibrosis (Zheng et al., 2012). We have shown that low expression of Pten is attributable to methylation on its promoter by DNMT1 (Bian et al., 2012). Smad4 hypermethylation and low gene expression are associated with pathogenesis of lung cancer and IPF (Takenaka et al., 2009). Scleroderma is associated with hypermethylation of FLI1, a suppressor of collagen expression (Wang et al., 2006). However, the hypomethylation of genes involved in regulation of inflammation (secreted phosphoprotein 1), apoptosis (serine/threonine kinase 17b), and histone modification (HIST1H2AH) was observed in lungs of IPF patients compared with controls (Rabinovich et al., 2012). Therefore, DNA methylation appears to occur in a gene-specific manner during fibrogenesis. Further study is required to determine the patterns of gene methylation during acute (inflammatory) and chronic (fibrotic) phases, which would reveal the mechanisms underlying the pathogenesis of fibrotic diseases, and provide clues on early intervention of these diseases.

Inflammation is linked to tissue fibrogenesis, despite inflammatory cells exhibiting both beneficial and detrimental functions during the repair response (Stramer et al., 2007; Lee and Kalluri, 2010). Indeed, the hypermethylation of PPARγ gene promoter is significantly associated with liver inflammation and fibrosis in patients with chronic hepatitis B (Zhao et al., 2013a). Hypomethylation of secreted phosphoprotein 1 gene, a proinflammatory mediator, is observed in fibrotic livers of CC14-treated mice (Komatsu et al., 2012). Nevertheless, further study is required to determine the common and differentiated genes between inflammation and fibrosis in terms of DNA methylation.

As the function of DNA methylation is linked to the distribution of methylation sites across in genome, the studies of genome-wide and loci-specific DNA methylation from fibrotic organs (e.g., liver, lung, heart, and kidney) will identify the common and differential DNA methylation biomarkers and pathways for different organ fibrosis. Interestingly, various genes hypermethylated [e.g., RASAL1, Smad4, Smad7, Pten, E-cadherin, Klotho, p14(ARF), Friend leukemia virus integration 1, Thy-1, and prostaglandin E receptor 2] during fibrogenesis are also hypermethylated in carcinogenesis (Diep et al., 2004; Wang et al., 2007; Sanders et al., 2008; Takenaka et al., 2009; Huang et al., 2010b; Calvisi et al., 2011; Fornari et al., 2012; Sun et al., 2013). It has been shown that the incidence of cancer (e.g., lung and liver) was increased in patients with fibrosis compared with controls (Chiesa et al., 2000; Hubbard et al., 2000; Wallace and Friedman, 2014). This raises intriguing questions: whether the aberrant DNA methylation of these genes is a shared mechanism for both fibrosis and cancer, and if these hypermethylated genes in fibrosis are the susceptible factors for the tumorigenesis.

As discussed above, aberrant DNA methylation is associated with fibroblast activation and fibrogenesis. This suggests the potential therapy for fibrosis of erasing DNA methylation. Indeed, the expression of DNMT enzymes DNMT1 and DNMT3B was increased in human cardiac fibroblasts with profibrotic phenotypes in response to hypoxia (Watson et al., 2014). Treatment with a DNMT inhibitor 5-aza-2′-deoxycytidine suppressed the profibrotic effects of TGF-β in human cardiac fibroblasts as well as a murine bleomycin-induced pulmonary fibrosis (Dakhllallah et al., 2013; Watson et al., 2014). Similarly, 5-aza-2′-deoxycytidine, a deoxyribos analog of 5-azacytidine, reduces the expression of collagen in SSc fibroblasts, which is associated with increased FLI1 transcription (Jungel et al., 2011). In addition, treatment with 5-azacytidine, a DNMTs inhibitor, attenuates folic acid–induced renal fibrosis as well as angiotensin II–induced cardiac fibrosis (Bechtl et al., 2010; Kim et al., 2014). Methyl CpG binding protein 2 (MeCP2) is able to specifically bind to methylated DNA, and forms a complex along with HDACs, thereby turning off gene expression. MeCP2 can also control the histone methyltransferase EZH2, as well as PPARγ, protein patched homolog 1, and α smooth muscle actin gene expression, thereby promoting myofibroblast transdifferentiation and fibrosis (Yang et al., 2006; Mann et al., 2010; Affo and Sancho-Brui, 2014). These findings suggest the therapeutic potential of DNMT inhibitors along with MeCP2 and HDAC modifiers for halting the development of fibrosis (Stramer et al., 2007). However, both 5-azacytidine and 5-aza-2′-deoxycytidine are nonspecific DNMT inhibitors. Thus the development of specific inhibitors of DNMTs that are...
augmented during fibrogenesis would be more beneficial to fibrosis with less potential side effects.

