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Epigenetic Regulation of Myofibroblast Phenotypes in Fibrosis

Thu Elizabeth Duong^{1,2} and James S. Hagood^{1,2}

¹Division of Pediatric Respiratory Medicine, University of California–San Diego, La Jolla, California

²Division of Respiratory Medicine, Rady Children's Hospital of San Diego, San Diego, California

Abstract

Purpose of Review—Myofibroblasts are the fundamental drivers of fibrosing disorders; there is great value in better defining epigenetic networks involved in myofibroblast behavior. Complex epigenetic paradigms, which are likely organ and/or disease specific, direct pathologic myofibroblast phenotypes. In this review, we highlight epigenetic regulators and the mechanisms through which they shape myofibroblast phenotype in fibrotic diseases of different organs.

Recent Findings—Hundreds of genes and their expression contribute to the myofibroblast transcriptional regime influencing myofibroblast phenotype. An increasingly large number of epigenetic modifications have been identified in the regulation of these signaling pathways driving myofibroblast activation and disease progression. Drugs that inhibit or reverse profibrotic epigenetic modifications have shown promise in vitro and in vivo; however, no current epigenetic therapies have been approved to treat fibrosis. Newly described epigenetic mechanisms will be mentioned, along with potential therapeutic targets and innovative strategies to further understand myofibroblast-directed fibrosis.

Summary—Epigenetic regulators that direct myofibroblast behavior and differentiation into pathologic myofibroblast phenotypes in fibrotic disorders comprise both overlapping and organ-specific epigenetic mechanisms.

Keywords

Myofibroblast; epigenetic; fibroblast; fibrosis

Corresponding Author: James S. Hagood, MD, University of California-San Diego, Biomedical Research Facility 2, Room 2113, 9500 Gilman Dr. MC 0731, La Jolla, CA 92093-0731, jhagood@ucsd.edu, Tel: (858) 822-7566.

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I. Introduction

Myofibroblasts are considered the primary cell type responsible for normal wound healing and tissue injury repair. Excessive or aberrant activation of repair processes, however, often leads to undesired, pathologic fibrosis (scarring) with subsequent organ dysfunction, and can cause premature mortality. In addition to fibrosis of specific tissues, myofibroblasts also feature prominently in diffuse disease processes such as systemic sclerosis (scleroderma). Furthermore, fibrosis provides an environment in which cancer is more likely to develop, as in hepatocellular carcinoma [1]. Myofibroblasts themselves have been shown to play a role in tumor development and invasion [2]. Despite the prominent role of fibrosis in diseases affecting many different organs, only two specific antifibrotic drugs are FDA-approved for idiopathic pulmonary fibrosis. Most clinicians have used anti-inflammatory or immunomodulatory agents to treat fibrosis, with mixed results. Therefore, elucidating the molecular etiologies for the activation of myofibroblasts is of great interest to researchers and clinicians. Epigenetic mechanisms are the principal regulators of cell phenotype or “identity,” and their role in the differentiation of myofibroblasts has been studied intensely but remains far from complete. As the discovery and development of epigenetic-directed therapies advance and the potential of epigenetic editing becomes reality, an improved understanding of the epigenetic regulation of myofibroblasts will provide promising new therapeutic targets to prevent, stop, or even reverse, fibrosis.

II. Myofibroblast-Origin, fibrotic phenotype, and drivers

Origin

The origin of myofibroblasts seems to be quite diverse, and can be organ or disease specific. Fibroblasts, smooth muscle cells, pericytes, mesenchymal stem/progenitor cells, bone marrow derived cells, adipose tissue cells, and epithelial and endothelial cells via epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (endoMT) have all been described as precursors, which can transdifferentiate into myofibroblasts when appropriately stimulated. Myofibroblasts have both fibroblast secretory and smooth muscle contractile characteristics. The myofibroblast phenotype is typified by the expression of smooth muscle α -actin (SMA) and the secretion of multiple pro-fibrogenic mediators such as TGF β 1, type I/III collagen, and tissue inhibitor of metalloproteinase-1 (TIMP-1), a collagenase inhibitor. SMA strengthens activated myofibroblast contractile activity and is commonly used as a myofibroblast marker though there is no known single marker to discriminate myofibroblasts, just as there are no specific markers to distinguish fibroblasts in general from other mesenchymal cells [3].

Myofibroblast Phenotype in Fibrosis

Activated myofibroblasts are the fundamental drivers of fibrosing disorders and there is great clinical value in better defining myofibroblast origin, fate, and characteristic markers and proteins. In response to injury or infection, the appearance and proliferation of myofibroblast results in deposition of collagen-rich extracellular matrix (ECM). Myofibroblast responses are attenuated as the repair process forms a scar in normal wound healing. They are cleared predominantly through apoptosis or the cells revert back to a more dormant fibroblast

phenotype. Pathologic fibrosis results if active myofibroblasts persist in tissues, with uncontrolled proliferation and excessive collagen deposition. It has been increasingly demonstrated that epigenetic mechanisms contribute prominently to this “hyperactive” phenotype [3].

Myofibroblasts are also the principal cell type involved in tumor stroma remodeling as they establish a microenvironment for epithelial tumor cells to thrive and drive cancer progression [2]. The epigenetic regulation of cancer-related myofibroblasts is not included in this review, but is an immensely important topic that should be considered separately.

Myofibroblast Drivers

Transforming growth factor $\beta 1$ (TGF $\beta 1$) drives profibrotic myofibroblast differentiation from precursor cells and stimulates expression of extracellular matrix and other genes essential to the pathogenesis of fibrosis. TGF $\beta 1$ induces fibrosis by activation of canonical (Smad-based) and non-canonical (non-Smad-based) signaling pathways. Downstream, complex interactions regulate myofibroblast activation and fibrogenesis. Multiple cytokines can suppress TGF $\beta 1$ -mediated fibrogenesis such as basic fibroblast growth factors (bFGF, FGF-2), interferon gamma (IFN γ), and Interleukin-1 (IL-1). Anti-fibrogenic antagonists of TGF $\beta 1$ generally downregulate myofibroblast SMA expression but others have also been shown to decrease collagen deposition and contraction and induce apoptosis [3].

Another pivotal myofibroblast activator is mechanical stress or stiffness of extracellular matrix. ECM strain and stiffness, generated in part by myofibroblast contraction, can be sensed by other mechanosensitive cells, and positively feed back to sustain profibrotic activation. This topic was well reviewed by Hinz in 2016 [3]. Along with TGF $\beta 1$ and mechanical stress, a myriad of other factors can also activate myofibroblasts. A list of recent publications on myofibroblast-inducing factors and an overall review on current myofibroblast literature is available in the aforementioned review [3]. A dominant transcription factor or group of regulatory factors has not been identified to globally direct MTD. Hundreds of genes and their expression can contribute to the myofibroblast transcriptional regime and influence MTD. How these are coordinately regulated to program myofibroblast appearance and behavior is not completely understood, but epigenetic mechanisms are thought to play a prominent role.

III. Epigenetic Regulation of Myofibroblasts

Epigenetic modifications, heritable changes in the genome that do not alter the DNA sequence, influence and regulate gene expression. Epigenetic mechanisms include stable chromatin modifications and interactions, changes in DNA or protein conformation, nuclear organization, and non-coding RNAs. These mechanisms interact in multiple ways to allow or restrict access of transcription factors, transcriptional machinery, and epigenetic modifiers to the DNA sequence, thus affecting promoter and enhancer function, and gene transcription. Non-coding RNAs have predominantly post-transcriptional effects, but also modify the function of other epigenetic modifiers.

