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REVIEW

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The epigenetic roles of pirfenidone - implication in liver disease management

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ABSTRACT

Liver diseases represent a major global health challenge, responsible for over two million deaths annually. Metabolic dysfunction-associated steatotic liver disease (MASLD) and its progressive form, metabolic dysfunction-associated steatohepatitis (MASH), are the primary contributors to liver fibrosis and hepatocarcinoma (HCC). Epigenetic mechanisms, including DNA methylation, histone modifications, and miRNAs, play a crucial role in the pathogenesis of liver disorders, presenting promising therapeutic targets due to their reversibility. Pirfenidone, an antifibrotic agent approved for idiopathic pulmonary fibrosis (IPF) and hepatic fibrosis in Mexico, has shown significant potential to modulate epigenetic pathways. This review discusses the molecular and epigenetic mechanisms by which PFD exerts hepatoprotective effects, including modulation of miRNA expression, restoration of DNA methylation patterns, and regulation of histone acetylation and methylation. Notable findings include PFD-mediated downregulation of pro-fibrotic miRNAs, hypermethylation of TGFB1, and inhibition of JMJD2B histone demethylase. Together, these findings suggest that PFD not only targets fibrotic and inflammatory pathways but also acts as a novel epigenetic regulator, positioning it as a promising therapeutic candidate for MASLD, MASH, and HCC.

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Pirfenidone; epigenetic; hepatic diseases; DNA methylation; histones; miRNAs

1. Introduction

Liver diseases constitute a significant global health burden, accounting for two million deaths annually and responsible for 4% of all mortality [1]. Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disorder worldwide, affecting more than one-third of adults, with a higher prevalence in obese and diabetic populations [2]. MASLD is characterized by the accumulation of liver fat exceeding 5% along with the presence of at least one cardiometabolic risk factor [3]. Approximately 20–35% of MASLD cases progress to metabolic dysfunction-associated steatohepatitis (MASH), characterized by hepatocyte ballooning, lobular inflammation, and fibrosis [4]. Persistent liver injury in MASH induces the activation of hepatic stellate cells (HSCs), which produce extracellular matrix deposition proteins, such as collagen I and III. If this fibrotic response remains unchecked, it can progress to cirrhosis, characterized by regenerative nodules and architectural distortion, significantly increasing the risk of hepatocellular carcinoma (HCC) development [5].

Epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNA regulation play a central role in the pathogenesis and progression of liver diseases. In recent years, the development of pharmacological agents, agonists, or inhibitors that target epigenetic modifications has emerged as a promising strategy for the treatment of hepatic diseases. Noteworthy, unlike genetic mutations, epigenetic alterations are potentially reversible, making them attractive therapeutic targets [6].

Pirfenidone, a synthetic pyridone drug also known as 5-methyl-1-phenyl-2-(1 H)-pyridone, is primarily metabolized by CYP1A2. This small molecule has demonstrated antifibrotic, antioxidant, and anti-inflammatory properties, making it an effective strategy to potentially treat fibrotic diseases by preventing or reducing excessive deposition of the extracellular matrix (ECM) in various organs, including the lungs, skin, eyes, kidneys, and liver [7]. Pirfenidone was first approved in Europe in 2011, followed by approval from the U.S. Food and Drug Administration in 2014 for use in treating idiopathic pulmonary fibrosis (IPF). In Mexico, pirfenidone has been authorized by COFEPRIS not only for pulmonary fibrosis, but also for the treatment of hepatic fibrosis, under brand formulations such as KitosCell LP.

While the precise mechanism of action of pirfenidone remains under investigation, it is currently recognized for its ability to inhibit TGF-β, PDGF, COL1A1, profibrotic molecules, and the inflammatory cytokines IL-6 and TNF. Notably, several recent studies have suggested that pirfenidone may also induce epigenetic modifications. In this review, we describe these studies, discuss the specific epigenetic mechanisms identified, and propose novel pathways that warrant further attention.