**miRNAs in Fibrotic Diseases.** Microarray analysis has identified a variety of miRNAs that are dysregulated in both murine models of fibrosis and patients with fibrosis (Lee and Kalluri, 2010; Murakami et al., 2011; Bowen et al., 2013; Lino Cardenas et al., 2013; Li et al., 2014) (Table 2). For instance, there are 11 miRNAs (let-7e, miR-125a-5p, -199a-5p, -199b, -199b*, -200a, -200b, -31, -34a, -497, and -802) correlating with the progression of liver fibrosis (Murakami et al., 2011). In CCl4-induced hepatic fibrosis, all miR-29 members (miR-29a, -29b, -29c) are significantly downregulated in liver tissues compared with the controls (James et al., 2012; Li et al., 2014). In dimethylnitrosamine-induced rat hepatic fibrosis, 16 miRNAs are augmented (the 10 most upregulated miRNAs are miR-34b, -34c, -34a, -221, -146b, -214, -199a-5p, -199a-3p, -223, -324-5p), while 22 miRNAs are reduced (the 10 most downregulated miRNAs are miR-150, -193a, -221, -222, -224, -225, -23a, -29a, -29c, and -30c).

<table>
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<th>Target</th>
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<td>Smad4</td>
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<td>He et al., 2012</td>
<td></td>
</tr>
<tr>
<td>miR-150</td>
<td>HSC activation</td>
<td>Liver</td>
<td>Venugopal et al., 2010</td>
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<tr>
<td>miR-194</td>
<td>HSC activation</td>
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<tr>
<td>miR-196a</td>
<td>Collagen</td>
<td>Skin</td>
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<tr>
<td>miR-200</td>
<td>TGF-β and EMT</td>
<td>Kidney and liver</td>
<td>Oha et al., 2010; Pogribny et al., 2010; Wang et al., 2011</td>
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<tr>
<td>miR-204</td>
<td></td>
<td>Kidney</td>
<td>Scian et al., 2011</td>
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<td>miR-211</td>
<td></td>
<td>Kidney</td>
<td>Scian et al., 2011</td>
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<tr>
<td>miR-214</td>
<td>Ncx1</td>
<td>Heart</td>
<td>Aurora et al., 2012</td>
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<tr>
<td>miR-335</td>
<td>HSC activation and proliferation</td>
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<td>miR-449a/b</td>
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<td>Kidney</td>
<td>Muth et al., 2010</td>
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<tr>
<td>miR-590</td>
<td>TGF-β signal</td>
<td>Heart</td>
<td>Shan et al., 2009</td>
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CTGF, connective tissue growth factor; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; HSC, hepatic stellate cell; MAPK, mitogen-activated protein kinase; TPS-1, thrombospondin-1.
whereas 7 miRNAs are downregulated (the three most downregulated miRNAs are miR-378, -193, -878) (Li et al., 2011). The role of these miRNAs in regulation of fibrogenesis is linked to their functions involved in fibroblast proliferation, migration, invasion, apoptosis, and differentiation into myofibroblasts (Yao et al., 2011; Turner and Porter, 2013). For example, in vitro overexpression of miR-19b, -29, -150, -194, and -355 inhibits cell migration and reduces both the myofibroblastic marker α-smooth muscle actin and collagen (Lakner et al., 2012; Zhou et al., 2012; Azrak et al., 2013; Masamune et al., 2014). The TGF-β receptor II is also a target of miR-19b (Lakner et al., 2012) and c-Fos as a putative target of miR-29b (Rossi et al., 2013).

Therefore, targeting these miRNAs may have implications for the therapy of hepatic fibrosis.

