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REVIEW
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Beyond genes: a clinician's guide to epigenetics

Petar Popov and Renata Z. Jurkowska

Division of Biomedicine, School of Biosciences, Cardiff University, Cardiff, UK.

Corresponding author: Renata Z. Jurkowska (JurkowskaR@cardiff.ac.uk)

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Epigenetic signalling provides a novel angle for understanding how chronic lung diseases develop and identifying novel biomarkers and therapeutic targets for respiratory diseases
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Abstract

Epigenetic mechanisms control how and when our genes are switched on or off. They are altered by chronic environmental exposures, like cigarette smoke or air pollution, contributing to disease development. Excitingly, they can also be manipulated with the potential to target disease. Thus, epigenetic signalling provides a novel angle for understanding how chronic lung diseases develop and identifying novel biomarkers and therapeutic targets for respiratory diseases. This educational review is meant as a guide for clinicians and other lung researchers interested in epigenetic regulation. We introduce the main epigenetic modifications and the general cellular machinery that introduces and removes them. We provide selected examples of epigenetic dysregulation in response to environmental exposures and in chronic lung diseases. Finally, we discuss the promise of epigenetic biomarkers and therapies for clinical practice, including new precision medicine epigenetic editing approaches.

Epigenetic modifications: a clever molecular code for regulating our genes

Different cells in our body carry the same genetic blueprint in the form of DNA (the genome). Yet, they can have dramatically different morphology (compare, for example, a mucus-secreting goblet cell with an immune cell like a neutrophil), distinct physiology and specialised functions. How can the same genetic instruction give rise to so many different cell types (61 identified to date in the human lungs [1])? The answer came with the seminal discoveries showing that, although all cells share the same genome, they possess distinct "epigenomes": unique sets of epigenetic modifications that play a key role in establishing and maintaining cell identity [2]. Epigenetic modifications are chemical groups on the chromatin that regulate how the cellular machinery reads and interprets the genetic information. They do not alter the DNA sequence but change how it is packed in the cell nucleus. If we compared our DNA to an instruction manual, the epigenetic modifications could be compared to highlighters of different colours. They would mark different parts of the instruction text as green (active) and others as red (inactive), guiding the cellular machinery regarding which DNA sections to read and convert into functional proteins and which to ignore. Hence, different cell types with specific gene expression programmes can be generated from the same genetic instruction. Although its definition has evolved over time, epigenetics is commonly defined as "heritable changes in gene function that cannot be explained by changes in the DNA sequence" [3].

Each human cell contains about 2 m of DNA that needs to fit in the cell nucleus. To achieve that, our DNA is tightly packaged into chromatin and folded into chromosomes. The basic unit of chromatin is the nucleosome, composed of eight histone proteins (two of each of the core histones H2A, H2B, H3 and H4), around which DNA is tightly wrapped. The tails of the core histone proteins, which extend from the nucleosome and the DNA itself, are subject to various chemical epigenetic modifications (figure 1). This molecular "epigenetic code" controls DNA accessibility to the transcriptional machinery and regulates the binding of transcriptional activators and repressors [4], thereby determining which genes are active and which are silenced. By modulating cell-type-specific gene expression programmes, epigenetic mechanisms play a central role in regulating cell fate, identity and function. The main epigenetic mechanisms include DNA methylation, post-translational modifications (PTMs) of histones, chromatin remodelling and

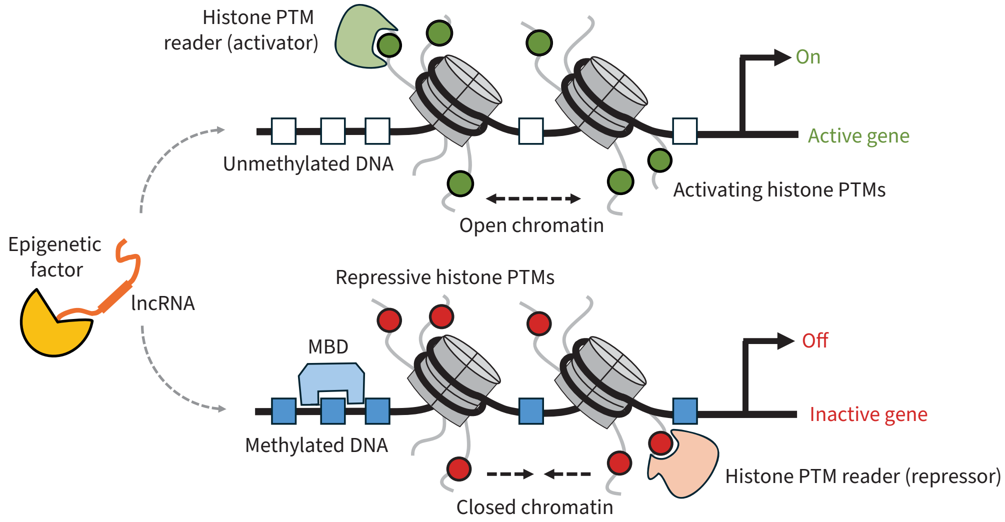

FIGURE 1 The main epigenetic mechanisms regulating chromatin structure and gene expression: mechanisms associated with active and repressed genes. These include DNA methylation (displayed as a pattern of methylated CpG sites (blue squares) and unmethylated CpG sites (white squares)), histone post-translational modifications (PTMs) (activating marks are shown as green circles, while red circles denote repressive marks) and long non-coding RNA (lncRNA) that can recruit additional epigenetic factors. Specific reader proteins can bind to epigenetic modifications and elicit biological responses. Examples include methylated DNA binding domain (MBD) protein, which binds methylated CpG sites, or specific reader proteins that bind activating or repressing histone PTMs. Active gene promoters are usually associated with activating histone PTMs (*e.g.* acetylation, or trimethylation of lysine 4 on histone H3 (H3K4me3)), lack DNA methylation and recruit transcriptional activators. In turn, repressed promoters are often methylated, associated with deacetylated histones (and other repressing histone PTMs, like H3K9me3 or H3K27me3), and recruit repressor complexes.

non-coding RNA (ncRNA) (figure 1). Epigenetic marks are added and removed by specific proteins known as writers and erasers, respectively. They are recognised by reader proteins, which contain conserved domains that bind to specific modifications and translate the epigenetic code into a cellular and biological response. Notably, all three classes of epigenetic factors (writers, readers and erasers) are druggable targets for clinical applications [5, 6].