DNA Methylation

The most common epigenetic change associated with gene silencing is methylation of 5-cytosine (5meC) at CpG islands at promoter sites by DNA methyltransferases (DNMTs). Inhibition of gene transcription may occur by direct blockage of transcription factor binding to methylated CpG sites, or indirectly through proteins such as methyl CpG binding protein (MeCP2). MeCP2 binds to methylated DNA through a methyl CpG-binding domain and is associated with additional epigenetic changes such as recruitment of histone modifying enzymes [4]. Conversely, hypomethylation at promoters in general is associated with gene activation, although there is no simple inverse relationship.

DNA methylation is considered a favorable therapeutic target, as it can be reversible; however global alteration of methylation lacks specificity. Ten-eleven translocation 1 (TET1) enzyme and its role in DNA demethylation was identified in 2009. TET enzymes convert 5meC to 5-hydroxymethylcytosine (5hmC) (i.e., DNA hydroxymethylation) facilitating reactivation of gene transcription [5]. Given its recent discovery, not much is known regarding TET protein involvement, nor about hydroxymethylation, in fibrosis. The relationship between cytosine methylation and gene function is complex; methylation at non-CpG sites is now known to play a role in gene and enhancer function in numerous cell types [6].

Histone Modifications

Histone proteins are critical in chromatin structure and packaging. Lysine acetylation by histone acetyltransferases (HATs) reduces histone charge, loosening its electrostatic attraction to DNA and locally opening chromatin structure allowing for activation of gene transcription. P300, a histone acetylase and transcriptional coactivator, illustrates this concept. Activated Smad2/3 and p300 hyperacetylation of histone H4 at the collagen type 1 alpha-2 (COL1A2) locus activates TGF β -induced collagen gene expression in myofibroblasts. Peroxisome proliferator-activated receptor γ (PPAR γ) inhibits the TGF β -induced recruitment of p300 to COL1A2 promoter [7]. Lysine acetylation can also create docking sites for bromodomains, where transcription-associated “reader” proteins can bind, creating multiprotein complexes that regulate transcription programs [8].

Deacetylation by histone deacetylases (HDACs) is associated with repression. HDAC4 has been described to most efficiently abrogate SMA expression and prevent TGF β 1-mediated morphological changes seen in myofibroblast differentiation in cultured human fibroblasts. HDAC4 also upregulates expression of the TGF β 1 signaling pathway repressors 5'-TG-3'-Interacting Factor (TGIF) and TGIF2 homeoproteins [9]. Histone hypoacetylation has been associated with profibrotic effects in pulmonary fibrosis [10] and systemic sclerosis [11, 12].

Histone methylation by specific histone methyltransferases (HMTs) can cause activation or repression of gene transcription. The most well-described histone methylations are histone H3 lysine 4 trimethylation (H3K4me3), an activator, and histone H3 lysine 9 di- and trimethylation (H3K9me2/3) and histone H3 lysine 27 trimethylation (H3K27me3) which are transcription repressors. Methylated histones function as a docking site for chromatin modifier proteins that facilitate downstream effects. For example, heterochromatin protein 1

(HP1) recruits additional HMT, DNMTs, and HDAC-containing complexes [13]. Methyl groups can be removed by histone lysine demethylases (KDMs). Exploration of KDM involvement in fibrosis is limited but have been demonstrated in liver HSCs activation to fibrotic myofibroblasts [14] and systemic sclerosis [15].

There is evidence to suggest that DNA methylation and histone modifications are closely interconnected in their regulation and promotion of gene silencing, which are also dependent on the physiologic environment and cell type [16]. The relationship between histone modification and DNA methylation seems to be bi-directional with each able to affect the other. This link may be facilitated by methyl-binding proteins that can recruit histone deacetylases to methylated areas [4].

Non-coding RNAs

A bi-directional relationship has also been described between non-coding RNAs (ncRNA) and DNA methylation. ncRNA are transcribed from DNA but not translated into proteins. Rather they are functional molecules that regulate gene expression at the level of gene transcription and post-transcriptional modifications. ncRNAs were recently reviewed by Beermann et al in 2016 [17].

MicroRNAs (miRs) are the most well described ncRNA involved in epigenetic regulation which bind to 3' UTR of target genes and block translation of the target gene, leading to breakdown of target mRNA and/or blocking of protein translation. Abnormal expression of miRs is associated with disease processes including calcific aortic valve disease [18], cardiac fibrosis [19, 20], liver fibrosis [4, 21–24], lung fibrosis [25–30], renal fibrosis [31–33] and systemic sclerosis [34–43]. Multiple miRs may simultaneously and synergistically affect complex cellular processes such as EMT and these miRs may play different roles in varying organs or developmental stages [44]. The influence of different miRs on the development of fibrosis in various organs was reviewed by O'Reilly in 2016 [45]. Given their implication in the pathogenesis of various diseases, they have become a target for promising therapeutic interventions. Long non-coding RNAs (lncRNAs) are longer than 200 nucleotides and likewise influence gene transcription and protein translation in developmental and disease processes [17].

Taken all together, seemingly separate epigenetic mechanisms construct complex epigenetic regulatory networks which shape whole gene expression profiles, and the disruption of these circuits interrupts normal functions and causes disease.

In this review, we highlight epigenetic mechanisms that have been elucidated to shape myofibroblast behavior in different organs and disease processes related to fibrosis. It can be difficult to discriminate myofibroblasts from their precursor cells, or even to measure myofibroblast de-differentiation into a more quiescent state. Thus, we assume that studies referring to fibroblasts that play a role in fibrosis without specifically mentioning myofibroblasts are essentially describing a myofibroblast phenotype. As hundreds of individual genes can be epigenetically altered in myofibroblasts, where relevant we have referred to recent reviews on epigenetically-regulated genes by organ, and here we focus on mechanisms. Table 1 is a collection of epigenetic regulators and the mechanism by which

they influence myofibroblast phenotype in different organ-specific fibrotic diseases. This table and review is by no means a complete compilation of all epigenetic mechanisms involved but we strive to present a starting point for readers to unravel overlapping and organ/disease-specific epigenetic drivers of myofibroblast phenotype in the context of fibrosis. (Table 1)

IV. Epigenetic Regulation of Myofibroblast Phenotypes in Organ Fibrosis

Heart

After injury or stress, initial reparative cardiac remodeling can adversely evolve into fibrosis, replacing normal heart tissue with non-functional fibrotic myocardium. This stiff tissue causes ventricular dysfunction and essentially heart failure. As heart failure is a leading public health problem with very high mortality rates, pursuing therapies directed at these fibrotic alterations is vital. Activated cardiac fibroblasts, fibroblast-like cells from endothelial-to-mesenchymal transition, and possibly circulating bone-marrow-derived fibrocytes transdifferentiate into myofibroblasts and are the principal drivers of cardiac fibrosis. Epigenetic changes including DNA methylation [46–49] and hydroxymethylation [46], histone modifications [50], and miRs [20] have been implicated in cardiac fibrosis.

