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Epigenetics in lung fibrosis: from pathobiology to treatment perspective

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Abstract

Purpose of review—Idiopathic pulmonary fibrosis (IPF) is a fatal disease with limited treatment options and extensive gene expression changes identified in the lung parenchyma. Multiple lines of evidence suggest that epigenetic factors contribute to dysregulation of gene expression in IPF lung. Most importantly, risk factors that predispose to IPF – age, gender, cigarette smoke, and genetic variants – all influence epigenetic marks. This review summarizes recent findings of association of DNA methylation and histone modifications with the presence of disease and fibroproliferation.

Recent findings—In addition to targeted studies focused on specific gene loci, genome-wide profiles of DNA methylation demonstrate widespread DNA methylation changes in IPF lung tissue and a substantial effect of these methylation changes on gene expression. Genetic loci that have been recently associated with IPF also contain differentially methylated regions, suggesting that genetic and epigenetic factors act in concert to dysregulate gene expression in IPF lung.

Summary—While we are in very early stages of understanding the role of epigenetics in IPF, the potential for the use of epigenetic marks as biomarkers and therapeutic targets is high and discoveries made in this field will likely bring us closer to better prognosticating and treating this fatal disease.

Keywords

DNA methylation; histone modifications; pulmonary fibrosis; gene regulation

Introduction

Idiopathic pulmonary fibrosis (IPF) is a late-age-of-onset lung disease with a median survival of only 3 years characterized by progressive scarring of the pulmonary parenchyma that leads to progressive loss of lung function with dyspnea and hypoxemia, ultimately

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Conflicts of Interest

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resulting in respiratory failure and death. The prevalence of IPF is estimated at 63 individuals in 100,000 in the United States (1), with the prevalence and mortality in pulmonary fibrosis increasing as our population ages (2). Treatment options for IPF are limited to two recently approved drugs that slow down disease progression (3, 4). We are therefore in need of prevention and additional treatment strategies for this fatal lung disease.

The paradigm about disease pathogenesis has shifted from beliefs that IPF is a result of chronic inflammation (5) to the idea that it results from excessive, sequential injury and/or aberrant wound healing of the alveolar epithelium (6) and to more recent suggestions that the distal airway epithelium may also be important in disease development (7–9). It is likely that the disease process underlying the IPF phenotype is heterogeneous and many different cell types (10–12) and molecular processes may be involved (7, 8, 13–27). Regardless of the specific pathways that lead to fibrosis, the end stage result is usual interstitial pneumonia (UIP) histological pattern of heterogeneous, subpleural regions of fibrotic and remodeled lung (28).

Gene expression profiling studies have demonstrated that extensive transcriptional changes are present in the lung parenchyma of individuals with IPF (29–35). Gene expression changes are dramatic and consistently have identified genes associated with extracellular matrix (ECM) formation, degradation, and signaling; smooth muscle markers; growth factors; developmental pathways; and genes encoding immunoglobulins, complement, chemokines, and other host defense/innate immune genes. We also recently identified two molecular subtypes of IPF based on a strong gene expression signature of cilium-associated genes (11). Expression of *MUC5B*, the strongest and most replicated genetic risk factor for IPF (8, 36–41), is highly correlated to expression of cilium genes. This is also the case for *MMP7*, an extracellular matrix gene that has emerged as the main expression biomarker for IPF (30, 33, 42) and was recently shown to play a role in attenuating ciliated cell differentiation during wound repair (43). While gene expression studies in aggregate have been successful in identifying molecular processes that are dysregulated in IPF lung, we know much less about how expression of these genes is regulated at the transcriptional, post-transcriptional, and post-translational levels.

Introduction to Epigenetic Mechanisms

Epigenetic processes translate environmental exposures into regulation of chromatin, which shapes the identity, gene expression profile, and activity of specific cell types that participate in disease pathophysiology (44). They are emerging as key mechanisms that mediate the effects of both genetics and the environment on gene expression and disease (45, 46). Traditionally epigenetic processes refer to DNA methylation and histone modifications. Although noncoding RNAs are often considered a part of the epigenome, this review will focus only on DNA methylation and histone modifications.

