**Review Article**



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# Epigenetics and Chromatin Remodeling Play a Role in Lung Disease

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Epigenetics is defined as heritable changes that affect gene expression without altering the DNA sequence. Epigenetic regulation of gene expression is facilitated through different mechanisms such as DNA methylation, histone modifications and RNA-associated silencing by small non-coding RNAs. All these mechanisms are crucial for normal development, differentiation and tissue-specific gene expression. These three systems interact and stabilize one another and can initiate and sustain epigenetic silencing, thus determining heritable changes in gene expression. Histone acetylation regulates diverse cellular functions including inflammatory gene expression, DNA repair and cell proliferation. Transcriptional coactivators possess intrinsic histone acetyltransferase activity and this activity drives inflammatory gene expression. Eleven classical histone deacetylases (HDACs) act to regulate the expression of distinct subsets of inflammatory/immune genes. Thus, loss of HDAC activity or the presence of HDAC inhibitors can further enhance inflammatory gene expression by producing a gene-specific change in HAT activity. For example, HDAC2 expression and activity are reduced in lung macrophages, biopsy specimens, and blood cells from patients with severe asthma and smoking asthmatics, as well as in patients with chronic obstructive pulmonary disease (COPD). This may account, at least in part, for the enhanced inflammation and reduced steroid responsiveness seen in these patients. Other proteins, particularly transcription factors, are also acetylated and are targets for deacetylation by HDACs and sirtuins, a related family of 7 predominantly protein deacetylases. Thus the acetylation/deacetylation status of NF-κB and the glucocorticoid receptor can also affect the overall expression pattern of inflammatory genes and regulate the inflammatory response. Understanding and targeting specific enzymes involved in this process might lead to new therapeutic agents, particularly in situations in which current anti-inflammatory therapies are suboptimal.

**Key words:** HDAC, Inflammatory cells, COPD, Asthma

**Abbreviations: AP-1**: Activator protein (AP)-1, **COPD**: Chronic Obstructive Pulmonary Disease, **CS**: Cigarette smoke, **GWAS**: Genome-wide association analysis, **HDAC**: Histone deacetylase, **HAT**: Histone acetylase, **HDM**: Histone demethylase, **HMTs:** Histone-methyltransferase, **NF-κB**: Nuclear factor (NF)-κB

## INTRODUCTION

Many lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, interstitial lung disease and acute respiratory distress syndrome, involve inflammation, with the coordinate expression of multiple inflammatory genes in the lungs. These inflammatory genes code for the expression of cytokines, chemokines, enzymes that synthesize inflammatory mediators, inflammatory mediator receptors and adhesion molecules, resulting in a regulated influx and activation of inflammatory cells and stimulation of resident structural cells. Many of these inflammatory genes are regulated by proinflammatory transcription factors, including nuclear factor kappaB (NF-κB) and activator

protein (AP)-1. These transcription factors orchestrate, amplify and perpetuate the inflammatory response and form the molecular basis of chronic inflammation (1-3). The term epigenetics; as currently used, refers to a change in gene expression which is heritable but that does not involve any change in DNA sequence.

Post-translational modifications of histones play an important role in epigenetic regulation of gene expression, and thus have critical effects on environment-mediated chronic lung diseases such as COPD and asthma (4,5). Since histones are post-translationally modified during disease progression, the identification of these patterns as well as the altered activity of the enzymes that 'write' and 'erase' these marks are important mechanisms for the understanding of human diseases. The most intensively studied modifications are histone acetylation and methylation which through the action of specific enzymes form marks that allow 'readers' of these marks to remodel chromatin producing the open chromatin structure associated with active gene transcription or a closed repressive chromatin state linked to a lack of active transcription (6). For example, an acetylated lysine residue forms a bromodomain that is read by many enzymes and transcription regulating factors (7)(Fig. 1).