During activation of fibroblasts, histone deacetylases (HDACs) are up-regulated and thereby likely take part in cardiac fibrosis; however, the exact molecular mechanisms linking specific histone modifications and fibrosis remain unknown. HDAC inhibitors have been used to infer mechanisms such as blockage of cardiac fibroblast proliferation and migration, induction of genes that suppress fibroblast ECM production, repression of pro-inflammatory markers, and endoMT prevention [51–55]. HDAC inhibitors also block collagen synthesis in isolated cardiac fibroblasts and fibrotic changes diminished in pressure-overload cardiac hypertrophy [56]. Lysine methyltransferase DOT1L belongs to the KMT4 family and seems to be involved in cardiac fibrosis and hypertrophy though it is unclear whether KMT directly orchestrates activation of fibroblasts in cardiac fibrosis [57].

After myocardial infarction (MI), a repair process engages fibroblasts to proliferate, differentiate, and deposit ECM for scar formation and stabilization. An imbalance in the breakdown and deposition of ECM can cause poor repair, aneurysm formation, infarction expansion, and even detrimental myocardial rupture. Myofibroblasts are the primary source of ECM and its modulators after an MI. Fibroblasts, endothelial cells, epithelial cells, smooth muscle cells, and pericytes are considered cardiac myofibroblast precursors. Regulation of precursor cell morphology, growth, proliferation, and migration to infarcted areas are crucial to appropriate healing [57].

Calcific aortic valve disease (CAVD) is a common cardiovascular disease that involves fibrosis and calcification of aortic valve leaflets for which there is no pharmacological treatment [58]. Aortic valve interstitial cells (AVICs) preserve the structure and function of the aortic valve. In healthy human valves, AVICs mainly take on a fibroblastic phenotype and produce ECM to retain normal valvular structure. In contrast, AVICs adopt a myofibroblastic phenotype and are abundant in calcified aortic valves and their augmented proliferative activity and overproduction of ECM protein cause valve thickening and fibrosis

[59]. miRs have been implicated as an epigenetic regulator of AVIC phenotypic transition [18].

Examples of multiple epigenetic mechanisms acting together to affect myofibroblast phenotype can be illustrated in cardiac fibrosis. Hydroxymethylation by TET3 induces DNA methylation which downregulates endoMT inhibiting fibrosis [46, 47]. In pressure overload-induced cardiac fibrosis, histone deacetylases HDAC1 and HDAC2 counteract miR133a antifibrotic signaling [60]. Additional epigenetic modifications involved in cardiac fibrosis and therefore, myofibroblast phenotypes can be found in recent reviews [56, 57].

Liver

Repetitive liver injury and almost all chronic liver diseases cause liver fibrosis. Progressive fibrosis leads to liver failure and in some circumstances, even hepatocellular carcinoma [1]. Liver fibrosis was previously regarded as a process that was beyond repair but compelling evidence now indicates that the removal of inciting triggers and apoptosis of activated hepatic stellate cells (HSCs) can reduce fibrosis. In the liver, myofibroblasts can originate from HSCs which drives the pathogenesis of liver fibrosis. Or, as suggested by Karin et al, the origin of myofibroblasts changes based on whether injury originates in the hepatic lobule such as in chronic hepatitis versus portal areas in biliary diseases. Activated HSCs are considered the “myofibroblast for hepatocytes” and activated portal myo(fibroblasts) the “myofibroblast for cholangiocytes” [61]. Recently, the epigenetic regulation of HSC activation in liver fibrosis was reviewed [1]. In 2016, Nwosu et al summarized DNA methylation and histone modifications described in liver fibroblasts and anti-fibrotic therapies that target these epigenetic mechanisms [62].

The epigenetic control of myofibroblast transdifferentiation was validated with the application of 5-aza-2'-deoxycytidine (5-azadC), a DNA methylation inhibitor, to cultured HSCs which suppresses MTD features [63]. Subsequently, DNA methylation of genes engaged in myofibroblast activation and fibrogenesis have been identified [62, 64–68]. Hepatic MTD seems to be epigenetically regulated by its DNA methylome through direct histone methylation and changes in chromatin structure [4, 63].

PPAR γ , a master antifibrogenic gene, negatively regulates myofibroblast transdifferentiation. MiR132, MeCP2, and EZH2, a lysine methyltransferase, guide an epigenetic relay pathway that transcriptionally represses PPAR γ . MeCP2 is absent in normal human and rat livers but strongly upregulated during culture-induced transdifferentiation of HSCs and diseased livers suggesting that MeCP2 is a key regulator of the myofibroblast phenotype [63]. Furthermore, MeCP2 leads to hypermethylation of Patched (PTCH1) whose repression is critical to maintain HSC activation and hepatic fibrosis [68]. A histone H3K9 demethylase Jumonji domain-containing protein 1A (JMJD1A) directs HSC activation through PPAR γ expression [14]. DNA methylation also inhibits Smad7 granting TGF β -mediated Smad2/3 phosphorylation activating fibrogenesis [66] and it governs I κ B α inflammatory effects. Attenuation of I κ B α is necessary to stimulate NF- κ B activity that leads to MTD with expression of pro-inflammatory genes and myofibroblast resistance to apoptosis [63]. Hence, DNA methylation controls key inflammatory (I κ B α) and fibrogenic (PPAR γ) transcriptional regulators of the myofibroblast phenotype.

Additionally, histone modifications are also operational during HSC activation and differentiation. Upregulated MMP9 causes collagen IV degradation and can therefore counter myofibroblast-stimulated ECM production. HDAC4 has been pinpointed as the culprit in the suppression of IL-1 induced MMP9/13 allowing for HSC transdifferentiation [69]. Hepatocyte growth factor (HGF) is involved in the fibrotic response to acute injury and cylindromatosis (CYLD) is an anti-fibrotic tumor-suppressor gene. HDAC7 inhibits HGF increasing HSCs susceptibility to fibrosis. CYLD removes HDAC7 from the HGF promoter preventing fibrosis [70]. Interestingly, ethanol can stimulate epigenetic modifications in hepatic stellate cells [71]. Excess alcohol consumption causes liver disease and fibrosis is a common feature of disease progression. Ethanol stimulates histone modifications changing chromatin structure and directly impacting HSC transdifferentiation with resultant ECM protein expression. Vitamin D receptor (VDR) ligands and histone modifications can also influence HSC transdifferentiation [72].

Lung

Idiopathic pulmonary fibrosis (IPF) is a progressive, eventually fatal disease with limited effective treatments. Fibrogenesis is driven in large part by the transdifferentiation of pulmonary fibroblasts into myofibroblasts [73]. Prevention of fibroblast transdifferentiation into myofibroblasts is an effective strategy to assuage pulmonary fibrosis. The epigenetic players in IPF have been reviewed [74]. A review of microRNAs that play a role in idiopathic pulmonary fibrosis was published in 2011 [75]. As the epigenetic modifications in IPF have been extensively reviewed, we will provide a limited overview of epigenetic regulators of myofibroblasts in lung fibrosis.

Altered DNA methylation in a large number of genes is associated with fibroblast activation and myofibroblast proliferation [73, 76], and global changes in DNA methylation are seen in IPF [77]. Starting in 2008 descriptions of epigenetic processes driving fibrosis in IPF began to appear, with Sanders et al demonstrating that DNA methylation at the Thy-1 promoter inhibited Thy-1 expression, stimulating MTD and myofibroblast apoptotic resistance [76]. Later in 2011, Sanders et al further demonstrated that histone acetylation affects Thy-1 expression and histone modifications can alter DNA methylation [78]. In 2013, Huang et al demonstrated that histone modifications led to the downregulation of FAS with resultant FAS-mediated apoptosis resistant fibroblasts in lung fibrosis [79]. In 2015, for the first time, Korfei et al demonstrated the involvement of specific histone deacetylases in IPF [80]. Since then, multiple studies have linked histone deacetylase enzymes to pathologic (myo)fibroblast behavior [81–83]. Bromodomain and extra-terminal domain-containing proteins (Brd) bind to acetylated histone residues triggering recruitment of components necessary for gene transcription. Brd2 and Brd4's role in influencing lung fibroblast phenotype in fibrosis was first demonstrated in 2013 [8, 84].