The tale of DNA methylation (or why a small chemical group matters)

DNA methylation is one of the best-studied epigenetic signals in mammals (reviewed in [7–10]). It plays important biological roles: it preserves the stability of our DNA by preventing repetitive elements (transposons) from moving around, contributes to X-chromosome inactivation in females and regulates gene expression during development and in response to environmental signals. The importance of DNA methylation for human health is highlighted by the growing number of diseases associated with altered DNA methylation signatures [11, 12], including lung diseases [13–15].

Methylation is introduced to the DNA by a class of enzymes known as DNA methyltransferases (DNMTs) and removed by ten-eleven translocation (TET) DNA demethylases [9, 16, 17] (figure 2). DNMTs transfer a methyl group from the cofactor S-adenosyl-L-methionine (SAM) to cytosine residues within CpG sites, where a cytosine is followed by a guanine. Notably, not all CpG sites in the human genome are methylated, resulting in cell-type-specific patterns of both methylated and unmethylated sites. Hence, the DNA methylation pattern, together with other epigenetic modifications, confers an epigenetic blueprint to the cells. CpG sites cluster in regions called CpG islands (CGIs), which serve as regulatory units for DNA methylation. About 70% of human genes have a CGI in their promoters [18]. CGIs usually exhibit low and relatively stable methylation levels across healthy tissues, while their aberrant methylation (referred to as hypermethylation) usually leads to gene repression [18]. For example, hypermethylation of CGIs in the promoters of tumour suppressor genes leads to their repression and is a key mechanism contributing to cancer development [19, 20]. The observed negative correlation between promoter DNA methylation and gene expression led to the classical view of DNA methylation as a repressive mark. However, with the advent of genome-wide methylation profiling across various tissues and disease states, it is now clear that

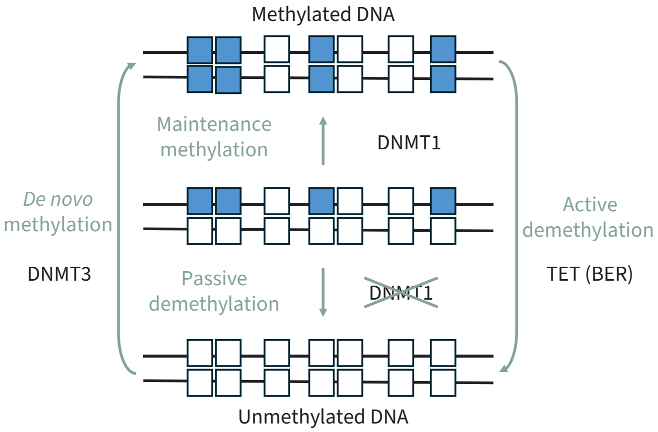

FIGURE 2 The DNA methylation cycle: establishment, maintenance and removal of DNA methylation. The DNA methylation pattern, which consists of methylated (blue squares) and unmethylated (white squares) CpG sites, is generated on unmethylated cytosines during embryonic development by DNA methyltransferases (DNMTs), specifically DNMT3 methyltransferases. It is maintained during cell divisions by a maintenance enzyme, DNMT1, which copies the methylation pattern onto the replicated, hemi-methylated DNA. DNA methylation can be lost *via* a passive mechanism when DNMT1 activity is absent, or can be removed actively by ten-eleven translocation (TET) enzymes, with the help of base excision repair (BER) enzymes, leading to the restoration of unmethylated DNA.

the role of DNA methylation is highly context-dependent. For instance, high levels of DNA methylation within gene bodies are often associated with active gene expression, whereas methylation at enhancers can be dynamically regulated to fine-tune gene activity in response to cellular and environmental signals [21]. These insights underscore the importance of considering genomic context when interpreting altered DNA methylation patterns in disease.

How are DNA methylation patterns established, maintained and erased?

Three active DNMTs (DNMT1, DNMT3A and DNMT3B) and three TET enzymes (TET1, TET2 and TET3) add and remove DNA methylation, respectively (reviewed in [9, 16, 17]). DNMT3A and DNMT3B establish initial DNA methylation patterns during embryonic development (figure 2). These patterns are then maintained during cell divisions by DNMT1, a molecular copy machine, which introduces methylation into newly synthesised DNA. The DNA methylation maintenance mechanism is quite clever. Because CpG sites are symmetric, methylation is present on both DNA strands. During DNA replication, the newly synthesised daughter strand is initially unmethylated, creating a hemi-methylated state, with the original strand still carrying the methylation pattern (figure 2). Hemi-methylated CpG sites are the preferred substrates for DNMT1, which quickly restores the original methylation pattern. Once established during embryonic development, DNA methylation patterns are largely maintained through cell divisions, with only minor tissue-specific changes. This robust inheritance mechanism preserves cell identity and function. However, it also has important implications for human health: if aberrant epigenetic patterns are acquired in lung cells in response to chronic environmental exposures, they will be propagated over cell divisions, potentially contributing to the development of lung diseases.