Figure 1. Histone acetyltransferases (HATs) acetylate (AC) histones on lysine residues to leave a bromodomain (acetylated lysine) residue as an epigenetic mark. This is read by a bromodomain containing protein such as those found in a chromatin remodeling enzymes which can then alter chromatin structure and allow gene transcription to occur. Acetylated tags are removed by erasers such as histone deacetylases (HDACs).

Abnormalities in histone acetylation and methylation resulting from an imbalance in histone acetylation (HAT)/histone deacetylase (HDAC) and histone methyltransferase (HMT)/histone demethylase (HDM) activities are associated with a change in gene expression (Fig. 2) (8-10). However, global abnormalities linking epigenetics to airways disease are only just being investigated.

Oxidative stress has been implicated in the pathogenesis of several inflammatory lung disorders including COPD due to its effect on pro-inflammatory gene transcription. In this regard it has been shown that NF-κB-dependent gene expression, at least in part, is regulated by gene-specific changes in the acetylation and methylation status of selective histone H3 and H4 residues (11).

Cigarette smoke (CS) and oxidants alter the activity of both HATs and HDACs and thereby enhance NF-κB-dependent gene expression (12). Furthermore, prolonged exposure of human airway epithelial cells to CS in vitro results in marked temporal changes in histone acetylation and methylation patterns, altered DNA methylation and modifies the cell phenotype (13).

CS/oxidants also reduce glucocorticoid sensitivity by attenuating HDAC2 activity and expression and this has been proposed to account, at least in part, for the relative glucocorticoid insensitivity seen in patients with COPD (14). Understanding the mechanisms of NF-κB regulation and the balance between histone acetylation and deacetylation may lead to the development of novel therapies in lung inflammation and injury. Importantly, bromodomain mimics such as JQ1 and I-BET have been shown to switch off specific sets of inflammatory genes in human macrophages and to completely suppress sepsis and cancer in murine models of disease and more recently to prevent multiple myeloma (15-17).

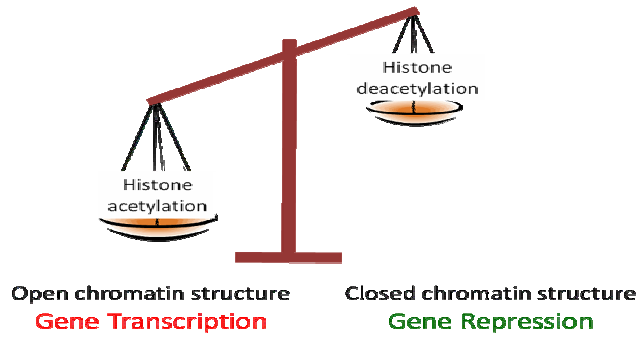


Figure 2. Schematic cartoon indicating how the balance between the gene transcription and gene repression is controlled by alterations in histone acetylation status.

The aim of this review is to highlight the immune-inflammatory responses linked to epigenetic chromatin alterations in lungs disease, and the importance and role of histone acetylation in modulating chronic lung diseases augmented by exposure to cigarette smoke and environmental agents such as airborne particulates and allergens. HMT regulation of inflammatory gene expression is more complex as the effect on gene transcription is dependent upon the specific methylated residue and the degree of methylation on each residue e.g. mono-, di- and tri-methylated lysine. As a result this will not be discussed here but the reader is directed to an excellent review (18). The role of small non-coding RNAs in the epigenetic control of inflammation is becoming increasingly apparent such as the differential expression of miR38-5p and miR146 in severe asthma (19). A review of the roles of miRNAs in airways disease is not discussed here but is discussed elsewhere (20, 21).