Kidney

Renal fibrosis occurs when physiologic wound healing fails to cease and transitions into pathologic scarring and progressive organ failure. In renal fibrosis, the proliferation of activated (myo)fibroblasts in addition to increased deposition of ECM and increased SMA expression is considered the hallmark of fibrosis. DNA hypermethylation has been shown to

be associated with fibroblast activation and proliferation of pro-fibrogenic myofibroblasts [85]. Remarkably, activated myofibroblasts can play a role in the development of anemia by stimulating epigenetic changes. By comparing two models of renal fibrosis, Chang et al found that fibroblast-like FOXD1+ pericytes differentiate into myofibroblasts and this transition is associated with decreased erythropoietin production and a dampened response by these cells to anemic stimuli. Methylation studies reveal hypermethylation of the erythropoietin gene and application of a demethylating agent on isolated myofibroblasts from these fibrotic kidneys restores erythropoietin expression at baseline and in response to hypoxia [86]. HDAC [87, 88] and miRNAs [31–33] also participate in myofibroblast activation and fibrosis. A review of epigenetic mechanisms regulating renal fibrosis was written by Tampe and Zeisburg in 2012 [89].

Systemic Sclerosis

System sclerosis (SSc) or scleroderma is a rheumatologic disease that causes systemic tissue fibrosis. The current hypothesis for the persistence of activated myofibroblasts in SSc is through profibrotic cytokine stimulation of epigenetic modifications in fibroblasts. In a recent review by Bergmann and Distler in 2017, the authors provide a thorough summary of DNA methylation, histone modifications, microRNAs, and long noncoding RNAs involved in myofibroblast activation along with a list of DNA methyltransferase inhibitors in different phases of drug development [44].

Eye

Epigenetic modifiers play a role in ocular disorders such as pterygia or pterygium, which is a common disease of the eye surface resulting in an abnormal triangular-shaped, fibrotic connective tissue and conjunctival epithelial growth implicating the involvement of fibroblasts [90]. Uncontrolled fibroblast activation and pro-fibrotic signaling causes a pterygium to migrate from the periphery to the center of the cornea causing irritation and can harm vision. Pterygia pathogenesis is multi-factorial and pathways involving fibroblasts and myofibroblasts are not well understood. A few epigenetic mechanisms have been described including aberrant DNA methylation [91] and miRNAs [92]. As surgical treatment is the current intervention of choice, targeting ocular (myo)fibroblasts with an epigenetic therapy is a hopeful avenue to avoid recurrence and potential side effects of eye surgery.

Therapeutic Targets

The successful application of medications that inhibit epigenetic modifications in cancer and the neoplastic-like behavior of myofibroblasts that drives fibrosis has sparked investigations into epigenetic targets that alter myofibroblast behavior. In vitro and a few in vivo studies have established the benefit of pharmacologic therapies that inhibit DNA methylation and histone deacetylation and methylation in the suppression of fibrosis-promoting myofibroblasts [4, 63, 93–101]. Additionally, a small molecule inhibitor of G9a, a lysine methyltransferase, attenuates fibroblast activation [16]; a bromodomain inhibitor prevents fibrosis after intratracheal bleomycin and reduces proliferation and contractile activity in cultured lung fibroblasts [8]; and a BRD4 inhibitor can reverse pre-existing liver fibrosis in a CCl4 mouse model [84].

As fibroblasts and myofibroblasts are the central mediators of fibrosis, epigenetic modifiers that stimulate their apoptosis are a possible therapeutic option, such as HDAC inhibitors which prevent histone modifications responsible for apoptosis resistance [79, 82]. Combinational therapies that incorporate more than one epigenetic mechanism to synergistically reverse or inhibit pro-fibrotic myofibroblast behavior is a promising route. Epigenetic modifiers may also serve as biomarkers of disease severity. For example, expression profiles of miRs in local and diffuse cutaneous SSc [44] and DNA methylation of the PPAR- γ promoter in fibrosis secondary to nonalcoholic fatty liver disease [102].

Future Research

“Epi-drugs” that target active myofibroblasts in fibrosing disorders are a promising direction in the treatment of a myriad of diseases; however, we are far from a comprehensive understanding of how epigenetic modulators influence each other and myofibroblast behavior. This is further complicated by organ and disease-specific variances in epigenetic networks. Future studies must also help us better understand myofibroblast origin and subpopulations.

Regulatory apparatuses involved in disease can be explored by mapping chromatin features to locate tissue-specific enhancers which work with promoters to regulate gene expression [103]. Emerging high throughput single-cell assays that utilize combinatorial indexing, microfluidics, droplet-based methods in combination with new bioinformatics pipelines can better evaluate epigenetic states and trace myofibroblast lineage, possibly identifying rare subpopulations [104]. Reconstructing “epigenetic landscapes” can elucidate candidate transcription factors for reprogramming and modulating cell fate [105]. Recent elegant work in macrophages illustrates how local environments influence enhancer utilization to drive context-dependent heterogeneity of macrophage phenotypes [106]; the same may be true of fibroblasts. Current large-scale efforts to develop multi-“omic” maps of human tissues and organs will provide important knowledge about how normal cell phenotypes are developed and maintained. Another interesting layer to the regulation of gene expression is how local environmental cues (e.g., mechanical stiffness) are transmitted to the nucleus and force-induced changes effect cell signaling and gene transcription [107]. This needs to be further explored as mechanical stress is a key driving factor of myofibroblast activation.

With the advent of bacterial clustered regularly interspaced short palindromic repeats (CRISPRs) system, genome engineering and editing has rapidly progressed and studies pursuing epigenome editing have surfaced including CRISPR-based targeted demethylation [108]. Targeted DNA demethylation is also possible through fusion of a plant 5-methylcytosine DNA glycosylase to sequence-specific DNA binding domains [109]. Applications of targeted epigenome editing would greatly enhance our understanding of how epigenetic modifications regulate gene expression in specific tissues and diseases, translating into targeted tissue and lesion specific anti-myofibroblastic therapies that will have immense clinical benefit.

Overall, the findings presented in this review highlight the complex epigenetic paradigms that directs cell differentiation into pathologic myofibroblast phenotypes in many fibrotic diseases. A more comprehensive understanding of the organ-specific epigenetic mechanisms

involved in fibrosis, and of how to target them for therapeutic benefit without significant off-target effects, will facilitate the development of novel antifibrotic therapies.