For DNA methylation to function as a regulatory mechanism, complementary erasing systems must exist. DNA methylation can be lost *via* a passive or an active process (figure 2). In the absence of DNMT1, DNA methylation is diluted over successive cell divisions (passive DNA demethylation). Active DNA demethylation involves several rounds of oxidation of the methylated bases by the TET DNA demethylase enzymes, followed by the removal of the oxidised cytosine bases by the DNA repair machinery, reverting DNA to an unmethylated state [9, 16]. Thus, a balance between DNA methylation writers (DNMTs) and erasers (TETs) shapes the final patterns of DNA methylation in human cells. Notably, changes in expression, localisation or activity of these proteins in response to environmental exposures can alter epigenetic patterns and contribute to the development of lung diseases (as illustrated later in this review).

How are DNA methylation patterns translated into a biological response?

The pattern of DNA methylation is interpreted by specific proteins interacting with the DNA. Methylation can directly modulate (attract or repel) the binding of sequence-specific transcription factors that regulate gene expression programmes [22]. It can also recruit specific methyl-binding domain proteins that act as transcriptional repressors (figure 1). They recruit additional epigenetic factors, like histone-modifying enzymes or chromatin remodelling enzymes, leading to reduced DNA accessibility to the transcriptional machinery and gene repression. Therefore, DNA methylation has traditionally been viewed as a repressive epigenetic signal, often linked to inactive genes and compacted chromatin. However, recent evidence suggests a more complex role for DNA methylation in gene expression regulation, where methylation in different parts of the genome may have varied effects on gene expression [21].

The histone code

The flexible tails of histone proteins that extend from the nucleosomes are subject to numerous PTMs, including acetylation, phosphorylation, methylation, ubiquitination and others (reviewed in detail in [23]). Multiple protein readers can interpret this molecular code as an activating or repressing signal for gene expression (figure 1). For example, acetylated histones mark active genes, whereas deacetylated histones are associated with repressed genes. Notably, the meaning of some histone modifications depends on their localisation and the specific residue that is modified. For example, trimethylation of lysine 9 on histone H3 (H3K9me3) correlates with repressed regions, whereas trimethylation of lysine 4 on the same histone (H3K4me3) marks active gene promoters. Crosstalk between adjacent modifications adds another layer of complexity to the histone code. Like DNA methylation, histone PTMs are added by writers and removed by erasers. For example, histone acetyltransferases add acetyl groups to lysine residues in histones, while histone deacetylases (HDACs) remove them. Specific protein readers recognise these modifications (for example, bromodomains bind acetylated lysines and chromodomains bind methylated lysines), translating epigenetic marks into downstream cellular signals. These “reading” domains, which are present in numerous proteins, are attractive targets for therapy (exemplified by bromodomain inhibitors), as blocking their binding stops the epigenetic signalling cascade.

Notably, histone modifications and DNA methylation do not operate in isolation but form an interconnected network that ensures precise control of chromatin structure and gene activity (reviewed in [17]). Histone modifications can recruit DNMTs to specific genomic regions through direct interaction. For example, DNMT3 methyltransferases bind to H3 tails, which allosterically activate the enzymes to methylate neighbouring DNA. Conversely, H3K4me3, which marks active gene promoters, blocks DNMT3 binding, thereby protecting these regions from DNA methylation.

Non-coding RNA

The information encoded in DNA is transcribed into messenger RNA, which can be translated into proteins. However, most RNAs in cells are ncRNAs that do not produce proteins. ncRNAs, classified as long (>200 bp) or short (<200 bp), serve as an additional mechanism of epigenetic regulation and play key roles in transcriptional regulation, silencing repetitive elements, and RNA modifications (for a recent review, see [24]). For example, long ncRNAs can recruit epigenetic factors to help establish active or repressed chromatin states (figure 1). MicroRNAs, the best-known class of short ncRNAs, negatively regulate gene expression by binding to messenger RNA and controlling its translation into proteins. Additionally, microRNAs influence chromatin structure and play a role in maintaining genome stability [24].

Epigenetics of environmental exposures

The lungs are a primary interface between the body and the external environment and are uniquely susceptible to environmental insults. Each breath delivers a wide array of pollutants, such as particulate matter (PM), smoke, and volatile organic or inorganic compounds, directly to the respiratory tract, where they can penetrate deep into the lungs. Notably, ultrafine particles can reach the gas exchange regions of the lungs and cross into the pulmonary circulation, entering the bloodstream. These toxic particles can induce epigenetic modifications and alter DNA methylation, histone modification patterns, and ncRNA expression. The resulting dysregulation fosters local inflammation and tissue injury and can trigger systemic effects through inflammatory and oxidative pathways, leading to lung disease development (figure 3) (reviewed in [25, 26]). Understanding how epigenetic alterations interface with clinical disease states is crucial for uncovering the pathogenesis of complex respiratory conditions and identifying epigenetic biomarkers and therapeutic targets to improve patient outcomes.