2. Pirfenidone for the treatment of liver diseases

In 2002, Garcia et al., utilizing a rat model of experimental cirrhosis, demonstrated a 70% reduction in fibrosis, accompanied by a decrease in HSC activation [8]. In a similar model, pirfenidone also enhanced antioxidant capacity through the induction of the gene expression of superoxide dismutase (SOD), catalase (CAT), and inducible nitric oxide synthase (iNOS) [9].

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Article highlights

- Pirfenidone modulates epigenetic pathways in hepatic diseases.
- Pirfenidone regulated DNA methylation, mainly targeting the TGF-β1 gene.
- Pirfenidone modifies chromatin state, such as H3K9me3 levels.
- Pirfenidone decreases the expression of profibrogenic genes.

In 2006, the first pilot study highlighting the improvement in steatosis and fibrosis in Mexican patients treated with pirfenidone was published. Fifteen patients with advanced liver disease due to chronic hepatitis C virus infection were administered 1200 mg/day of pirfenidone for 12 months. The findings showed steatosis reduction in 60% of the cases and decreased fibrosis in 30% of the cases [10]. The extended administration of pirfenidone over a two-year period enhanced its therapeutic effects, resulting in a decrease in fibrosis in 67% of the patients and 82% showing a decrease in necroinflammation [11].

A novel formulation of pirfenidone, known as prolonged-release pirfenidone (PR-PFD), was developed in Mexico. This formulation was designed to maintain optimal plasma concentrations of pirfenidone with a dosage administered every 12 h (600 mg), in contrast to the standard release-PFD, which requires administration every 8 h [12]. PR-PFD may improve treatment adherence by reducing dosing frequency while maintaining plasma exposure and therapeutic efficacy comparable to those of standard-PFD. Later on, PR-PFD was evaluated in a cohort of 281 patients with advanced liver fibrosis of various etiologies, including NAFLD, HCV infection, autoimmune hepatitis (AIH), and ALD in the context of additional standard of care. Noteworthy, fibrosis was significantly reduced in 35% of patients with PR-PFD compared to just 4.1% in those without PR-PFD [13]. These hallmarks recapitulated and extended the findings by Armendariz-Borunda in 2006.

The efficacy of pirfenidone has recently been reaffirmed in a placebo-controlled randomized clinical trial. In this study, 180 patients with compensated cirrhosis were assigned to receive either a placebo or PR-PFD at doses of 1200 mg or 1800 mg/day for 24 months, in addition to standard of care treatment. The authors demonstrated that a dose of 1200 mg significantly reduced liver fibrosis, as evidenced by a decrease in liver stiffness (24.2 ± 2.4 vs. 15.4 ± 2.4 kPa, $p < 0.001$) measured by elastography, accompanied by improvements in liver function tests, Model for End-Stage Liver Disease (MELD) score, and quality of life. Unexpectedly, 1800 mg/day did not result in significant changes in liver stiffness and FibroTest. The authors hypothesized that this could be attributed to cytochrome P450 saturation. Consequently, treatment regimens of up to 1200 mg administered for as long as two years have been reported in clinical studies [14].

3. Molecular mechanism of pirfenidone

Over the past year, research has focused on elucidating the molecular mechanisms by which pirfenidone enhances its therapeutic effects on liver pathology. While the efficacy of pirfenidone in humans has been evaluated primarily for hepatic and lung fibrosis, its effectiveness has been demonstrated in animal models across a broad range of liver diseases, as well as in the molecular pathways through which it exerts its effects.

In a murine model of MASLD, treatment with pirfenidone over a high-fat diet resulted in a reduction of steatosis and serum levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). The anti-inflammatory effects of pirfenidone were evidenced by a decrease in interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 levels, as well as a reduction in the phosphorylation of nuclear factor kappa-B (p-NF-κB p65). Additionally, Pirfenidone CAT activity and upregulated nuclear factor erythroid 2-related factor 2 (Nrf2), inducing antioxidant capacity [15].