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Table 1

Epigenetic Regulation and Mechanisms directing Myofibroblast Phenotype in Organ Fibrosis

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
Heart	Fibrosis	DNA methylation Hydroxymethylation	EndoMT → myofibroblast ↓EndoMT	[TGFβ1, hypoxia → aberrant methylation RASAL1 gene promoter ∩ RASAL1] → ↑ Ras-GTP activity BMP7 → ↑ TET3 hydroxymethylation of induced RASAL1 promoter methylation → RASAL1	[46, 47]
	Fibrosis	DNA methylation	Overactive fibrotic phenotype	Hypoxia → human cardiac fibroblasts express hypoxia-inducible factor (HIF-1α) → DNMT1/3b → DNA methylation → pro-fibrotic genes	[48]
	Fibrosis	DNA methylation	Fibroblast activation	[↑ DNMT3a → DNA methylation ∩ Ras association domain family 1 isoform A (RASSF1a)] → ERK1/2	[110]
	Myocardial infarction and myocardial rupture	DNA methylation	Proliferation and differentiation (myofibroblast-like cells → ECM degradation and fibrogenesis)	Deficient NEIL3 → dysregulation methylation in cardiac epigenome	[58]
	Fibrosis and hypertrophy	DNA methylation inhibitor 5-azacytidine (5AZ)	∩ fibroblast activation → ↓myocyte size	5AZ ∩ myofibroblast differentiation → ↓ TGFβ1-induced collagen I/III and SMA mRNA expression and ↓myocyte size 5AZ → ↑MYBP-C3 gene expression in myofibroblasts	[99, 100]
	Fibrosis		Fibroblast proliferation	HDAC6 ∩ RASSF1a → ERK1/2	[50]
	Fibrosis		∩ Angiotensin II dependent cardiac fibrosis, fibroblast activation and differentiation ∩Differentiation bone marrow-derived fibrocytes	[HDAC1 inhibitor → p15, p57 ∩ cyclin-dependent kinase] ∩ retinoblastoma protein phosphorylation ∩ G1-to-S phase transition → ↓ECM-producing cells Class I HDAC inhibitor ∩ ERK1/2	[51]
	Congestive heart failure after MI	HDAC1 inhibitor (Mocetinostat)	[Reversal of cardiac myofibroblast phenotype to fibroblast and induction cell cycle arrest/apoptosis] → ↓ ECM production, ↓interstitial fibrosis, ↓scar size	Mocetinostat → 1. Reversal of myofibroblast phenotype (↑SMA, ↓collagen III, ↑MMP-2) 2. ↑E-cadherin and surface translocation β-catenin → CD 90+ fibroblast transformation to epithelial-like phenotype 3. ↓phosphorylation Akt (↓EMT/EndoMT) in CD 90+ fibroblast 4. Induction cell cycle arrest/apoptosis (fibroblasts in vivo) → ↓reverse fibrosis, ↓ ECM production	[53]
	Systolic Heart Failure (HF)	HDAC inhibitor (MPT0E014)	∩ Cardiac fibroblast proliferation and migration	MPT0E014 on isolated primary cardiac fibroblasts from HF rats → ↓TGFβ1, ↓angiotensin II type I receptor (AT1R), ↓migration and proliferation cardiac fibroblasts (↓↓ in presence of TGFβ1)	[54]
	Atrial fibrosis and arrhythmias in cardiac hypertrophy	TSA (non-selective HDAC inhibitor)	Reverses atrial fibrosis	HDAC → cardiac hypertrophy TSA → reverses atrial fibrosis, connexin 40 remodeling and ↓ atria arrhythmia vulnerability independent of angiotensin II	[55]
	Myocardial disease and failure		→ fibroblast survival and fibrosis	↑ miR21 in cardiac fibroblasts of failing heart ∩ sprout homologue 1 (Spry1) → ERK-MAP kinase pathway → fibroblast survival and growth factor secretion → interstitial fibrosis and cardiac hypertrophy [miR21 antagonist in mouse pressure-overload induced disease ∩ ERK-MAP kinase activity ∩ interstitial fibrosis] → ↑cardiac function	[111]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
	Myocardial infarction (MI) and fibrosis	miR-29 family	Q miRNAs involved in ECM production and fibrosis	MI → ↓ miR-29 in regions adjacent to infarct → ↑ collagen expression (COL1A1, COL1A3, COL3A1 and fibrillin 1-FBN1) ↑ miR-29 in fibroblasts → ↓ collagen	[19]
	MI		Cardiac fibroblasts → myofibroblast phenotype and mature collagen → scar healing and contracture	Coronary occlusion and hypoxia → ↓ miR145 then ↑ miR145 miR145 → MTD with iSMA	[112]
	Fibrosis and hypertrophy		Pro-fibrotic	[Q miR199b] → normalized Dyrk1a expression → ↓ nuclear NFAT activity → inhibition and reversal of hypertrophy and fibrosis in mouse heart failure model Dyrk1a Q [cardiac stress → calcineurin/NFAT signaling → cardiac hypertrophy/remodeling and failure] miR199b + NFAT Q Dyrk1a	[20]
	Calcific Aortic Valve Disease		Aortic valve interstitial cells → myofibroblast phenotype	↑ miR-486 → ↓ phosphatase and tensin homolog (PTEN) expression, ↑ phosphorylated protein kinase B (AKT) phosphorylation in AVICs → ↑ SMA expression	[18]
	Pressure overload-induced cardiac fibrosis	HDAC1, HDAC2, and miR133a	↓ miR133a → fibrotic phenotype	HDAC1, HDAC2 on miR133a enhancer regions → ↓ miR133a → ↑ connective tissue growth factor expression, ↑ fibrosis, ↑ left atrium diameter (↓ diastolic function)	[60]
Liver	Fibrosis	DNA hypermethylation	HSC → myofibroblast phenotype → fibrosis	Methylation by DNMT3a → loss Septin 9 expression	[64]
	Fibrosis	DNA methylation	Methylation CTGF gene promoter Q [HSCs → myofibroblast]	[↑ methylation at connective tissue growth factor (CTGF) gene promoter Q CTGF] Q HSCs phenotype changes into myofibroblast (iSMA)	[65]
	Fibrosis	DNA methylation	HSC → myofibroblast phenotype → fibrosis	[DNA methylation Smad7 gene Q Smad7 repression] → phosphorylation and activation TGFβ-mediated Smad2/3 pathways	[66]
	Fibrosis	DNA hypermethylation	HSC → myofibroblast phenotype → fibrosis	DNMT1 methylation of phosphatase and tension homolog deleted on chromosome 10 (PTEN) gene promoter → PI3K/AKT and ERK pathways	[67]
	Fibrosis	DNA methylation and MeCP2	Sustain fibroblast activation → fibrosis	[MeCP2 and hypermethylation Patched (PTCH1) Q PTCH1] → perpetuation fibroblast activation and fibrosis	[68]
	Fibrosis and wound healing	DNA methylation and MeCP2 HDAC,HMT	Loss of IxIa and PPARγ expression → proinflammatory and profibrotic myofibroblast characteristics	[MeCP2 interacts with IxIa promoter via methyl-CpG sites + CBF-1 bound to NF-κB (κB2/CBF-1 site) → HDAC,HMT recruited to IxIa promoter → histone modification → transcriptional repression	[63]
	Fibrosis	DNA methylation, MeCP2, and miR132 Methylation histone 3 lysine 27 (H3K27me3) and histone 3 lysine 9 (H3K9me3)	HSC → myofibroblast phenotype → fibrosis	miR132 downregulated → MeCP2 translation → MeCP2 binds to PPARγ → H3K9me3 → HPIα recruitment → chromatin condensation Q PPARγ MeCP2 → EZH2 expression → H3K27me3 → binding site for Polycomb repressor 1 complex → chromatin condensation Q PPARγ	[4]
	Fibrosis		HSC transdifferentiation and Q collagen degradation	[HDAC4 → ↓ histone acetylation Q IL-1 induced MMP promoter activity and MMP9 expression] → HSC activation IL-1 → MMP 9 → collagen IV degradation	[69]
	Fibrosis		→ pro-fibrotic HSCs	[HDAC7 Q HGF in HSCs] → fibrosis	[70]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
				CYLD removes HDAC7 from HGF promoter ⊗ fibrosis	
	Alcohol-induced liver disease and fibrosis	MLL1 (H3K4 methyltransferase)	→ pro-fibrotic HSC transition with ↑ECM production	Ethanol → MLL1 recruitment to elastin gene promoter→ H3K4me3 → ↑ elastin expression in transiting HSCs ↑ MLL1 → ↑telastin/collagens in alcoholic liver disease explants Ethanol → ↑profibrogenic/ECM gene expression	[71]
	Fibrosis	Absent, small or homeotic disc 1 (ASH1) – histone methyltransferase of lysine 4 at histone H3 (H3K4) and MeCP2	HSC → myofibroblast phenotype → fibrosis	ASH1 binds to regulatory regions of SMA, collagen I, TIMP1, TGF-β1 in activated HSCs → H3K4 methylation and gene activation MeCP2 → ASH1 expression	[113]