Methylation of cytosine residues in CpG dinucleotides within the context of CpG islands is the simplest form of epigenetic regulation. DNA methyltransferases (*de novo* DNMT3A/B and maintenance DNMT1) are enzymes responsible for DNA methylation while the TET family of enzymes actively demethylate DNA though the 5-hydoxymethylcytosine

intermediate (47). Traditional view of DNA methylation is that hypermethylation of CpG islands in gene promoters leads to gene silencing while hypomethylation leads to active transcription (48, 49) but we now know that methylation of less CpG dense regions near islands ('CpG island shores') (50, 51) and within gene bodies (52, 53) is also important in regulation of gene transcription and alternative splicing. While the canonical inverse relationships of methylation and expression are predominant in the genome, direct relationships also exist especially for methylation marks in gene bodies and for those associated with alternative splicing (53, 54).

Acetylation and methylation are the most common modifications of histone tails that occur at specific sites and residues, and control gene expression by regulating DNA accessibility to RNA polymerase II and transcription factors. Histone acetyltransferases (HATs) acetylate histone tails, histone deacetylases (HDACs) remove acetyl groups from histone tails, and bromodomain (Brd) proteins are chromatin readers that recognize and bind acetylated histones and play a key role in transmission of epigenetic memory across cell divisions and transcription regulation (55, 56). Similarly, histone methyltransferases (HMTs) add the methyl groups to histone tails while histone demethylases (HDMs) remove them (55, 56). A number of modifications at specific sites residues regulate chromatin accessibility (57, 58).

Epigenetics and IPF – the Potential Links

Given what we know about IPF and epigenetic marks, several lines of evidence support a critical role for control of gene expression in IPF lung by DNA methylation and histone modifications (Figure 1). Firstly, IPF is a disease of the elderly and changes in DNA methylation, histone modifications, and gene expression occur as we age (59–61). Genome-wide studies in aging cells and tissues have revealed the occurrence of stochastic changes in DNA methylation, also referred to as drift (62). Stochastic profibrotic DNA methylation drift could predispose to the development of the disease in susceptible individuals (63). Similarly, gender is known to play a role in tissue specific DNA methylation patterns (64–68) and being of male gender is a risk factor for development of IPF (1).

Secondly, IPF is an environmental lung disease (69, 70). It is well established that environmental exposures strongly influence epigenetic marks (71). Cigarette smoke, the main environmental risk factor for IPF, has an influence on the methylome (72–74) and on methylation of specific promoters in genes involved in pathogenesis of IPF such as *WNT7A*(75). Recent work identified extensive genomic changes in DNA methylation in small airway epithelium (SAE) of smokers compared to smokers with corresponding modulation of gene expression (76). Other recent studies have shown how cigarette smoke influences histone modifications and chromatin accessibility (77–79).

Thirdly, IPF is also a genetic disease (38, 39, 80), and genetic factors also influence epigenetic marks. An individual's genetic background influences epigenetic marks in two ways – by direct inheritance (imprinted loci) (81) and by genetic variants that segregate with disease exerting their effects through epigenetic modifications, such as the case of haplotype-specific methylation. In addition to investigation at specific loci, genomewide

studies demonstrate a strong genetic component to inter-individual variation in methylation (82–84) and histone modification profiles (85–87).

Finally, epigenetic marks are crucial in lung development and aberrant recapitulation of the developmental program following injury is a hallmark feature of IPF (27). DNA methylation and histone modifications determine cell fate during organ development by controlling tissue-specific expression (88). Epigenetic control of gene expression is also involved in lung epithelial cell differentiation (89) and developmental pathway signaling (90, 91).

Targeted Studies of DNA Methylation and Histone Modifications in Lung Fibrosis

Several targeted studies have shown that epigenetic modulation (both DNA methylation and histone marks) regulates expression of genes and miRNAs involved in pathogenesis of IPF (Table 1), namely, cyclooxygenase-2 (COX2) (92, 93), chemokine IP-10 (94), Thy-1 (CD90) (95, 96), p14(ARF) (97), α-smooth muscle actin (α-SMA) (98, 99), and miR-17~92 cluster (100). Similarly, molecular processes of high relevance to pulmonary fibrosis are also epigenetically regulated; this has been demonstrated specifically for fibroblast apoptosis (101, 102), cell senescence (103), and innate immunity (104) in IPF. These studies of DNA methylation and histone modifications in specific genes, miRNAs, and molecular processes have also shown a direct link to fibroproliferative phenotypes. For example, Dakhlallah et al. identified a DNMT1-controlled feedback loop that contributes to the IPF fibroblast phenotype and ECM deposition (100). Another study has linked TGF-β1 signaling, lung development and histone modifications (105). Taken together, these targeted studies have provided crucial information on the role of DNA methylation and histone modifications in regulation of gene expression in some of the key genes, miRNAs, pathways, and molecular processes that are hallmarks of IPF.