Pirfenidone maintained the reduction in hepatic steatosis in a MASH model by enhancing the expression of genes involved in fatty acid oxidation [16]. Notably, pirfenidone induced the polarization of M2 macrophages (CD11c-CD206+) and decreased the number of M1 macrophages (CD11c+CD206-) [17]. The efficacy of pirfenidone in modulating the immune system was also demonstrated in cirrhotic Wistar rats. Administration of pirfenidone over a three-week period resulted in the downregulation of GATA3 and IL-4 expression, which are associated with the T helper type 2 (Th2) phenotype linked to fibrosis [18].

TGF-β1 is pivotal in phosphorylating Smad2/3, thereby promoting HSC activation, matrix deposition, and fibrosis progression. Pirfenidone demonstrated significant anti-TGF-β1 activity in hepatic models, contributing to its antifibrotic efficacy. In cultured HSCs activated by TGF-β1, pirfenidone inhibited the accumulation of type I collagen [19]. In vivo, pirfenidone markedly reduced TGF-β1 and procollagen-1 transcript levels and improved histological indices of fibrosis in models of liver injury induced by carbon tetrachloride (CCl_4) and dimethyl nitrosamine [8,20]. More recently, in a diet-induced MASH model, pirfenidone inhibited MAPK (p38/ERK1/2) and NF-κB signaling, thereby attenuating Smad-mediated TGF-β1 activation and reducing hepatic inflammation, stellate cell activation, and fibrosis [17]. These findings consistently demonstrate that pirfenidone disrupts TGF-β1/Smad-driven fibrogenic pathways in the liver.

Pirfenidone has been demonstrated to inhibit Wnt/β-catenin signaling pathways, which are critical regulators of cell proliferation, survival, and fibrogenesis. In HCC cell lines such as HepG2, pirfenidone reduced both total and phosphorylated βcatenin levels in a dose-dependent manner, leading to decreased proliferation and increased apoptosis. These effects were reversed by the βcatenin activator SB216763, indicating a direct inhibition of canonical Wnt signaling [21]. In vivo, in a model of hepatocarcinoma (HCC) induced in rats by diethylnitrosamine (DEN) and 2-Acetylaminofluorene (2-AAF), pirfenidone treatment was shown to reduce both the quantity and size of tumors, as well as lowering the expression of TGB-β1 and α-SMA. Remarkably, pirfenidone demonstrated an anti-inflammatory effect on NF-κB p65 and translocation of p65 to the nucleus [22].

4. Pirfenidone as an epigenetic modulator in liver diseases

4.1. miRNAs expression

MicroRNAs (miRNAs) are small RNA molecules that do not encode proteins but play a crucial role in the post-transcriptional regulation of gene expression. miRNAs degrade or inhibit the translation of specific mRNA by binding through base pairing. Various miRNAs have been implicated in the dysregulation of hepatic lipid metabolism, fibrosis, and progression to HCC, becoming potential therapeutic targets [23,24].

Escutia-Gutiérrez and colleagues investigated the effects of pirfenidone on hepatic microRNAs implicated in the development of MASLD/MASH, specifically miR-21a-5p, miR-34a-5p, miR-122-5p, and miR-103-3p. In C57BL/6J mice fed a high-fat carbohydrate diet, daily administration of PR-PFD (300 mg/kg) for eight weeks resulted in histological improvements in inflammation, steatosis, and fibrosis scores. Notably, RT-PCR analysis showed that pirfenidone significantly reduced the expression of miR-21a-5p and miR-122-5p, whereas miR-34a-5p and miR-103-3p showed a strong tendency to decrease. Notably, these changes were accompanied by increased expression of miRNA target genes, including Fatty Acid Binding Protein 1 (Fabp1), Sirtuin 1 (Sirt1), Peroxisome Proliferator-Activated Receptor Delta (Ppard), and Carnitine Palmitoyltransferase 1A (Cpt1a). This upregulation was assessed using microarray analysis that examined 22,000 genes [25].