**Chromatin modification and inflammatory gene expression**

Gene expression in all tissues including the lung is regulated, at least in part, through coordinated changes in the pattern of histone post translational modifications (22-26). The specific role of histone acetylation via the actions of histone acetylases (HAT) and histone deacetylases (HDAC) has been extensively described (22-26). In a simplistic form increased histone acetylation is associated with increased inflammatory gene expression. Increased HAT activity and decreased HDAC activity have been reported in asthma along with reduced HDAC activity in COPD. In both cases this is linked to the altered inflammatory response seen in these diseases (27). For example, there is increased acetylation of histone-4 in asthma, consistent with increased expression of multiple inflammatory genes (28). In peripheral lung, airway biopsies and alveolar macrophages from COPD patients there is increased acetylation of histones within inflammatory gene promoter regions such as that of CXCL8/IL-8 (29). CXCL8 is regulated by NF-κB and the degree of CXCL8 promoter acetylation correlates CXCL8 mRNA and with disease severity (30).

**Histone deacetylases (HDAC) and histone acetylases (HAT)**

Eukaryotic DNA is highly organized and packaged into the nucleus. The organization and packaging are achieved through the addition of proteins, including two of each core histones H2A, H2B, H3 and H4 which, together with DNA, form the nucleosome structure (31). HAT enzymes, now known as writers of epigenetic marks, acetylate the ε-amino groups of lysine residues located near the amino termini of core histone proteins. These acetyl marks (bromodomains) are detected by epigenetic readers which are found in transcription factors, transcriptional co-factors and chromatin remodeling enzyme complexes. These later complexes alter the local chromatin structure by allowing nucleosomal movement in an ATP-dependent manner and recruitment of the basal transcriptional machinery (32). Thus, the level of acetylation is related to transcriptional activity due to the formation of an open chromatin confirmation (31). Histone acetylation must be reversed to prevent uncontrolled gene expression. This is performed by histone deacetylases (HDACs) which are therefore associated with gene silencing (6)(Fig. 1). It is important to note that HDACs are also involved in the reversible acetylation of non-histone proteins including transcription factors such as NF-κB (33) and the glucocorticoid receptor (6) and inflammatory enzymes such as p38 MAPK adding another layer of control to cellular function by HAT/HDAC activity (Fig. 3)(34, 35).

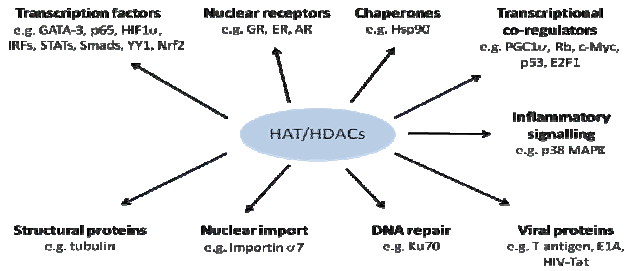


Figure 3. The regulation of the acetylation status of histone and non-histone proteins is essential for a wide variety of cell functions.

For example, steroid resistance has been linked to changes in p38 MAPK activity and recent evidence indicates that acetylation of this enzyme results in a greater activation than phosphorylation providing a link between these two processes underlying steroid responsiveness (35).

Thus, expression of inflammatory genes is determined by a balance between histone acetylation (which activates transcription) and deacetylation, which switches off transcription. There are 11 HDAC isoenzymes that deacetylate histones within the nucleus, and specific HDACs appear to be differentially regulated and to regulate different groups of genes (36)(Fig. 4).

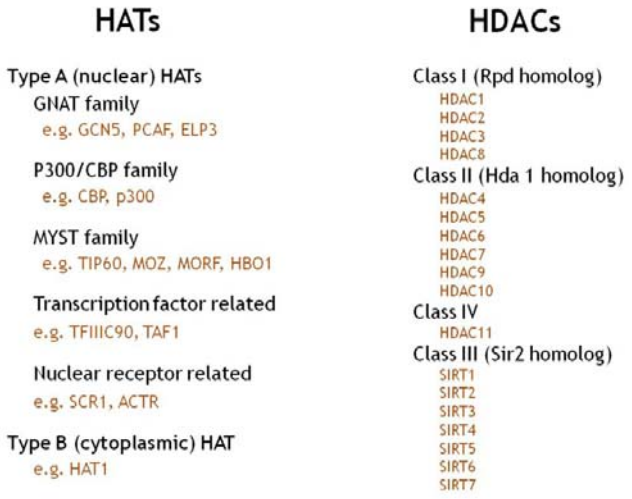


Figure 4. HATs and HDACs exist in distinct families.