	Fibrosis	HAT and coactivator p300	→ HSC activation and ↑collagen	TGF-β → p300 /CBP → histone H3 hyperacetylation at VDR/SMAD COL1A1 regulatory region→ HSC activation Calcipotriol (synthetic VDR agonist) ⊗ VDR/SMAD interaction → ↓collagen deposition and fibrotic gene expression	[72]
	Fibrosis	Jumonji domain-containing protein 1A (JMJD1A) – H3K9 demethylase	Modulates HSCs activation →	Small interfering RNA → knockdown JMJD1A in HSCs→ H3K9me2 PPAR γ gene promoter → ↓PPAR γ mRNA and protein → ↑SMA/COL1A expression, strengthened collagen production, enhanced necrosis	[14]
	Fibrosis	Histone methyltransferase inhibitor 3-deazaneplanocin A (DZNep)	⊗ fibrosis progression in vivo despite sustained liver damage	Myofibroblast targeting antibody-liposome- DZNep → ↓EZH2 → ↓ H3K27me, H3K4me3, H3K9me3, and H3K36me3	[98]
	Fibrosis	TSA (non-selective HDAC inhibitor)	⊗ EMT and fibrosis	[TSA ⊗ TGF-β1-induced EMT] ⊗ fibrosis TSA ⊗ p300 binding to SMAD3 ⊗ activation of type I collagen promoter ⊗ type I collagen deposition TSA → Friend leukemia virus integration 1 ⊗ type I collagen gene	[101]
	Fibrosis	miR17-5p	→ HSC activation and proliferation	TGF-β1-treated HSCs and CCI4-induced rat fibrosis → ↑ miR17-5p ⊗ [SMAD7 ⊗ TGF-β1] ⊗ miR17-5p ⊗ HSC proliferation, apoptosis resistance	[24]
	Fibrosis		↓ miR29 → HSCs → fibrosis	↓ miR29 in murine carbon tetrachloride-induced hepatic fibrosis and human fibrotic livers TGF-β, NF-κB, lipopolysaccharide (LPS) → ↓ miR29 in HSCs → ↑ extracellular matrix genes ↑ miR29 in HSCs → ↓ collagen expression	[21]
	Fibrosis		HSC activation → fibrosis	TGF-β1 → [↑ miR33a ⊗ P13K/Akt pathway, PPAR-α mRNA/protein] → HSC activation and extracellular matrix production	[23]
	Fibrosis		↓collagen synthesis in HSCs	TGF-β → ↓ miR133a in HSCs during fibrogenesis ↑ miR133a → ↓collagen expression	[22]
	Fibrosis	JQ1 (BRD4 inhibitor)	↓ [cytokine-induced activation of HSCs → myofibroblasts]	JQ1 ⊗ TGFβ-mediated activation pro-fibrotic gene expression JQ1 reversed pre-existing liver fibrosis in CCI4 mouse model	[114]
Lung	Fibrosis	DNA methylation and MeCP2	HEL F cells → myofibroblast → Fibrosis	MeCP2 → methylation of tuberous sclerosis TSC1, TSC2 promoter regions ⊗ TSC1, TSC2 protein expression	[73]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
	IPF	DNA methylation TSA (non-selective HDAC inhibitor)	→ fibrogenic phenotype	Thy-1 $\oslash$ MTD from fibroblast DNA hypermethylation at Thy-1 promoter → fibrogenic myofibroblast → fibrosis DNA methyltransferase inhibitors → restoration Thy-1 expression in Thy-1(−) fibroblasts TSA → [↑H3K27me3 → demethylation at Thy-1 promoter, restoration Thy-1 expression] and ↑histone acetylation (H4) and ↑H3K4me3	[76] [78]
	IPF	Methylation histone 3 lysine 9 (H3K9me3) and histone 3 lysine 27 (H3K27me3)	Promotes fibrogenic phenotype, increases collagen production	[G9a- and EZH2-binding → H3K9me3 and H3K27me3 ↔ recruitment DNNMTs and HDAC-containing complexes to cyclooxygenase-2 (COX-2) promoter] $\oslash$ COX-2 → ↓ loss prostaglandin E2	[16]
	IPF		Normal human lung fibroblasts → myofibroblasts	[HDAC4 $\oslash$ protein phosphatase-mediated dephosphorylation AKT] → TGF- β 1 → phosphorylated AKT	[81]
	IPF	Elevated HDAC Class I (HDAC1 , HDAC2 , HDAC3 , HDAC8) and Class II (HDAC4 , HDAC5 , HDAC7 , HDAC9)	Increase fibroblast proliferation, fibroblast-to-myofibroblast differentiation, apoptosis-resistant myofibroblast phenotype	Upregulation of myofibroblasts in fibroblastic foci HDAC 4 → AKT signaling pathways and TGF- β 1 induced differentiation of normal lung fibroblasts into myofibroblasts LBH589 (pan-HDAC inhibitor) on primary fibroblast → ↓expression of ECM synthesis, ↓proliferation and cell survival, ↓HDAC7 genes, and [endoplasmic reticulum stress → apoptosis] VPA (class I HDAC inhibitor) → partial attenuation profibrotic and apoptosis-resistant phenotype of IPF fibroblasts	[80]
	IPF	Histone acetylation and H3K9Me3	↓ apoptosis resistance in fibroblasts	↓histone acetylation, ↑ H3K9Me3 at Fas promoter → ↓Fas-mediated apoptosis resistant fibroblasts	[79]
	IPF	Suberoylanilide hydroxamic acid (SAHA , HDAC inhibitor)	↑myofibroblast apoptosis susceptibility	SAHA → ↑histone modifications (acetylation H3K9, H3, H4 and methylation H3K9me3), ↓DNMT1/3a mRNA → ↓pro-apoptotic Bak with promoter hypomethylation, ↓anti-apoptotic Bcl-xL→ (myo)fibroblast apoptosis	[82]
	IPF	Spiruchostatin A (selective Class I HDAC inhibitor)	$\oslash$ fibroblast proliferation and collagen production	Spiruchostatin A → ↑histone 3 acetylation, ↑cell-cycle inhibitor p21 ^{waf1} , ↓ SMA and interstitial collagen expression	[83]
	IPF	Histone hypoacetylation	Uncontrolled fibroblast proliferation	[↓ histone acetyltransferases→ ↓histone H3 and H4 acetylation] and ↑transcription of HDAC-containing corepressor complexes to COX-2 promoter → ↓ transcription factor binding → ↓ COX-2→ ↓ prostaglandin E2 (antifibrotic mediator)	[10]
	IPF	G9a (KMT1C)	Activation fibroblasts → fibrosis	G9a → methylates H3K9 ↑ H3K9me3 , HMT , heterochromatin protein 1 (HP1) to gamma interferon-inducible protein of 10 kDa (IP-10) promoter in IPF lung fibroblasts → repressive chromatin → ↓anti-fibrotic (IP-10) Small molecule inhibitor G9a → ↓hypermethylation and ↑HP1/CoREST binding to H3K9 promoter → restored expression IP-10	[13]
	Fibrosis	Brd2 and Brd4	Pro-fibrotic myofibroblast phenotype	TGF β and PDGF stimulation lung fibroblasts → histone acetylation → Brd2/4 binds → enhanced transcription → SMA induction of fibroblasts Brd inhibitor → prevent fibrosis after intratracheal bleomycin, [↓ PDGF- and TGF β -stimulated IL-6 secretion, collagen and SMA expression, and proliferation/contraction cultured lung fibroblasts]	[8, 84]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
	IPF	miR=17~92 cluster and DNMT1	Fibrotic gene expression → pro-fibrotic phenotype	↓ miR=17~92 → ↑DNMT1 → ↑DNA methylation miR=17~92 promoter	[29]
	IPF	Increased miR21 expression primarily in myofibroblasts	Promotes fibrosis	TGF-β1 → miR21 expression → Smad7 inhibition, Spry1 expression → TGF-β1 induced fibrogenic activation of pulmonary fibroblasts bFGF → miR21 expression in human primary fibroblasts	[25]
	Fibrosis	miR-29	↓ miR-29 → fibrotic phenotype with ECM deposition and remodeling	TGFβ → ↓ miR-29 → ↑ expression profibrotic target genes (collagens, ECM-associated, remodeling, laminins, integrins), ↑ severity of fibrosis miR-29 targets and negatively regulates SMAD3 in TGFβ pathway and CTGF expression	[27] [28]
	IPF	let-7d (miR)	↓ let-7d → EMT, ↑ collagen deposition	TGF-β1 → SMAD3 binding to let-7d promoter → ↓ let-7d → ↑ mesenchymal markers N-cadherin, vimentin, SMA, HMG A2	[26]
	IPF		Fibroblast → myofibroblast → fibrosis	↑ miR199a-5p in myofibroblasts from injured mouse lung and fibroblastic foci ecrotic miR199a-5p → pulmonary fibroblasts proliferation, migration, invasion, and differentiation into myofibroblasts TGFβ → ↑ miR199a-5p → TGFβ-induced leucolin-1 (CAV1)	[30]
Kidney	Fibrosis	DNA hypermethylation	Fibroblast → myofibroblast → fibrosis	DNMT1 → hypermethylation RASAL1 → irreversible transcriptional repression	[85]
	Fibrosis and renal anemia	DNA methylation	Demethylating 5-azacytidine → myofibroblast re-differentiation into pericytes	Renal erythropoietin-producing cells (REPCs) in fibrotic kidneys Kidney pericytes can differentiate into collagen-producing myofibroblasts Fibroblast-like FOXD1+ progenitor-derived kidney pericytes produce erythropoietin [FOXD1+ pericytes differentiate into myofibroblasts → hypermethylation erythropoietin] ⊗ erythropoietin production Demethylating 5-azacytidine on myofibroblasts 1. → erythropoietin ⊗ anemia 2. re-differentiate myofibroblasts into pericytes	[86]