Genomic Profiles of DNA Methylation in IPF

Genome wide assessments of epigenetic marks in IPF are limited to DNA methylation profiles at the present time (Table 1). The first two studies of genomic methylation profiles of IPF lung tissue used arrays with probes covering CpG islands and promoters. Despite the limited coverage of early array platforms the first two studies of genomic methylation profiles identified extensive DNA methylation changes in IPF lung tissue (106, 107). A more recent study used the same platform as in Sanders et al. (107) to also show substantial changes in DNA methylation in fibroblasts, the key effector cell in fibrosis, of patients with IPF compared to controls (108).

The most comprehensive study of IPF lung tissue to date was led by our group and interrogated 4.6 million CpG sites distributed across the human genome in lung tissue of 94 subjects with IPF and 67 controls (109). This analysis identified 2130 significant differentially methylated regions (DMRs), of which 60% are in CpG island shores, similar to published findings in cancer (110). 738 DMRs are associated with significant changes in gene expression and enriched for canonical inverse relationship between methylation and expression. An additional analysis of the relationship of methylation marks to expression

changes identified methylation marks that control both cis and trans regulation of gene expression, with an enrichment for cis relationships. This analysis also identified five trans relationships where a methylation change at a single DMR is associated with transcriptional changes in a substantial number of genes; four of these DMRs are near transcription factors. Taken together, these findings suggest not only widespread DNA methylation changes in IPF lung tissue but also a substantial effect of these methylation changes on gene expression. While it is unknown whether the methylation changes we have identified are are the result of the disease or are causative, given that several risk factors for IPF are independently associated with changes in DNA methylation, it is likely that the latter at least contributes to the methylation pattern.

In addition to replication of the published findings, one of the most important directions for the field of IPF epigenomics is to begin to understand cell specific patterns of DNA methylation and gene expression in the lung. For example, our study showed hypermethylation and reduced expression of the CASZ1 transcription factor in whole lung tissue but the same DMRs were hypomethylated in alveolar type II cells isolated from IPF lungs (109). In accordance with these findings, immunohistochemical staining showed loss of expression in airway epithelium and concomitant increase in expression of CASZ1 in the alveolar epithelium in IPF lung tissue sections compared to histologically normal lung (109). Isolation of specific cell types would also allow for profiling of histone modifications to paint a more complete picture of the role of epigenetic regulation of gene expression in IPF lung.

Methylation Changes within the IPF Genetic Loci

Recent work in the field of genetics of IPF has made it clear that there is a strong genetic component to this disease. Common genetic variants in the 10 loci identified by Fingerlin et al using a genome-wide association study (GWAS) explain ~30% of the disease risk (38). We intersected the 2130 IPF-associated DMRs with recently loci identified by the two published GWAS in IPF (38, 39) and identified methylation changes in genes within 5 of these loci (109). Of special interest are genes that are differentially expressed in IPF lung and whose expression may be regulated by both genetic variants and DNA methylation. As more genetic discoveries are made and the loci that have been already been associated with fine mapped, it is highly likely that additional candidate genes will emerge.

MUC5B

The strongest genetic candidate gene is MUC5B whose expression appears to be regulated at least in part by the IPF-associated promoter polymorphism rs35705950 (8, 111). This *MUC5B* promoter variant is associated with a 34.1-fold increase in MUC5B expression in lung tissue among unaffected subjects and a 5.3-fold increase among IPF patients, with IPF patients expressing 14.1-fold more MUC5B than unaffected controls. While our genomic methylation study did not identify DMRs near the *MUC5B* gene, there is reasonable evidence for the potential role of DNA methylation in regulation of MUC5B expression. The variant is approximately 3kb upstream of the MUC5B transcriptional start site, in an area of open chromatin, a dense region of ChIP-seq hits, in a highly conserved genomic region and within a CpG island, strongly suggesting that this region is important for gene