Similar to the findings in hepatic fibrosis, miR-29 is downregulated in human IPF (Cushing et al., 2011; Xiao et al., 2012). Further study demonstrates that miR-29 targets the TGF-β1 coding sequence region, thereby inhibiting TGF-β1 signaling and subsequent extracellular matrix deposition and remodeling in lung and heart (Qin et al., 2011; Zhang et al., 2014). miR-200 family members were downregulated in both experimental and idiopathic pulmonary fibrosis (Yang et al., 2012), which is similar to its change in renal fibrosis (Wang et al., 2011; Xiong et al., 2012), but in contrast to its alteration in hepatic fibrosis (Simic et al., 2013). miR-21 is increased in lung epithelial cells, and promotes epithelial mesenchymal transition during lung fibrosis (Yamada et al., 2013). The expression of miR-21 is increased in both pulmonary and renal fibrosis, which amplifies TGF-β signaling, and anti–miR-21 reverses kidney injury (Chau et al., 2012; Glowacki et al., 2013; Yamada et al., 2013; Zerr et al., 2014). miR-155 upregulation correlates with the extent of pulmonary fibrosis, and it targets keratinocyte growth factor (FGF7) (Pottier et al., 2009). A recent study has shown that miR-199a-5p is the best miRNA candidate associated with bleomycin response, and its expression is increased during pulmonary, renal, and hepatic fibrosis (Lino Cardenas et al., 2013). Interestingly, certain miRNAs (e.g., miR-29) are associated with severity of systemic sclerosis and pulmonary fibrosis (Cushing et al., 2011; Zhu et al., 2013). These findings suggest specific miRNAs as potential biomarkers during fibrosis, despite differential patterns of miRNA expression in different fibrotic organs. Further study is needed to screen the miRNA profile in different organs with fibrosis, and to discover and validate the target genes involved in the progression of fibrogenesis. The two main pharmacological strategies to regulate aberrant miRNA (fibro-miRs) during fibrosis are: oligonucleotide-based miRNA antagonists and mimics. However, certain issues are raised regarding the usage of oligonucleotides as pharmaceutical agents. This includes instability in serum and difficulties of targeted delivery of fibromiRs, which are the focus of current research (Pottier et al., 2014).

### DNA Methylation and miRNAs Interaction in Fibrotic Diseases

It is known that miRNAs can be encoded in intronic or exonic DNA regions and are regulated by DNA promoter elements, including DNMT-mediated DNA methylation. Treatment with 5-aza-2’-deoxycytidine enhances miR-17-92 cluster expression, which is associated with the attenuation of bleomycin-induced pulmonary fibrosis (Dakhllallah et al., 2013). Additionally, miR-152 can directly target the 3’-untranslated regions of the DNMT1 transcript, and downregulate DNMT1 gene expression. Interestingly, treatment of 5-aza-2’-deoxycytidine, or depletion of DNMT1 increases miR-152 expression (Huang et al., 2010a; Ji et al., 2013). These findings suggest an epigenetic feedback loop between aberrant DNA methylation and miRNA expression in fibrotic diseases (Sun et al., 2013; Kwon et al., 2014). Further identification and discovery of the miRNA-DNA methylation regulatory loop would decipher the epigenetic roadmap for understanding the pathogenesis of fibrosis.

### Histone Post-Translational Modifications in Fibrotic Diseases

Aberrant histone acetylation occurs on the promoters of genes involved in the pathogenesis of fibrosis (Table 3). For example, ethanol treatment causes dose-dependent increase in histone H3K9 acetylation in hepatic stellate cells (Kim and Shukla, 2005). Similarly, in vivo exposure of liver to acute ethanol-induced phosphorylation (H3ser10 and H3ser28) and phosphoacetylation (H3K9S10) of histone H3 implicates histone acetylation and phosphorylation in ethanol-induced liver fibrosis (James et al., 2012). The class I HDAC inhibitor MS-275 (3-pyridinylmethyl-[[4-[[2-aminophenyl]amino]carbonyl]phenyl]methyl]carbamate) inhibits renal fibrosis via TGF-β and epidermal growth factor receptor signaling (Liu et al., 2013a). Suberoylanilide hydroxamic acid, an HDAC inhibitor, attenuates bleomycin-induced lung fibrosis

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<td>Methylation</td>
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<td>TGF-β1-treated rat mesangial cells</td>
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<td>H3K27me3</td>
<td>Skin fibroblasts from systemic sclerosis patients</td>
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<td>Acetylation</td>
<td>H3K9me2 and H3K9me3 on the promoters of ECM-associated genes</td>
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<td></td>
<td>H3K9</td>
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<td>H3Ac</td>
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<td></td>
<td>H4Ac</td>
<td>Lungs of IPF patients</td>
<td>Coward et al., 2009</td>
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ECM, extracellular matrix.  