Elevated levels of miR-21a-5 were reported in both the biopsy and serum of patients with MASH and were correlated with a reduction in nuclear receptor peroxisome proliferator-activated receptor α (PPARα) expression, and the inhibition of miR-21 reduced inflammation and fibrosis in mouse models [26,27]. Studies have demonstrated that transforming growth factor β1 (TGFβ1) activates SMAD2/3, promoting their nuclear translocation and subsequent binding to the miR-21 gene promoter, thereby enhancing its transcription [28,29]. Pirfenidone is a well-known inhibitor of TGFβ1, recognized as its primary antifibrotic pathway [9]. Additionally, TGFβ1 has been associated with the expression of other miRNAs involved in hepatic fibrosis, such as miR-181b, which is upregulated in HSCs following TGF-β1 stimulation [30]. Together, these findings suggest that pirfenidone may exert epigenetic effects by the inhibition of TGF-β1-driven downregulation of key miRNAs.

Recently, the ability of pirfenidone to restore altered miRNAs was confirmed in patients with advanced liver fibrosis. Thirty-eight patients with advanced residual fibrosis and sustained virologic response (SVR) following HCV infection received PR-PFD (1200 mg/day) for 12 months, in addition to the standard of care treatment. Of these, 28.94% exhibited a reduction in at least one fibrosis stage based on liver biopsies, whereas 44.73% showed a 30% reduction in liver stiffness, as measured by FibroScan. In contrast, within the control cohort of 20 patients with SVR who received only the standard of care treatment, only 15% exhibited a 30% reduction in liver stiffness (FibroScan). Remarkably, intervention with pirfenidone was associated with a decrease in miR-34a-5p, miR-16-5p, miR-192-5p, and miR-200a-3p and an increase in miR-122-5p in liver biopsies, as determined by RT-PCR [31]. All these miRNAs have been implicated in the progression of fibrosis [32]. Specifically, miR-16 suppresses hepatocyte growth factor (HGF) and Smad7, both of which are recognized as antifibrotic molecules [33]. Furthermore, miR-192 and miR-200 family members enhance the expression of TGF-β1 by inhibiting ZEB1 and ZEB2 proteins, which function as repressors of TGF-β1 [32,34].

These findings suggest that pirfenidone may have a significant impact on miRNA regulation in liver fibrosis, potentially contributing to its therapeutic effects. The observed changes in miRNA expression could be indicative of a shift toward a less fibrotic state in the liver tissue.

4.2. DNA methylation

DNA methylation is an epigenetic modification characterized by the addition of methyl groups to cytosine bases in DNA, predominantly at CpG sites. Generally, methylated CpG islands impede the access of DNA-binding proteins to their target sites, potentially leading to chromatin condensation and subsequent inhibition of gene expression. These changes affect gene expression without modifying the DNA sequence. Aberrant DNA methylation patterns are associated with the activation of HSCs and progression of liver fibrosis [35].

Zeybel et al. identified an increase in the hypermethylation of PPARα and PPARδ, as well as hypomethylation of TGFβ1, in patients with MASLD when comparing those with mild fibrosis to those with advanced/severe fibrosis [36]. In Cerda-Reyes' research on patients with advanced residual liver fibrosis who received PR-PFD for a year, the effect on DNA methylation of key genes was also evaluated. Notably, PR-PFD treatment resulted in hypermethylation at three key previously reported TGFB1-CpG sites by Zeybel et al., indicating a potential decrease in the transcription of this profibrogenic cytokine and a possible mechanism by which PR-PFD reduced the levels of TGF-β1, which would result in decreased stimuli for collagen exacerbation [31]. Additionally, DNA methylation patterns in circulating cell-free DNA (ccfDNA) have been proposed as potential biomarkers for several diseases. In cirrhotic patients, three CpG sites within the PPARγ promoter showed increased methylation in ccfDNA [37]. Cerda-Reyes et al. examined the percentage of PPARγ methylation in ccfDNA and observed a trend toward decreased methylation at two of the three CpG sites following pirfenidone intervention [31].