Mechanisms of epigenetic alterations induced by environmental exposures

Environmental studies have shown widespread DNA methylation changes in tissues exposed to inhaled hazardous particles (see the following sections for examples). A common alteration is an overall reduction in DNA methylation (DNA hypomethylation), which is primarily due to decreased DNA methylation at repetitive parts of the genome [26]. This leads to the activation of repetitive elements (*e.g.* transposons) and genomic instability, a hallmark of cancer cells [19]. In addition to global DNA methylation loss,

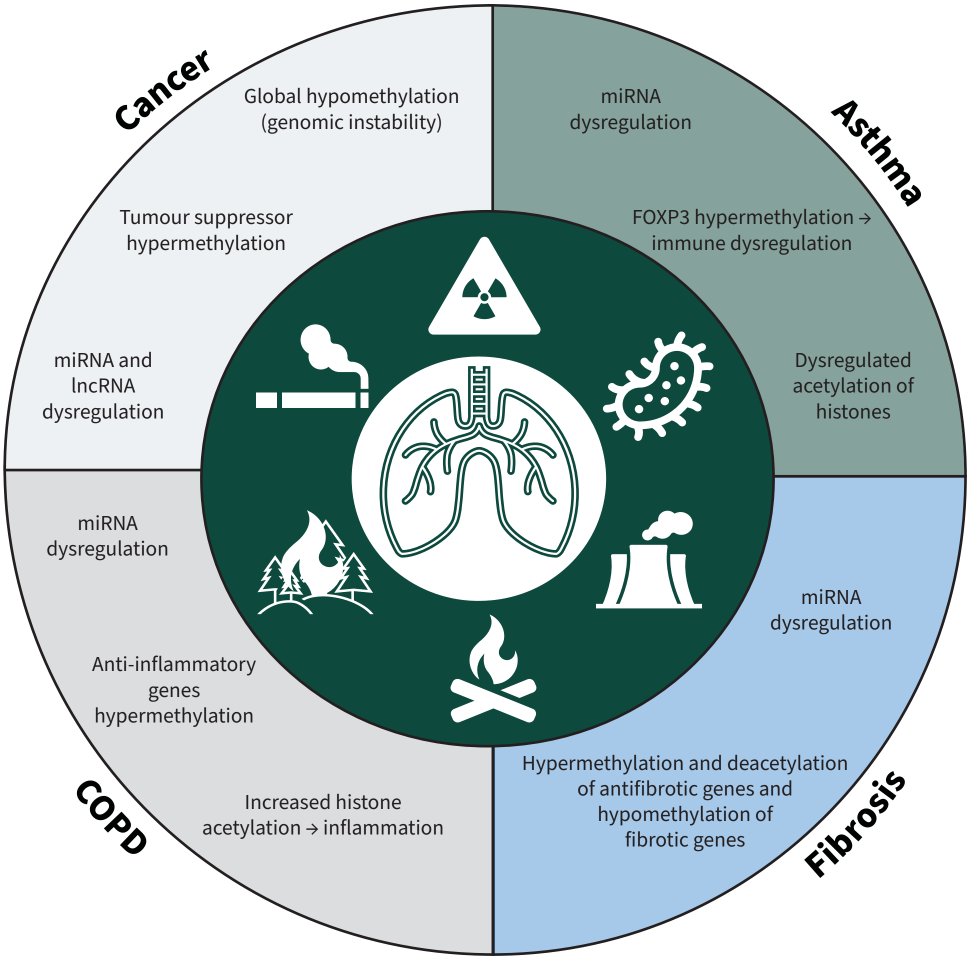

FIGURE 3 Environmental exposures modify the epigenetic landscape of cells, leading to lung disease development. Environmental exposures (*e.g.* cigarette smoke, air pollution, wildfire smoke, radiation and toxic chemicals), schematically depicted in the inner circle of the diagram, trigger dysregulation of multiple layers of epigenetic modifications in lung cells, including changes in DNA methylation, histone acetylation/methylation, microRNA (miRNA) and long non-coding RNA (lncRNA) expression, and lead to increased inflammation, immune dysregulation, tissue fibrosis, remodelling and tumorigenesis.

environmental pollutants can also cause aberrant methylation or demethylation of specific genes, altering their expression [26]. An example of this is the hypermethylation of tumour suppressor, DNA damage repair or antifibrotic genes, leading to their silencing and disease development [19, 20]. Finally, genes involved in DNA methylation, such as DNMT3A methyltransferase and TET2 DNA demethylase, are frequently dysregulated or mutated in various cancers, disrupting normal epigenetic regulation. Importantly, aberrant epigenetic signatures can persist through cell divisions and be maintained after the exposure ends, providing cellular memory of past exposures. Consequently, profiling the epigenetic landscape of individuals exposed to environmental pollutants may reveal robust signatures of past (or even prenatal) exposures, leading to the development of biomarkers of exposure [27, 28].

How can we measure epigenetic changes in biological samples?

Epigenetic analyses in epidemiology are most commonly used in epigenome-wide association studies. These population-based studies investigate the association between the levels of epigenetic modifications and a specific exposure (*e.g.* air pollution or cigarette smoke), disease or phenotype [29]. DNA methylation is the most extensively studied epigenetic modification in epidemiology, largely due to the availability of robust methods for large cohort profiling. Illumina methylation arrays (*e.g.* 450K or EPIC

Array) have become the method of choice for genome-wide DNA methylation analysis because of their cost-effectiveness and versatility. They rely on DNA hybridisation to specific probes immobilised on beads that distinguish methylated and unmethylated sequences (reviewed in [13]). They are compatible with formalin-fixed paraffin-embedded samples, allowing analysis of archived collections. Despite their widespread use, DNA methylation arrays cover only a fraction of the genome (up to 1 million of the 28 million CpG sites in the human genome). Whole-genome methylation sequencing can be achieved using next-generation sequencing-based approaches, for example, whole-genome bisulfite sequencing, whole-genome enzymatic methylation sequencing or long-read nanopore technologies [30]. However, the high sequencing cost makes them more suitable for discovery research, complementing array-based large-scale cohort profiling.

The link between environmental exposures and epigenetics is well established. Numerous studies have demonstrated widespread remodelling of the epigenetic landscape of tissues exposed to cigarette and wildfire smoke, PM, indoor pollution, diesel exhaust fumes, toxic chemicals and nanoparticles. Selected examples are highlighted in the next sections, but the reader is referred to recent reviews for a more comprehensive overview [25, 31–34].