regulation. DNase hypersensitivity assays indicate areas of open transcriptionally active chromatin (112). In the ENCODE project, 19 of the 125 analyzed cell lines, including the lung epithelial carcinoma cell line A549, have open chromatin in the chromosomal region overlapping the MUC5B promoter polymorphism (chr11:1241201–1241350 for A549) (112). Areas of open chromatin are often associated with binding of enhancer, silencer and other regulator elements (112, 113). Large scale ChIP-seq analysis, also part of the ENCODE project, has demonstrate the binding of at least 20 transcription factors to the A549 specific DNase hypersensitivity region described above, with 18 transcription factors predicted to bind in the region overlapping the common polymorphism (114–116).

Moreover, the promoter polymorphism is located within a ~200bp CpG island motif (chr11:1241162–1241364), which is of particular interest given that the variant (G to T transversion) allele at rs35705950 disrupts a CpG motif and therefore directly affects methylability of the adjacent cytosine. Although rs35705950 provides a very pointed example of a potential epigenetic regulator, more global changes in DNA methylation have also been associated with MUC5B expression. Vincent et al. previously showed that *in vitro* exposure to 5-azacytidine, a global DNA demethylating agent, can alter MUC5B expression (117, 118). We also know that the region of chromosome 11 becomes differentially methylated in some forms of cancer (119). Understanding regulation of MUC5B expression by a combination of DNA methylation in IPF lung and the rs35705950 polymorphism is an important future direction.

TOLLIP

Toll interacting protein (TOLLIP), a gene involved in innate immunity and inflammation, has emerged as a potential genetic candidate in addition to MUC5B on chromosome 11 (39), is 1.6 fold downregulated, and we identified two intronic DMRs in TOLLIP that are ~11% hypermethylated in IPF compared to controls. Hypermethylation of TOLLIP has recently been observed in synovial fibroblasts of patients with rheumatoid arthritis (120).

DSP

Desmoplakin (DSP), a key component of tight junctions, is located in one of the most precisely mapped IPF-associated GWAS loci (38), is 1.8 fold upregulated and has two hypomethylated intronic DMRs with one of the DMRs in the first intron. DSP expression is regulated by DNA methylation in non-small cell lung cancer (121).

Promise for Treatment Options

Identification of key epigenetic marks that are shaped by the genetics and environment and influence transcription of specific genes will not only help us have a better understanding of etiology and heterogeneity of IPF but will also empower us to develop biologically driven therapeutics and biomarkers for secondary prevention of this disease. DNA methylation changes have been shown to drive tumor formation and malignant progression (122), and as such have established basic mechanisms for disease pathogenesis, as well as targets for intervention in cancer. DNMT inhibitors have been approved for the treatment of myelodysplastic syndrome (123, 124) and are in clinical trials for treatment of solid tumors

(125, 126). While currently available DNMT inhibitors lack specificity for gene(s) of interest, locus-specific therapies are currently being developed using genome editing technologies (122, 127) or taking advantage of recently discovered DNMT1-interacting noncoding RNAs (128). Additionally, current FDA-approved and in development histone mark modifying drugs are effective in targeting specific gene loci and pathways (55, 129) and treating diseases such as lung cancer (130). As a proof of principle for IPF, profibrotic phenotypes have been reversed in primary fibroblasts and the bleomycin mouse model by the Brd4 inhibitor JQ1 (131) as well as HDAC inhibitors Spiruchostatin A (SpA) (132) and suberoylanilide hydroxamic acid (SAHA) (102).

Conclusions

While we are in very early stages of using epigenetic marks as biomarkers and therapeutic targets in IPF, the potential is high and the next several years are likely to bring many exciting discoveries in this field that will hopefully bring us closer to better prognosticating and treating this fatal disease.

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Key Points

- Epigenetic marks, just like pulmonary fibrosis, are affected by ageing, environmental exposures, genetic variants, and are important in developmental pathways whose aberrantly recapitulated in IPF lung.
- Expression of genes and pathways that are key in the fibroproliferative response is regulated by DNA methylation and histone modifications.
- Genomic studies of DNA methylation and histone modifications in IPF lung tissue and specific cells from the IPF lung are just emerging.
- Epigenetic marks that are shaped by the genetics and environment and influence transcription of specific genes will empower us to develop biologically driven therapeutics and biomarkers for secondary prevention of this disease.