Aberrant DNA methylation is a characteristic feature of HCC, encompassing global DNA hypomethylation, particularly in repetitive elements and oncogenic regions, as well as gene-specific hypermethylation of tumor suppressor genes. DNA methyltransferases (DNMTs) are responsible for establishing and maintaining DNA methylation patterns [35]. DNMT1 is primarily responsible for maintaining DNA methylation patterns during DNA replication, a process that requires UHRF1 in mammals. In contrast, DNMT3A is considered a de novo methyltransferase that establishes new methylation patterns [38]. Miranda-Robledo et al. reported that PR-PFD restores global methylation and upregulates DNMT1, DNMT3a, and UHRF1 in a HCC mouse model. Additionally, PR-PFD decreased

the expression of Glypican-3, β-catenin, and c-Myc in the nuclear fractions, where hypomethylation of c-Myc has been associated with HCC, suggesting that pirfenidone restores hypermethylation in key oncogenes. Remarkably, the authors demonstrated through molecular docking that PPARγ exhibits binding sites for pirfenidone, proposing a potential mechanism by which pirfenidone functions as an agonist of PPARγ. This interaction facilitates its binding to the BAH1 domain of DNMT1, thereby enhancing its methyltransferase activity [39].

4.3. Histones

In chromatin, DNA is packaged into nucleosomes, each comprising approximately 150 base pairs of DNA wrapped around a histone octamer containing one pair each of histones H2A, H2B, H3, and H4. Histones play a crucial role in regulating gene expression through several post-translational modifications, such as methylation, acetylation, phosphorylation, ubiquitinylation, and sumoylation, among others. These modifications can either condense or decondense the chromatin structure, thereby allowing transcription factor binding or rendering DNA less accessible for transcription, respectively [40].

Histone methylation is particularly important in regulating liver diseases; histone methylation is catalyzed by histone methyltransferases (HMTs) and histone demethylases (HDMs) [40]. Jumonji domain-containing protein 2 B (JMJD2B) is an HDM that removes methyl groups primarily from histone H3K9 and H3K4 trimethylation marks (H3K9me3 and H3K4me3), which are associated with transcriptional repression. In hepatic steatosis, JMJD2B expression increases, leading to a loss of H3K9me3 and H3K4me3 at the promoter of peroxisome proliferator-activated receptor gamma 2 (PPARγ2) and at LXR response elements of lipogenic genes, stimulating their transcription [41,42]. Lately, Rodriguez-Sanabria et al. demonstrated that pirfenidone reduced JMJD2B protein levels and restored H3K9me3 in a mouse model of MASH and in palmitic acid-treated HepG2 cells. Chromatin immunoprecipitation revealed that pirfenidone induced enrichment of the repressive mark H3K9me2/3 at the promoters of *Pparg*, *Fasn*, and *Srebp1*, correlating with the decreased expression of these lipogenic genes. Notably, authors suggested that pirfenidone may exert its effects by inhibiting JMJD2B, as they identified several potential binding sites for pirfenidone within the catalytic domain of JMJD2B, supporting a mechanism of direct enzymatic inhibition [43].

Among deacetylases, Sirtuin 1 (SIRT1) stands out as a significant deacetylase that pirfenidone may regulate. SIRT1 is a NAD±dependent histone/protein deacetylase and serves as a crucial regulator of metabolism [44]. SIRT1 interacts with peroxisome proliferator-activated receptor alpha (PPAR-α), inducing the expression of PPAR-α target genes and consequently promoting beta-oxidation [45]. Therefore, decreases in SIRT1 have been associated with the development of hepatic steatosis. In a rat model of MASH, Sandoval-Rodriguez et al. demonstrated that pirfenidone enhanced phosphorylation at serine 47 of SIRT1, inducing its enzymatic activation and subsequent translocation to the nucleus. Authors demonstrated that SIRT1 deacetylates liver kinase B1 (LKB1), leading to the activation of the adenosine monophosphate – activated protein kinase (AMPK) pathway, which is implicated in lipid metabolism. Authors suggest that pirfenidone likely influences the expression of SIRT-1 through peroxisome PPAR-α. Regarding this, molecular docking experiments demonstrated that pirfenidone binds to PPAR-α, promoting its activation and subsequent binding to PPAR-α response elements (PPREs) in the SIRT-1 promoter, thereby enhancing their expression. Thus, pirfenidone appears to modulate SIRT1 activity indirectly via PPAR-α activation, establishing a positive feedback loop between these two proteins that contributes to reduced hepatic lipid accumulation [16].