Cigarette smoke profoundly remodels the epigenetic landscape of tissues

Cigarette smoke is the best-understood example of environmental exposure with a profound impact on the epigenetic landscape. Multiple studies have demonstrated genome-wide alterations in DNA methylation in smokers (for recent reviews on epigenetics of smoking, see [35–37]). Smoke-related changes were observed in different biological samples, including blood, lung tissue, airway epithelium, sputum and buccal mucosa. Interestingly, while smoking cessation restored blood DNA methylation signatures at most regions within 5 years, some changes persisted over 30 years, providing evidence for a long-term memory of smoking exposure [38]. Additionally, acquired DNA methylation changes from prenatal exposure to maternal smoking may provide a molecular explanation for the increased lung disease risk in later life [39].

The identification of robust DNA methylation sites associated with smoking status, cumulative exposure and time since quitting has led to the development of DNA methylation-based predictors of smoking behaviour, which may prove useful in clinical practice. They exemplify the promise of DNA methylation as a sensitive and robust biomarker of exposure [28].

Epigenetic impacts of wildfire, particulate matter and indoor air pollution

Air pollution and climate-related environmental stressors, including wildfires, extreme weather events and shifts in atmospheric composition, have emerged as key contributors to respiratory diseases. They induce epigenetic alterations in the lung, driven by exposures to PM, gaseous pollutants and oxidative stress. The cumulative effects of these changes exacerbate inflammation, impair lung function, and heighten the susceptibility to chronic respiratory conditions, cancer and infections (figure 3) [25, 31].

Wildfire smoke, a significant source of PM and polycyclic aromatic hydrocarbons, was shown to induce DNA methylation changes in genes regulating immune responses [40]. PM also contains harmful components, such as arsenic, cadmium and lead, which can alter multiple layers of epigenetic modifications, leading to the development of lung diseases (reviewed in [25, 31]). Hence, it is not surprising that epigenetic changes have been found in individuals exposed to PM. Altered DNA methylation contributes to impaired immune responses, dysregulation of cellular repair mechanisms and carcinogenesis. For example, increased DNA methylation of immunoregulatory genes, like interleukin (IL)-10 or Foxp3, impairs immune regulation and perpetuates inflammatory states. In turn, dysregulation of histone acetylation plays a critical role in promoting inflammation and suppressing anti-inflammatory pathways in individuals exposed to PM, heavy metals and other particulate toxins. PM exposure also disrupts several microRNAs, contributing to heightened oxidative stress and inflammatory responses [25].

Indoor air pollution, resulting from biomass combustion or poor ventilation, mirrors many of the epigenetic disruptions caused by PM exposure. The hypermethylation of tumour suppressor genes, increased histone acetylation amplifying pro-inflammatory signalling pathways, and ncRNA changes induced by indoor pollutants can drive fibrosis and inflammation, further increasing the health risks associated with prolonged exposure [31].

Plastics, nanoparticles and engineered nanomaterials influence the epigenome

The rise of nanoparticles and engineered nanomaterials, including titanium dioxide, carbon nanotubes and silver nanoparticles, has revolutionised modern industrial and medical applications. However, their widespread use has raised concerns about potential health impacts. These materials are small enough to penetrate biological barriers and influence cellular processes in the lungs. For example, inhalation of nanoparticles like titanium dioxide and carbon nanotubes can lead to significant epigenetic changes in DNA methylation and histone modifications, which can be used as toxicity biomarkers (reviewed in [34]).

The mechanisms by which environmental or occupational exposures drive epigenetic dysregulation in lung cells are poorly understood. Environmental exposures can alter the expression of epigenetic enzymes (*e.g.* DNMTs or TETs) or influence their activity by regulating the availability of essential cofactors. The epigenetic effects of nanoparticles, for example, are likely to be linked to their ability to generate reactive oxygen species, which induce DNA lesions, preventing their methylation by DNMTs and leading to DNA hypomethylation [34]. Heavy metals, in turn, may alter DNA methylation through modulating the availability of SAM, dysregulation of DNMT expression or changes in chromatin accessibility [26].

In addition, oxygen levels affect the activity of DNA and histone demethylases, while levels of SAM, a universal cofactor for DNA and histone methyltransferases, are regulated by folate availability, linking diet to epigenetic regulation. Mitigating oxidative stress and inflammation is, therefore, crucial to minimising the impact of environmental exposures, and promising strategies are emerging to counteract the resulting epigenetic alterations. Further research into the long-term effects and mechanisms of different environmental exposures on the epigenome is vital for identifying novel therapeutic targets and refining preventive strategies.

Epigenetic dysregulation is prominent in chronic lung diseases

Widespread DNA methylation changes have been identified in lung diseases, including COPD, asthma, lung cancer and idiopathic pulmonary fibrosis (IPF). They are associated with the dysregulation of key pathways driving inflammation, tissue remodelling, fibrosis and cancer progression, demonstrating that epigenetic dysregulation is central to lung disease development (reviewed in [13–15, 25, 41, 42]).

Numerous studies have identified altered DNA methylation signatures in blood, sputum, oral mucosa, lung tissue, bronchial brushings and isolated lung cells of COPD patients (reviewed in [13, 15]). Methylation changes were associated with pathways involved in COPD pathology, indicating their potential role in contributing to aberrant gene expression programmes in COPD cells [43, 44].

Similarly, changes in DNA methylation have also been identified in IPF patients, providing initial evidence of dysregulated epigenetic signalling in lung fibrosis (reviewed in [13, 15, 41]). Examples of genes with epigenetic changes include Thy-1 antigen (THY1), prostaglandin E receptor 2 (PTGER2), caveolin 1 (CAV1) and α-smooth muscle actin (α-SMA). These are key genes implicated in IPF pathology, and their epigenetic dysregulation may drive amplified fibroblast activation and tissue remodelling.