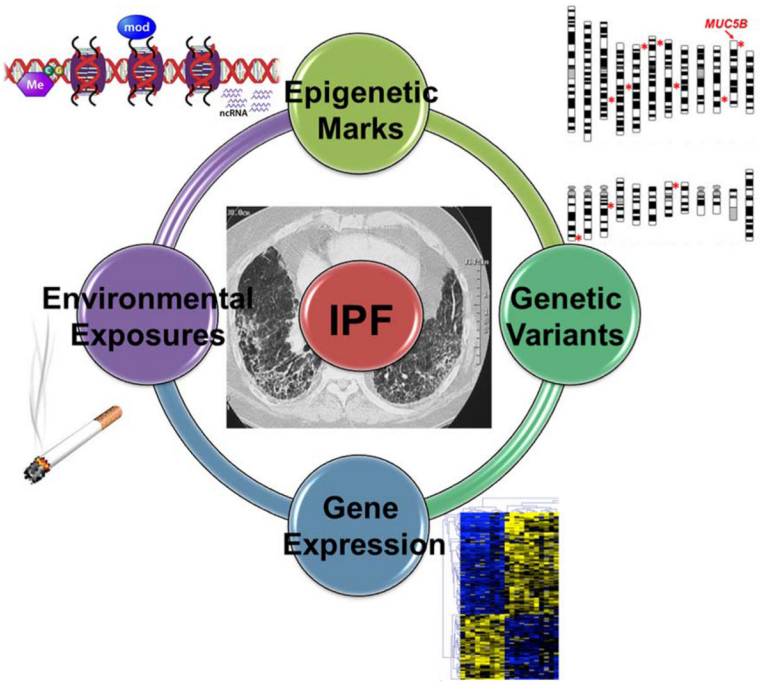

Figure 1.

An overview of epigenetic regulation of gene expression in IPF lung. Environmental exposures such as cigarette smoke and genetic variants are associated with changes in epigenetic marks which in turn are associated with altered gene expression. Because the causative nature of these associations has not been established, these molecular processes are drawn as a circle with no arrows. * symbols on the chromosome view plot indicate approximate location of IPF-associated genetic loci from the two published GWAS (Fingerlin. Nat Genet. 2013;45:613–20 and Noth. Lancet Resp Med. 2013;1:309–17).

Table 1

Summary of studies of DNA methylation and histone modifications performed to date in pulmonary fibrosis.

Genes/Genomic Loci	Epigenetic Mechanism	Tissue or Cell	Phenotypes and Outcomes
cyclooxygenase-2 (COX2) (92, 93)	DNA methylation; histone H3 and H4 acetylation; H3K9me3 and H3K27me3; DNMTs, HATs, and HMTs,	Human lung fibroblasts	IPF
chemokine IP-10 (94)			
Thy-1 (CD90) (95, 96)	DNA methylation; H3K4me3 and H4 acetylation	Human and rat lung fibroblasts	Myofibroblast differentiation
p14(ARF) (97)	DNA methylation	Human lung fibroblasts	IPF; apopotosis
α-smooth muscle actin (α-SMA) (98, 99)	DNA metylation; methyl CpG binding protein 2 (MeCP2)		Myofibroblast differentiation; collagen deposition
miR-17~92 cluster (100)	DNA methylation; DNMT1	Human lung tissue and human lung fibroblasts	IPF; collagen deposition
BAK, BCL-XL, FAS (101, 102)	DNA methylation (no difference); histone H3 acetylation; H3K9Me3	Human and mouse lung fibroblasts	Fibroblast apoptosis
NOX4 (103)	H4K16Ac and H4K20Me3;	IMR-90 human fetal lung fibroblast cell line	Cell senescence
TLR9 (104)	hypomethylated CpG DNA (ligand)	Human lung fibroblasts, mouse lung tissue, and A549 lung epithelial cell line	Innate immunity; EMT
Mmp9 (105)	histone acetylation; SIRT1	Mouse lung tissue	TGF-β1 signaling and lung development
CpG islands (106, 107)	DNA methylation	Human lung tissue	IPF
CpG islands (108)	DNA methylation	Human lung fibroblasts	IPF
2.1M CpG motifs in the genome (109)	DNA methylation	Human lung tissue	IPF