Although the most studied role of SIRT has been linked to fatty acid oxidation, it is also noteworthy that SIRT1 can induce epigenetic modifications through its histone deacetylase activity. Monroy-Ramírez et al. demonstrated that administering PR-PFD increased the translocation of SIRT1 to the nucleus, leading to the deacetylation of H3K9 and H3K14 in a model of HCC. Monroy-Ramírez and colleagues propose that this modification may occur in tumor suppressor genes, potentially linked to a reduction in anaplastic cells by Pirfenidone. However, this hypothesis requires further validation through comprehensive molecular experiments [46]. Additionally, Pirfenidone has been shown to upregulate the expression of SIRT1 in mouse models of multiple sclerosis [47] and acute kidney injury [48].

On the other hand, Histone deacetylases (HDACs) also play a central role in liver pathophysiology. Class I HDACs (HDAC1, HDAC2, and HDAC3) deacetylate lysine residues, leading to chromatin condensation and transcriptional repression. In activated HSC, HDAC1 and HDAC2 are overexpressed, repressing Smad7 and enhancing TGFβ/Smad signaling, inducing liver fibrosis [49,50]. Regarding steatosis, deletion of HDAC3 results in excessive lipid accumulation due to dysregulation of genes involved in lipogenesis and β-oxidation [51]. Although the direct inhibition of HDACs by pirfenidone has not yet been demonstrated in liver tissue, studies on idiopathic pulmonary fibrosis demonstrated that pirfenidone downregulated HDAC1, HDAC2, and HDAC3 and restored histone acetylation levels in fibroblasts [52]. This finding raises the possibility that a similar epigenetic modulation by pirfenidone may occur in the liver; however, this requires further verification.

5. Conclusion and future perspectives

Accumulated evidence highlights the epigenetic modulatory potential of pirfenidone in the management of liver diseases, as it affects miRNA expression, histone modification, and DNA methylation (Figure 1). These studies analyzed the effects of pirfenidone in isolation; however, it is plausible that pirfenidone exerts its epigenetic effects through a coordinated regulatory network. We hypothesized that the hypermethylation of TGF-β1 induced by pirfenidone may be mediated by the upregulation of DNMT1, thereby limiting TGF-β1 protein production. This reduction could potentially decrease the expression of fibrosis-related miRNAs, such as miR-21 and miR-181b. In particular, miR-21 downregulates TGF-β1 signaling by repressing SMAD7 and PTEN, potentially establishing a positive feedback loop that enhances antifibrotic regulation.