Asthma is another example of a chronic lung disease driven by complex interactions between host genetic factors and environmental exposures, including early-life exposure to cigarette smoke, viral infections or maternal diet. Widespread changes in DNA methylation patterns are associated with allergy and atopy in children and adults (reviewed in [42, 45]). Examples of well-replicated genes with altered DNA methylation signatures include IL-5 receptor subunit alpha (IL5RA), eosinophil peroxidase (EPX), SMAD family member 3 (SMAD3) and RUNX family transcription factor 3 (RUNX3), key genes involved in T-cell maturation, immunity and asthma pathology.

Current epigenetic profiling approaches have important limitations. Most lung disease studies profile DNA methylation in complex samples, such as blood or lung tissue, which consist of many different cell types (each with a specific DNA methylation pattern). Hence, while they reveal disease-associated pathways, they cannot pinpoint the specific cell populations driving epigenetic changes. Future research using isolated lung cells or single-cell approaches is required to identify disease-driving populations and unravel epigenetic mechanisms underlying lung disease pathology.

The examples described here demonstrate that DNA methylation can serve as a sensitive biomarker for lung disease detection and patient stratification. However, most studies have analysed relatively small numbers of samples, primarily from patients with end-stage disease. Additional well-designed longitudinal studies in larger cohorts are needed to validate these results and uncover specific epigenetic biomarkers associated with different disease subtypes and trajectories. Combining epigenetic profiling with other sequencing-based omics approaches (*e.g.* genomics, proteomics, metabolomics and transcriptomics) and imaging holds promise for identifying disease subtypes driven by common pathological mechanisms (endotypes), which is essential for the development of curative therapies.

Epigenetic biomarkers for diagnosis, patient stratification and therapy monitoring

DNA methylation changes induced by environmental exposure may serve as sensitive biomarkers of exposure and could be used to develop DNA methylation-based predictors of health and disease [28]. This concept is exemplified by the development of DNA methylation-based predictors of smoking behaviour, as described earlier. Epigenetic biomarkers also hold promise for identifying individuals at higher risk of developing lung diseases and helping to stratify patients based on disease severity, enabling tailored interventions to mitigate disease progression [46]. Increased methylation of serpin family A member 1 (SERPINA1), for example, is associated with reduced α_1-antitrypsin expression and increased susceptibility to COPD, particularly in smokers [47].

Epigenetic biomarkers could help identify patients most likely to benefit from specific therapies and enable real-time monitoring of therapeutic efficacy. For instance, aberrant methylation of cyclin-dependent kinase inhibitor 2A (CDKN2A) and Ras association domain family 1 isoform A (RASSF1A) genes is a hallmark of nonsmall cell lung cancer and can be detected in circulating tumour DNA, offering a noninvasive diagnostic tool [48]. Such biomarkers can provide valuable insights into disease biology and enable real-time assessment of therapeutic efficacy in patients receiving epigenetic therapies. The future development of strategies for biomarker monitoring may enable personalised therapeutic adjustments and improved patient outcomes.

The strong link between epigenetic changes and environmental exposures or disease states has led to the interest in using DNA methylation signatures as biomarkers. However, complex interactions exist between environmental exposures, diet and hormone signalling, highlighting the challenges of epigenetic biomarker studies. DNA methylation is also influenced by other confounding factors, such as age, sex, ethnicity, smoking and corticosteroid use, which must be accounted for in analyses [49]. Additionally, establishing causality is difficult because there is often a long latency between the initial exposure and the disease onset. Functional validation of the identified epigenetic changes in cellular and animal models using innovative epigenetic editing approaches (described later in this review) is therefore important.

To advance the epigenetic biomarker field, longitudinal studies involving well-characterised, ethnically diverse cohorts (with detailed nutritional and environmental exposure data) are needed. Study design must be tailored to the specific research question and the availability of clinical materials. Beyond cohort size, cell type heterogeneity within complex clinical samples (*e.g.* blood, epithelial brushings or lung tissue) must be considered, as each cell type carries distinct epigenetic profiles. Bulk tissue profiling averages these signals, masking cell-specific changes, and even small shifts in cell populations can influence the results independently of true epigenetic changes [49, 50]. This limits both the reliability of findings and our understanding of the cell-type-specific contributions to disease. To overcome this, studies using purified lung cell populations or single-cell epigenetic technologies are essential to uncover the precise roles of epigenetic alterations in driving chronic lung diseases.

Epigenetic therapy

Contrary to genetic mutations, epigenetic changes are reversible, making them attractive targets for therapeutic intervention. Epigenetic therapies aim to correct aberrantly activated or silenced genes by restoring their normal epigenetic state, without affecting the underlying genetic sequence. Cancer is the best-studied example of a disease with a clinically demonstrated benefit of epigenetic therapy. Multiple small-molecule inhibitors targeting DNMTs or histone-modifying enzymes have been developed (reviewed in [5, 6]). Several are currently available for standard-of-care treatment and are used in clinics, mostly for haematological malignancies (table 1). Although no US Food and Drug Administration (FDA)-approved epigenetic drugs currently exist for chronic lung diseases, the field is rapidly advancing, with many epigenetic compounds undergoing clinical trials.

The DNMT inhibitors azacitidine and decitabine were the first epigenetic drugs developed to target aberrant DNA methylation of tumour suppressor genes and restore their expression (table 1) [5, 6]. They incorporate into DNA, leading to the trapping and inactivation of DNMTs, and resulting in DNA hypomethylation and re-expression of silenced genes. These compounds effectively reduced tumour proliferation and enhanced chemosensitivity in preclinical and clinical settings. New DNMT inhibitors with improved stability and pharmacokinetics have been developed and are currently in clinical trials, although they have not yet reached clinical practice [5, 6].