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**TABLE 3**  
Histone modifications involved in organ fibrosis
by promoting fibroblast apoptosis (Zhang et al., 2013a; Sanders et al., 2014; Shenderov and Midtvedt, 2014). Suberoylanilide hydroxamic acid also inhibits choroid Plexus–induced peritoneal fibrosis (Io et al., 2014). Similarly, an HDAC inhibitor, largazole, decreases liver fibrosis and angiogenesis by inhibiting TGF-β and vascular endothelial growth factor signaling (Liu et al., 2013b). However, the acetylation of histone H3 and H4 is reduced on cyclooxygenase 2 (COX-2) promoter, which results in decreased expression of this antifibrotic gene in IPF (Coward et al., 2009). Therefore, histone acetylation is aberrant in a gene- and cell-specific manner, and this raises concerns about the safety of a global HDAC inhibitor to treat fibrosis. Further study is required to determine which and how HAT(s) cause histone acetylation in the gene promoter in fibrotic conditions.

The HDAC superfamily has four classes with around 20 members. In particular, sirtuin 1, a type III HDAC, protects against collagen synthesis in fibroblasts and tissue fibrosis (He et al., 2010; Simic et al., 2013; Huang et al., 2014a; Zerr et al., 2014). In contrast, HDAC2 silencing reduces profibrotic TGF-β1 responses in fibroblasts, and induces regression of fibrotic plaques in a rat model of Peyronie’s disease (Ryu et al., 2013; Kwon et al., 2014). The roles of other HDAC members in rectifying aberrant histone acetylation in profibrotic and antifibrotic genes during fibrogenesis remain elusive.

Epigenetic histone methylation modulates profibrotic gene expression. Histone H3K4 methylation (mono-, di-, tri-) positively correlates, whereas histone H3K9 methylation (di- and tri-) negatively correlates with profibrotic gene expression (Sun et al., 2010). Several histone methyltransferases and demethylases are involved in aberrant histone methylation during the development of fibrosis (Zhang et al., 2011; Perugorria et al., 2012). A recent study has shown that significant reduction of H3K9me3, H3K27me3, and DNA methylation was observed at the COX-2 promoter in lung fibroblasts from IPF patients compared with those from nonfibrotic lungs (Coward et al., 2014). This is associated with reduced recruitment of histone methyltransferases G9a, EZH2, and DNMTs as well as binding proteins heterochromatin protein 1, polycomb protein complex 1, and MeCP2 on the COX-2 promoter (Coward et al., 2014). Numerous histone H3K4 methyltransferases, including mixed-lineage leukemia 1 (MLL1), MLL5, Set1, and absent, small, or homoeotic 1 (ASH1), are upregulated during hepatic stellate cell transdifferentiation (Perugorria et al., 2012). 3-Deazaneplanocin A has been identified as a potent inhibitor of polycomb repression complexes that abrogates H3K27me3, which is increased in IFP patients. Treatment with 3-deazaneplanocin A alone is sufficient to induce fibrosis by inhibiting H3K27me3 (Kramer et al., 2013). JMJD3 is a histone H3K27 demethylase, while JMJD2A catalyzes the demethylation of histone H3K9 and H3K36. JMJD3 transfection reduces collagen synthesis in fibroblasts (Guo and Sime, 2012), whereas JMJD2A overexpression promotes cardiac fibrosis (Zhang et al., 2011). Further study on the profile of histone post-translational modifications and their recruitment on the specific gene promoters using the mass spectrometry and chromatin immunoprecipitation-sequencing technologies will reveal the common and differential patterns among different organs with fibrosis. Nevertheless, the development of highly selective inhibitors of histone methyltransferases/ demethylases and HATs/HDACs will be the potential therapeutics for fibrotic diseases.


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