Mammalian HDACs have been classified into three classes. Class I (HDACs 1, 2, 3 & 8; each of which contains a deacetylase domain exhibiting from 45% to 93% identity in amino acid sequence) are homologs of yeast RPD3 and are localized to the nucleus (37, 38). Class II (HDACs 4, 5, 6, 7, 9 & 10) are homologs of yeast Hda1 and are found in both the nucleus and cytoplasm. The molecular weights of which are all about twofold larger than those of the class I members, and the deacetylase domains are present within the C-terminal regions, except that HDAC-6 contains two copies of the domain, one within each of the N-terminal and C-terminal regions (39). HDAC11 has properties of both class I and class II HDACs. Class III (Sirt1 - Sirt7) are homologs of yeast Sir2 and form a structurally distinct class of NAD-dependent enzymes found in both the nucleus and cytoplasm. Histone acetylation not only regulates inflammatory gene expression but plays a role in diverse functions such as DNA repair and cell proliferation and apoptosis (40, 41) and are therefore implicated in many types of cancer (42, 43)(Fig. 3). However, the actions of HDAC inhibitors used in cancer therapy probably relates to their effects on non-histone proteins (44).

**Role and function of epigenetic modifications in pathogenesis of lung diseases**

*A) Lung cancer:* Changes in DNA methylation are also described in lung cancer (45). The CpG dinucleotide, which is usually underrepresented in the genome, is clustered in the promoter regions of some genes. These promoter regions have been termed CpG islands (45). CpG islands are protected from methylation in normal cells, with the exception of genes on the inactive X chromosome and imprinted genes. This protection is critical, since the methylation of promoter region CpG islands is associated with a loss of expression of these genes. The following three different alterations in DNA methylation are common in human cancer: (1) global hypomethylation, often seen within the body of genes; (2) dysregulation of DNA methyltransferase I, the enzyme involved in maintaining methylation patterns, and potentially other methyltransferases; and (3) regional hypermethylation in normally unmethylated CpG islands particularly those associated with tumor suppressor genes.

As indicated earlier, gene expression is regulated by a dynamic balance between HAT and HDAC activities and changes in histone acetylation patterns have been reported in many human diseases, particularly cancer (46) and investigators have used HDAC inhibitors against many malignancies (47). HDAC inhibitors induce apoptotic cell death in a number of tumor cell types (40, 41), probably through targeting non-histone proteins, whereas normal cells are usually resistant to cell death caused by HDAC

inhibitors (48-50). The discovery of bromodomain (Brd) mimics has enabled more selective suppression of this HAT/HDAC/gene expression nexus and a Brd4 mimic has recently been reported to be effective in multiple myeloma (50, 51).

*B) Asthma:* Post-translational modifications of histones play a key role in epigenetic regulation of gene expression and may therefore play an important role in environment-mediated chronic lung diseases like asthma (9,10). Asthma is a chronic inflammatory disease of the airways characterized by reduced airway patency, which is regulated by bronchodilators such as β-agonists, and by the infiltration of inflammatory and immune cells, which is treated by corticosteroids (52). Asthma phenotypes are highly heritable and the subject of many genetic researches. The occurrence of patients with an asthma cluster in their family indicates that a genetic component is likely operating. Twin studies represent a useful first step to determine whether a given trait or disease has a measurable genetic component. In a large twin study with 7,000 same-sex twins born between 1886 and 1925, the concordance rate for self-reported asthma in monozygotic twin pairs was 19%, which is four times higher than the 4.8% rate in dizygotic twins (53).