Another class of epigenetic drugs used in oncology targets histone-modifying enzymes such as HDACs. Several FDA-approved HDAC inhibitors are currently used in the clinic (table 1) [5, 6]. By restoring histone acetylation, these compounds can reactivate silenced genes (*e.g.* tumour suppressor genes). Clinical

TABLE 1 Examples of epigenetic drugs used in the clinics and selected novel epigenetic modulators in development

Compound	Epigenetic target	Clinical indication	FDA approval
Azacytidine	DNA methyltransferases	Myelodysplastic syndrome, acute myeloid leukaemia	Yes
Decitabine	DNA methyltransferases	Myelodysplastic syndrome, acute myeloid leukaemia	Yes
Vorinostat/SAHA	Histone deacetylases	Cutaneous T-cell lymphoma	Yes
Romidepsin	Histone deacetylases	Cutaneous T-cell lymphoma	Yes
Belinostat	Histone deacetylases	Peripheral T-cell lymphoma	Yes
Tucidinostat	Histone deacetylases	Peripheral T-cell lymphoma, adult T-cell leukaemia/lymphoma	Only in China
Tazemetostat	Histone methyltransferase EZH2	Follicular lymphoma, epithelioid sarcoma	Yes
Enasidenib	Mutant isocitrate dehydrogenase	Acute myeloid leukaemia, cholangiocarcinoma	Yes
Ivosienib	Mutant isocitrate dehydrogenase	Acute myeloid leukaemia	Yes
OTX-2002	Precision epigenomic modulator targeting MYC oncogene	Hepatocellular carcinoma	Not yet, IND cleared, in phase 1/2
EPIC-321	Precision epigenomic modulator targeting DUX4 gene	Facioscapulohumeral muscular dystrophy	Not yet, IND cleared
EPIC-341	Precision epigenomic modulator targeting α_1-antitrypsin gene	α_1-antitrypsin deficiency	Not yet, EpicBio pipeline

FDA: US Food and Drug Administration; SAHA: suberoylanilide hydroxamic acid; EZH2: enhancer of zeste homolog 2; DUX4: double homeobox 4; IND: investigational new drug.

trials have shown that HDAC inhibitors can work synergistically with chemotherapy and immunotherapies, highlighting their potential as combination treatments in lung cancer management.

Importantly, epigenetic therapies may benefit not only lung cancer, but also chronic lung diseases, like COPD or IPF. Targeting DNMTs led to the demethylation of the peroxisome proliferator-activated receptor-γ (PPAR-γ), a potent antifibrotic gene, and alleviated lung fibrosis [51]. Treatment with HDAC inhibitors reduced pro-fibrotic gene expression, restored antifibrotic gene activity and apoptosis sensitivity in fibrotic fibroblasts, and decreased lung fibrosis and matrix deposition in animal models (reviewed in [52]), supporting HDAC inhibitors as a promising strategy for targeting lung fibrosis.

In addition to epigenetic writers and erasers, epigenetic reading domains are the third group of potential targets for epigenetic therapy [5, 6]. Bromodomain inhibitors disrupt the interaction of bromodomain proteins with acetylated histones, leading to reduced expression of oncogenes, modulation of the tumour microenvironment and enhanced anti-tumour immunity. The integration of epigenetic therapies with immune checkpoint inhibitors has emerged as a promising approach to overcoming resistance and enhancing immune responses in lung cancer.

Epigenetic drugs demonstrate poor pharmacokinetics and high toxicity, mainly due to their lack of genomic and cell-type specificity [5, 6]. DNMT and HDAC inhibitors, while effective in reversing aberrant epigenetic marks, often act on non-disease-related genes, leading to unintended side-effects such as haematological toxicity, gastrointestinal disturbances and immunosuppression. Hence, novel approaches with increased precision are needed to advance the field of epigenetic therapy. Advances in targeted delivery systems, like inhalable formulations and nanoparticle-based carriers, are being explored to localise therapeutic effects to the lungs, reducing systemic exposure and improving safety [52]. Direct delivery to lung tissue offers key benefits for treating respiratory diseases, including lower toxicity, improved pharmacokinetics and increased drug bioavailability.

Epigenetic editing

Epigenome-wide association studies have linked DNA methylation changes to reduced lung function, environmental exposures and chronic lung diseases, providing strong evidence that epigenetic processes contribute to the pathogenesis of lung disease. However, the key limitation of the current epigenetic studies is that they provide only correlative data. Therefore, we do not know which epigenetic alterations are the cause and which are the consequence of the disease process.

Innovative epigenetic editing technologies have recently been developed, opening the possibility for the functional study of epigenetic regulation and future precision medicine approaches. Epigenetic editing