	Interstitial fibrosis secondary to obstructive nephropathy	TSA (non-selective HDAC inhibitor)	Prevent activation and accumulation fibroblasts	TSA ⊗ SMA and fibronectin expression, phosphorylated STAT3 and accumulation activated renal interstitial fibroblasts after obstruction	[87]
	Interstitial fibrosis secondary to obstructive nephropathy	Class III HDAC sirtuin (SIRT1 and SIRT2)	Renal fibroblast activation, proliferation and fibrogenesis	SIRT1/2 expressed in cultured renal interstitial fibroblasts Sirtinol (SIRT1/2 inhibitor), EX527 (SIRT1 inhibitor), AGK2 (SIRT2 inhibitor), small interfering SIRT1/2 RNA → ↓fibroblast activation markers (SMA, fibronectin, collagen I) and proliferation markers (proliferating cell nuclear antigen, cyclin D1/E) Sirtinol → dephosphorylation EGFR, PDGFRβ, STAT3	[88]
	Fibrosis secondary to obstructive nephropathy		⊗ TGF-β1/SMAD3 mediated fibrosis ⊗ [TGFβ → ADAM12/19] mediated fibrosis	TGF-β1 → SMAD3 binding to miR29 promoter → ↓ miR29 in cultured fibroblasts → ↑ collagen I/III expression miR29 gene delivery before or after obstructive nephropathy ⊗ fibrosis miR2a/b/c ⊗ [TGFβ-mediated upregulation ADAM12/19 → epithelial to fibroblast phenotype differentiation with ↑Col1a/Col3a] miR29 family has no effect on SMA/MMP1	[32] [33]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
	Fibrosis in diabetic nephropathy		◊EMT	[↑ miR-129 ◊ ZEB1/2] ◊ TGFβ-mediated downregulation E-cadherin in proximal tubular cells ↓ miR-129 → tubulointerstitial fibrosis and low GFR	[31]
	Fibrosis in diabetic nephropathy	HAT p300	Tubulointerstitial fibrosis	High glucose → p300 requires myocardin-related transcription factor MRTF-A a potent pro-fibrotic co-factor for serum response factor) → ECM genes → type 1 collagen ◊ MRTF-A → disappearance acetylated H3K18/K27 and trimethylated H3K4 MRTF-A recruits p300 and WD repeat-containing protein 5 (key H3K4 methyltransferase complex) to collagen promoters → transcription	[115]
Other	Systemic Sclerosis	DNA hypermethylation	Persistent fibroblast activation	[Dickkopf-1 (DKK1) and secreted frizzled-related protein-1 (SFRP) hypermethylation ◊ DKK1/SFRP] → canonical Wnt signaling	[116]
	Systemic Sclerosis	DNA hypermethylation, histone hypoacetylation, and histone deacetylation	Fibroblast activation→ profibrotic effects	[Friend-leukemia integration factor 1 (FLI1) hypermethylation and H3/H4 hypoacetylation ◊ FLI1] → ↑collagen type 1 gene expression 2-deoxy-5-azaC (DNMT inhibitor) and Trichostatin A (TSA , non-selective HDAC inhibitor) → ↓ type 1 collagen expression	[11]
	Systemic Sclerosis	DNA hypermethylation and histone hypoacetylation	Sensitizes fibroblasts to profibrotic stimuli	Kruppel-like factor 5 (KLF5) hypermethylation and histone H3/H4 hypoacetylation ◊ KLF5 Epigenetic downregulation KLF5 and FLI1 synergistically →↑ connective tissue growth factor	[12]
	Systemic Sclerosis	TSA (non-selective HDAC inhibitor)	Inhibits SSc fibroblast phenotype ◊fibroblast proliferation and extracellular matrix accumulation	[TSA ◊ Wnt inhibitor factor 1 (WIF-1) loss] ◊ collagen accumulation WIF-1 deficiency → Wnt → collagen production TSA treatment of SSc skin fibroblasts →↓ collagen type 1 alpha-1 (COL1A1) and fibronectin →↓ collagen protein TSA → ↑cell-cycle inhibitor p21	[117] [118]
	Systemic Sclerosis	HDAC7 (Class II HDAC)	◊ HDAC7 →↓ECM	TSA ◊ HDAC7 →↑constitutive and cytokine-induced production type I/III collagen, no ↑ profibrotic molecules (ICAM-1, CTGF) TSA , in addition to anti-fibrotic effects, also →↑ ICAM-1/CTGF, ↑ HDAC3 HDAC7 more specific to SSc → silencing HDAC7 more effective and safer than TSA	[119]
	Systemic Sclerosis	MeCP2 and miR135b	MeCP2 → SSc fibroblasts with increased ECM production miR135b → ↓ collagen	[↑ MeCP2 → hypermethylation miR135b promoter ◊ miR135b] → STAT-6-mediated ECM induction miR135b → ↓ IL-13 mediated collagen induction	[45]
	Systemic Sclerosis	HAT and transcriptional coactivator p300	Persistence and progression of fibrotic response by SSc fibroblasts	TGFβ in presence of p300 → TGFβ-induced collagen gene → ↑ collagen production TGFβ → early-immediate TF (Egr-1) expression → transcription COL1A2, [p300 → COL1A2 promoter histone acetylation → SMAD2/3 transcription activation]	[120] [7]
	Systemic Sclerosis	Class III histone deacetylase sirtuin 1 (SIRT1)	Fibroblast enhanced release of collagen Antifibrotic effects	SIRT1 →TGFβ/Smad signaling →release of collagen Conflicting outcome: SIRT1 →↓ p300 histone acetyltransferase →↓ collagen	[121] [122]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
	Systemic Sclerosis	Mitochondrial deacetylase sirtuin 3 (SIRT3)	Mitigates activated SSc fibroblast phenotype	SIRT3 ⊗ intracellular TGFβ signaling and fibrotic responses SIRT3 reduced in SSc skin and fibroblasts Downregulation SIRT3 → mRNA profibrotic genes In vivo, hexafluoro SIRT3 agonist) →↓ bleomycin-induced skin and lung fibrosis	[123]
	Systemic Sclerosis	H3K27me3 3-deazaneplanocin A (DZNep) – H3K27me3 inhibitor	DZNep → activation of fibroblasts and myofibroblast differentiation	[DZNep ⊗ H3K27me3] → release of collagen from fibroblasts, ↑ profibrotic transcription factor fos-related antigen 2 (FRA-2) DZNep → ↑FRA-2→ TIMP→ fibroblast-to-myofibroblast differentiation	[124] [125]
	Systemic Sclerosis	H3K27me3 demethylase Jumonji domain containing protein 3 (JMJD3)	Fibrosis phenotype	⊗ JMJD3 →↑ H3K27me3 , ↓FRA-2, ↓contractile fibers, ↓SMA, ↓collagen secretion, ↓myofibroblast differentiation TGFβ→ JMJD3 expression ⊗ JMJD3 →↓ fibrosis in bleomycin-, Topo- and TBR ^{act} -induced experimental models	[15]
	Systemic Sclerosis		↓collagen and anti-fibrotic phenotype	↑ thrombospondin-2 (TSP-2) protein in SSc dermal fibroblasts → → ↑ miR7 ⊗ TSP-2 mRNA miR7 ⊗ collagen expression	[34]
	Systemic Sclerosis	miR29a and miR29b	Anti-fibrotic phenotype with collagen degradation and ↓ ECM/collagen production	miR29a ⊗ [TGFβ activated kinase 1 binding protein 1 (TAB-1) → TIMP-1 production → ECM deposition] ↓ miR29a → TGFβ, PDGF⊗ miR29a miR29a → matrix metalloproteinase-1 (MMP-1) → collagen degradation miR29a targets COL3A1 post-transcription →↓ collagen production SSc skin/fibroblasts and TGFβ-stimulated fibroblasts → → ↓ miR29b →cCOL1A1 mRNA	[35, 36] [37]
	Systemic Sclerosis		↓collagen and ↓fibrotic characteristics	↓ miR129-5p in SSc fibroblasts IL-17A →→↑ miR129-5p →→↓connective tissue growth factor, ↓α1(I) collagen	[38]
	Systemic Sclerosis	miR150 and DNA methylation	↓ miR150 → fibrosis with ↑ collagen	DNA methylation →↓ miR150 in SSc dermal fibroblasts → integrin 3 → collagen type 1 overexpression	[39]
	Systemic Sclerosis		↓ miR196a → fibrosis with ↑ collagen	TGFβ-stimulated Fibroblasts and Ssc fibroblasts →→↓ miR196a ↓ miR196a →↑ collagen type 1 expression	[40]
	Systemic Sclerosis		↓ miR206 → fibrosis	SSc skin → → ↓ miR206 miR206 regulates 15 genes related to SSc pathogenesis: TGFβ1, TGFβ2, SMAD5, FGF2, FGF5, FGF3, IL1β, IL1R AP (receptor accessory protein), IL21, integrinα2, cyclinT2, FGFβ (fibrinogen β chain), COL6α3, COL29α2 and CD28	[41]
	Systemic Sclerosis	miR21 miR145	Fibrosis phenotype	Fibroblasts exposed to TGFβ → 1, ↑ miR21 →↓SMAD7 mRNA 2, ↑ miR145 →↓SMAD3 mRNA Conflicting outcome: ↓ miR145 and ↑ SMAD3 mRNA in SSc skin biopsies and SSc fibroblasts	[37]