Since this does not fully account for the heritability of asthma other mechanisms including epigenetics have been implicated in the pathogenesis of asthma (52). In bronchial biopsies from patients with asthma, there is a marked increase in HAT activity and a small reduction in HDAC activity compared with normal airways, thus favoring increased inflammatory gene expression (26). Interestingly, in patients with asthma who smoke, there is a significantly greater reduction of HDAC activity in bronchial biopsies than in nonsmoking asthmatic patients (26) and this may account for why these smoking asthmatics have more severe asthma and resistance to steroids (54).

*C) COPD:* Pulmonary inflammation including infiltration of neutrophils and macrophages plays a central role in the etiology of COPD as evidenced in the emphysematous lungs of smokers and in mice exposed to cigarette smoke (55). Pulmonary inflammation in COPD is associated with fibrosis and irreversible narrowing of small airways and destruction of the lung parenchyma or emphysema (56). It is generally accepted that genetic predisposition plays a role in COPD development in susceptible individuals. Many candidate genes that could be linked to the development of disease have been examined in COPD and more recent GWAS analysis has been performed (57).

However, inconsistent results in different study populations have limited this approach and suggest that other factors such as epigenetics may be important in understanding the gene-environment aspects involved in the susceptibility to COPD in smokers.

In COPD patients' peripheral lung and airway biopsy specimens, and alveolar macrophages, there is an increase in the acetylation of histones associated with the promoter region of inflammatory genes, such as CXCL8, that are regulated by NF-κB and the degree of acetylation increases with disease severity (27).

This increased acetylation of inflammatory genes is not due to any global increase in histone acetyltransferase activity in the lungs or macrophages but a reduction in HDAC activity in alveolar macrophages of cigarette smokers compared to nonsmokers, and this is correlated with increased expression of inflammatory genes in these cells (58, 59). There is also a reduction in total HDAC activity in peripheral lung, bronchial biopsy specimens and alveolar macrophages from COPD patients and this is correlated with disease severity and with increased gene expression of CXCL8 (27).

There is a selective reduction in the expression of HDAC-2, with lesser reductions in HDAC-3, -5 and -8 and an increase in HDAC-4 and -6 (27).

In patients with very severe COPD (Global Initiative for Chronic Obstructive Lung Disease stage 4) the expression of HDAC-2 was < 5% of that seen in normal lung. The reasons for the reduction in HDAC, particularly HDAC-2, in COPD are not yet completely understood. However, there is increasing evidence that this may be due to

inactivation of the enzyme due to the presence of oxidative and nitrative stress (60, 61). HDAC-2 shows increased tyrosine nitration in macrophages and peripheral lung specimens of COPD patients and this is correlated with increased expression of CXCL8 (62). HAT activity is increased and HDAC2 activity is reduced in lungs of rats exposed to cigarette smoke, which show increased NF-κB activation and expression of inflammatory genes (63).

In addition, alveolar macrophages from normal smokers also show a reduction in HDAC activity and expression of HDAC2, and this is correlated with an increase in release of TNF-α and CXCL8 in response to an inflammatory stimulus (58) and a failure to respond to the presence of exogenous dexamethasone.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that is activated in response to oxidative stress and as a result switches on the expression of anti-inflammatory genes such as haemoxygenase (HO)-1 (64). Down-regulation of Nrf2 expression in COPD (64,65) may account for the enhanced oxidative stress seen in this disease. Nrf2 is an acetylated protein and enhanced acetylation leads to reduced Nrf2 stability and an impaired anti-oxidant response providing a feed forward mechanism to enhance inflammation and reduce steroid responsiveness in COPD (64).