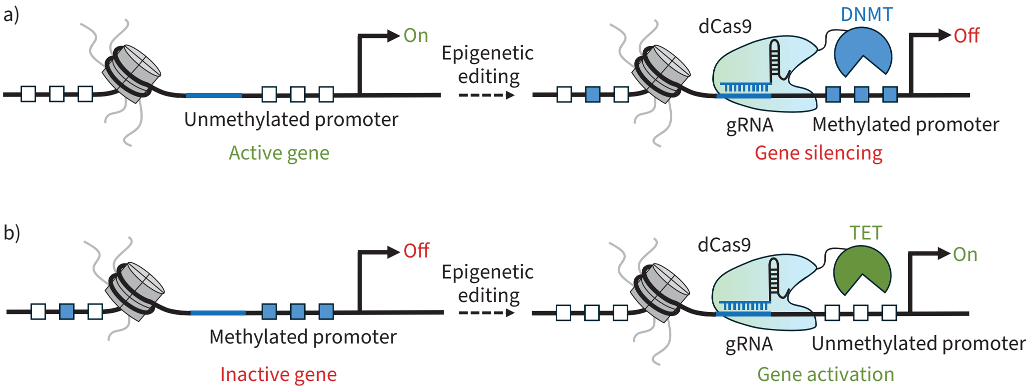

FIGURE 4 Targeted DNA methylation and demethylation using CRISPR-based epigenetic editing for a) gene silencing and b) gene activation. A DNA-targeting domain consisting of the catalytically dead Cas9 (dCas9) is fused to an epigenetic effector domain, like DNA methyltransferase (DNMT) or DNA demethylase (ten-eleven translocation (TET)). The fusion domain can be directed to a desired genomic region (*e.g.* gene promoter) through interaction with guide RNAs (gRNAs) specific for the target regions. Once targeted, the epigenetic effector domain a) introduces or b) removes DNA methylation, leading to subsequent a) gene silencing or b) activation. gRNA sequences can be designed to target any desired gene, and a combination of different epigenetic effector domains can be used to modify different layers of epigenetic regulation.

enables precise re-writing of epigenetic modifications at a genomic region of interest (reviewed in [13]). It employs synthetic epigenetic tools, consisting of a targeting domain fused to an epigenetic effector domain (figure 4). Once delivered into cells, the epigenetic programmer is recruited to the target region (*e.g.* gene promoter) through its sequence-specific DNA-binding domain, directing the fused epigenetic domain (*e.g.* DNMT or TET demethylase) to that site. The epigenetic effector domain then modifies the epigenetic state of the region, leading to gene activation or silencing. Catalytically inactive (or dead) Cas9 (dCas9) nuclease from the CRISPR system is the most widely used targeting domain because it can be recruited to specific genomic regions by designing a guide RNA (gRNA) complementary to the target sequence (figure 4). Precise targeting of DNMTs or TET enzymes to specific genomic regions provides powerful tools to investigate the causal role of DNA methylation on gene expression and explore the potential role of epigenetic editing for epigenetic therapy [44, 53].

While currently used as a research tool, precision epigenetic editing approaches also hold promise for future clinical applications. One of the most attractive aspects of epigenetic editing is that it could be used as a single intervention. Once the epigenetic signal is established, it should theoretically be inherited through cell divisions by the cellular machinery. The first-in-class epigenetic modulator OTX-2002 from Omega Therapeutics is already in clinical trials for hepatocellular carcinoma (ClinicalTrials.gov identifier NCT05497453) [54], and several others are in preclinical development (table 1). Excitingly for the chronic lung disease field, EpicBio is developing an epigenetic programmer (EPIC-341) to epigenetically suppress the mutated α_1-antitrypsin gene and replace it with a functional version of the gene, targeting the genetic cause of COPD/emphysema.

Preventive interventions

Public health interventions can reduce lung disease risk through modulating the epigenome. Smoking cessation, even after prolonged exposure, has been shown to partially reverse tobacco-induced DNA methylation changes, restoring the expression of key protective genes [38]. This highlights the plasticity of the epigenome and its responsiveness to behavioural changes. Nutritional interventions, such as diets rich in folate and other methyl donors, promote DNA methylation homeostasis and mitigate the impact of environmental exposures. These strategies have been shown to reduce the epigenetic burden of oxidative stress and inflammation, improving respiratory health and preventing the onset of chronic diseases [55]. Leveraging epigenetic biomarkers to guide targeted public health strategies can help mitigate the effects of environmental exposures, reduce the incidence of lung diseases, and improve long-term health outcomes in vulnerable populations.

Conclusions and future directions

Although the role of DNA methylation and other epigenetic mechanisms in the pathology of chronic lung diseases is increasingly recognised, and significant progress has been made in mapping these changes across various lung conditions, the field remains in its early stages. Many foundational questions remain unresolved. We still do not understand how aberrant DNA methylation patterns (and other epigenetic alterations) are established in specific lung cells in response to chronic environmental exposures, because the molecular mechanisms by which DNMTs and TET enzymes are targeted and regulated in diseased lungs remain poorly investigated. Similarly, most studies provide only associations between altered epigenetic states and environmental exposures or disease phenotypes. Hence, it remains unknown whether epigenetic alterations can be targeted to reverse disease-associated phenotypes and serve as a novel therapeutic strategy for chronic lung diseases.

These unanswered questions and challenges reflect the fact that lung epigenetic research is still in its infancy, but they also underscore its potential for clinical translation and point to promising directions for future studies. To advance the translation of epigenetic research in the lung field, it is essential to develop a detailed mechanistic understanding of how epigenetic dysregulation in specific cell types contributes to the pathology of chronic lung diseases. This requires the generation of high-resolution, genome-wide reference epigenetic profiles from healthy and diseased lung cells, isolated from clinically well-characterised tissues. In parallel, functional validation of candidate epigenetic changes is crucial to establish causal relationships. Finally, current epigenetic therapies often lack genomic and cell-type specificity, highlighting an urgent need to develop next-generation therapeutics with improved precision and pharmacokinetic properties.

Key points

- Epigenetic modifications regulate which genes are active and which are silenced, regulating the transcriptional profile of the cells.
- Chronic environmental exposures alter the epigenetic landscape of cells, contributing to aberrant gene expression programmes and lung disease development.
- Epigenetic modifications may provide sensitive biomarkers for exposure, early disease diagnosis, patient stratification and response to therapy.
- Epigenetic modifications are reversible and, therefore, constitute attractive targets for therapy and precision medicine approaches.

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