Organ	Disease	Epigenetic Regulator(s)	Effect	Mechanism and Findings	References
	Systemic Sclerosis		→ collagen synthesis and skin, lung, and systemic fibrosis	↑ miR155 in fibrotic skin from localized scleroderma and SSc ⊗ miR155 in primary skin fibroblasts → 1. ⊗ collagen synthesis 2. ⊗ Wnt/β-catenin and Akt pro-fibrotic pathways miR155 targets 1. casein kinase 1α (CK1α) ⊗ β-catenin pathway 2. Src homology 2-containing inositol phosphatase-1 (SHIP-1) ⊗ Akt signal pathway topical anagomir-155 treats bleomycin-induced skin fibrosis in mice ((β-catenin pathway and -phosphorylated Akt staining in fibroblasts) miR155 (−/−) mice resistant to bleomycin-induced skin fibrosis (β-catenin pathway, ↓phosphorylated Akt) miR155 (−/−) protected from bleomycin-induced pulmonary fibrosis	[42] [43]
	Systemic Sclerosis	lncRNA TSIX	fibrosis with ↑ collagen	TGFβ → ↑ TSIX → stabilizes COL1A1 mRNA → ↑ type I collagen	[126]
Eye	Pterygium	miR200 family	→ EMT	miR-200a/-200b/-429 and miR-200c/-141 → EMT, ↑ fibronectin (FN1)	[91, 92]

By organ-specific fibrotic diseases, epigenetic regulators, their effect on myofibroblast phenotype, and mechanism of action. Epigenetic regulators in **green** and **red** illicit an antifibrotic versus profibrotic effect respectively.

→ = leads to or stimulates, ⊗ = inhibits, ↑ = upregulates, ↓ = downregulates, [ ] groups events in a mechanism