Epigenetic mechanisms can affect the transcriptional activity of specific genes, at different points in time and in different organs. Therefore, unlike genetic analysis that can use blood cells the analysis of epigenetic profiles in airways disease must be performed in samples obtained from the airways of subjects with disease. Mapping and understanding global epigenetic changes in cell and tissue samples from bronchial biopsies, brushings and alveolar macrophages in stable disease and potentially in exacerbations is now an active area of research (65-67). In addition, the effect of environmental stimuli such as diesel particles and cigarette smoke on epigenetic profiles is being investigated at the cell and epidemiological levels to further appreciate the potential for gene-environment effects on airway inflammation and disease (68-71). Of note, monozygotic twins who have a greater degree of pro-inflammatory epigenetic changes as a result of environmental stressors for over 50 years (72,73) are more susceptible to chronic disease.

Since histones are post-translationally modified during disease progression, the identification of these patterns is important for the understanding of human epigenetic marks in disease conditions. Although the majority of information currently available relates to changes in histone acetylation and methylation along with DNA methylation changes in tumor suppressor genes in lung cancer, it is likely that other modifications may prove to be important in the regulation of inflammatory gene expression in these diseases as more unbiased techniques such as proteomics are applied (6).

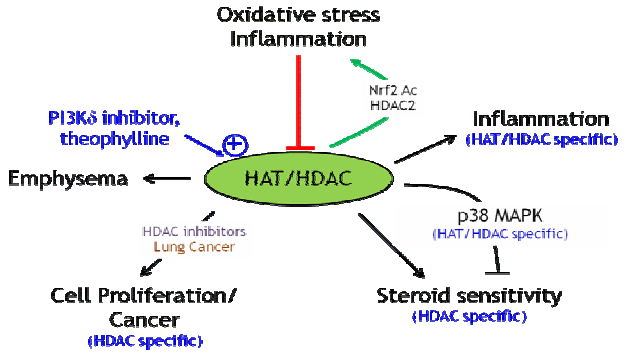


Figure 5. Oxidative stress and inflammation modulate the HAT/HDAC ratio and thereby control cell death (emphysema), cell proliferation and inflammation along with alterations in steroid function. The later process may also involve acetylation of p38 MAPK. Feedback mechanisms, such as Nrf2 acetylation, also exist to control oxidative stress and inflammatory processes. Drugs that modify the HAT/DAC ratio may be useful in modifying these cellular functions (see text for details).

There is emerging evidence supporting a role of epigenetics in the regulation of inflammatory genes in diseases such as COPD. Moreover, recent studies suggest that the currently used treatments including corticosteroids may work through epigenetic mechanisms. Epigenetic regulation can be reprogrammed, potentially affecting the risk, etiology and treatment of various disease states. The epigenetically influenced phenotype could be reversed

with demethylating or deacetylating agents, consistent with epigenetic plasticity. The postnatal reversibility of these methylation or acetylation events may therefore provide good opportunities for intervention. Furthermore, the development of bromodomain mimics and their utility in models of inflammation and cancer suggest that similar drugs may prove useful in COPD. The recognition of the role of epigenetic mechanisms in the development of COPD may identify novel targets that result in new therapies for patients with COPD (Fig. 5).

## CONCLUSION

In many patients with pulmonary disease there is a selective reduction in the activity and/or expression of specific HDAC isoforms in the peripheral lung, airways and in alveolar macrophages. This may worsen as the disease becomes more severe. For example, this may account for the increased pulmonary inflammation and resistance to corticosteroids observed in COPD and in severe asthma. In COPD, there is a selective reduction in HDAC2 expression and this may be due to oxidative and nitrative stress which is increased in the airways of these patients. Therapeutic options aimed at increasing HDAC activity, such as antioxidants, iNOS inhibitors and theophylline may be beneficial. Alternatively, bromodomain mimetics may prevent aberrant inflammatory gene expression. Reduced HDAC activity may also occur in other inflammatory diseases like in asthmatic patients who smoke, patients with lung fibrosis and those with rheumatoid arthritis and inflammatory bowel disease. This area of research may lead to the development of novel anti-inflammatory therapies aimed at increasing modulating HAT/HDAC activity in the future.

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