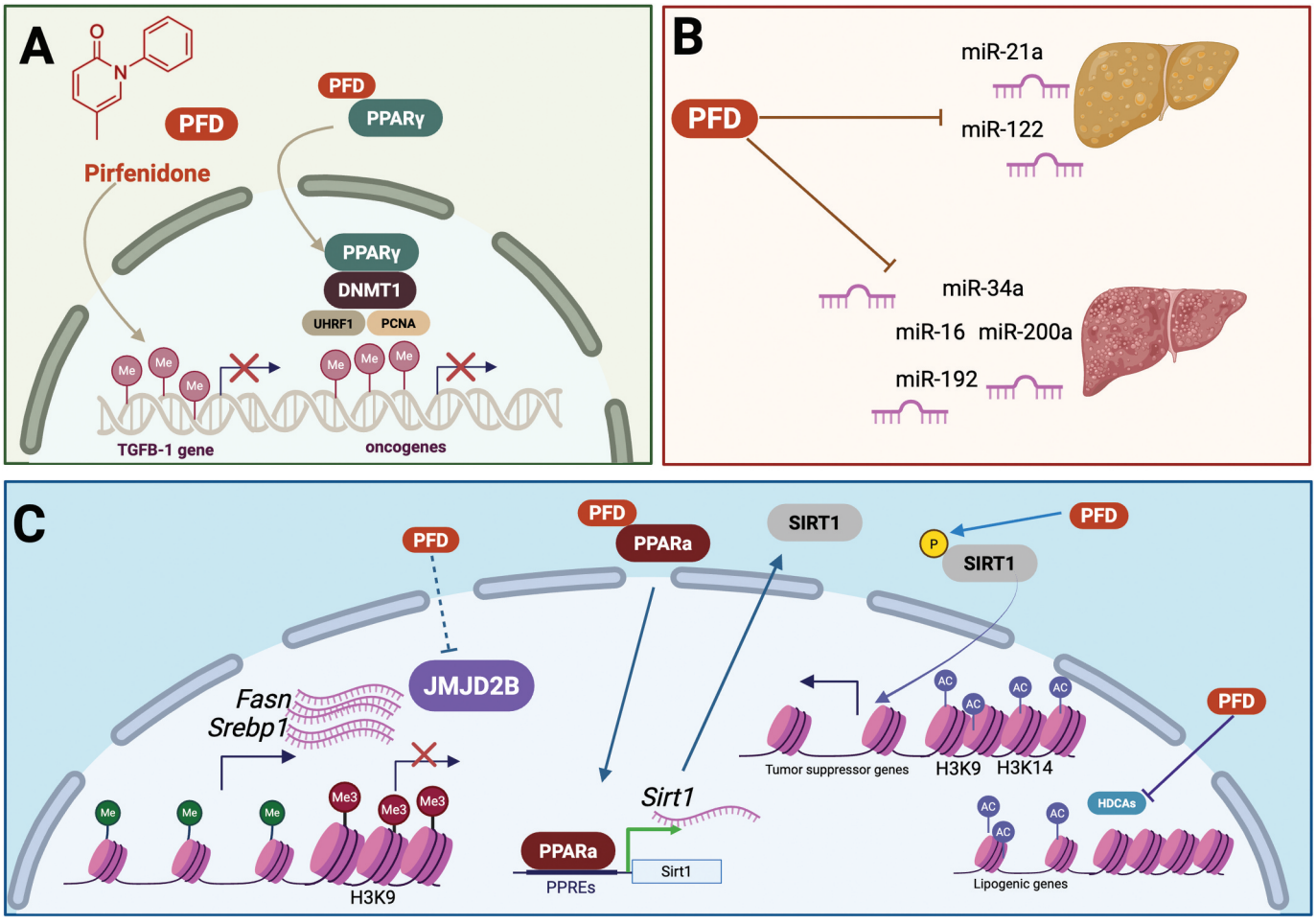

Figure 1. Epigenetic modulation by pirfenidone in liver disease. (A) Dna methylation: pirfenidone promotes hypermethylation of profibrotic genes such as *TGFB1* in liver fibrosis. Additionally, in HCC, pfd activates PPARγ, which forms a complex with DNMT1, UHRF1, and PCNA, contributing to the hypermethylation of oncogenes and repression of their transcription. (B) miRNA expression: pfd reduces the expression of miR-21a and miR-122 in the steatotic liver. In fibrotic tissues, pfd downregulated additional miRnas involved in fibrogenesis, including miR-34a, miR-16, miR-192, and miR-200a. (C) Histone modifications: pfd inhibits the histone demethylase JMJD2B, resulting in increased H3K9me3 levels and transcriptional repression of lipogenic genes, such as *fasn* and *Srebp1*. Moreover, pfd enhanced SIRT1 levels via PPARα activation, promoting H3K9 and H3K14 deacetylation. pfd downregulates class I HDACs, restores histone acetylation, and represses lipogenic gene expression.

Concurrently, pirfenidone may modulate chromatin structure through the regulation of JMJD2B, SIRT1, and HDACs. Specifically, JMJD2B contributes to chromatin condensation by increasing H3K9me3 methylation, thereby preventing the transcriptional activation of pro-lipogenic genes. However, these findings were obtained from different models, including MASLD, fibrosis, and HCC; therefore, further research is necessary to validate these potential interactions.

On the other hand, epigenetic modifications are cell-type dependent. For instance, mir-21 stimulates the ERK1 pathway, leading to HSC activation [53]. Similarly, DNA methylation and the methyl-CpG-binding protein (MeCP2) regulate the trans-differentiation of HSCs into myofibroblasts, a critical step in the progression of fibrosis [54]. However, the studies previously discussed, which evaluated the impact of pirfenidone on epigenetic mechanisms, were conducted on total liver tissue, thereby reflecting a general impact rather than cell-specific effects. Moreover, the specificity of the epigenetic effects of pirfenidone on different cell types within the liver microenvironment, including hepatocytes, HSCs, and immune infiltrates remains unclear. Future studies employing single-cell epigenomic approaches or cell type-specific isolation will be essential to elucidate this specificity.

In addition, it is crucial to emphasize that pirfenidone does not achieve complete inhibition of the epigenetic pathways analyzed. Instead, its effect appears to restore or normalize expression patterns toward levels comparable to those observed under control conditions, without entirely suppressing them. Nonetheless, it is pertinent to investigate potential off-target effects resulting from prolonged modulation of epigenetic mechanisms, particularly in non-hepatic tissues. Although some of the targets mentioned have not demonstrated adverse effects upon sustained silencing, such as miR-21, where miR-21 knockout mice did not exhibit phenotypic alterations [55], other targets play a significant role in physiological conditions. Further studies are warranted to comprehensively assess these aspects.

Although pirfenidone has been shown to decrease the expression of specific profibrogenic miRNAs, further research is needed to elucidate the specific mechanisms by which pirfenidone influences miRNA expression and how these changes correlate with improvements in liver fibrosis and

overall liver function. In this review, we propose a plausible mechanism influenced by TGB-1, which could be an interesting initial approach to study. Regarding DNA methylation, the influence of pirfenidone on DNMTs has become evident. However, integrative multi-omics approaches, such as Whole-Genome Bisulfite Sequencing (WGBS), are necessary to evaluate all CpG sites across the genome. This will help determine whether the effect of pirfenidone is specific to certain genes and their functions.

Finally, the combination of pirfenidone with other epigenetic drugs to synergistically target fibrosis, steatosis, and tumor progression in MASLD, MASH, and HCC should be studied. For instance, in IPF, Korfei et al. compared pirfenidone with the pan-HDAC inhibitor panobinostat, and observed that both agents reduced extracellular matrix gene expression and increased histone acetylation; however, this was more pronounced with panobinostat [52]. For the treatment of liver diseases, various epigenetic modifiers have been tested, including HDAC inhibitors (e.g., vorinostat, belinostat, panobinostat), DNMT inhibitors (e.g., vidaza, azacitidine, decitabine), and miRNAs [44,56,57]. A significant concern with these agents is their lack of specificity; for instance, DNMT inhibitors alter global methylation patterns [57]. In contrast, pirfenidone targets more specific epigenetic pathways, such as hypermethylation of the TGF-β1 promoter. Additionally, pirfenidone exerts a more moderate modulation compared to the potent effects of HDAC or DNMT inhibitors, which may limit its efficacy in reversing advanced fibrosis. However, the therapeutic advantage of pirfenidone is its reduced off-target toxicity, which is a common issue associated with other epigenetic modifiers. Future comparative studies are necessary to determine whether pirfenidone could complement or synergize with these established epigenetic agents to achieve more effective antifibrotic and antisteatotic outcomes.

Elucidating the epigenetic mechanisms underlying liver diseases presents an opportunity for the development of targeted therapies. Pirfenidone exhibits promising modulatory epigenetic effects, which may account for its beneficial effects on fibrosis regression, lipid metabolism, and hepatocellular transformation. In conclusion, the integration of epigenetic insights into the management of MASLD, MASH, and HCC could result in more precise and effective treatment strategies, with pirfenidone emerging as a pivotal molecule in the epigenetic-based therapeutic arsenal for chronic liver diseases.

Author contributions

Rosas-Campos Rebeca and Franco-Acevedo Adriana were responsible for data collection and selection. Rosas-Campos Rebeca, Franco-Acevedo Adriana, and Armendariz-Borunda Juan wrote the initial manuscript draft. Armendariz-Borunda Juan reviewed and edited the manuscript.

Disclosure statement

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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