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# Role of epigenetic mechanisms in the pathogenesis of chronic respiratory diseases and response to inhaled exposures: From basic concepts to clinical applications

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ABSTRACT

Epigenetic modifications are chemical groups in our DNA (and chromatin) that determine which genes are active and which are shut off. Importantly, they integrate environmental signals to direct cellular function. Upon chronic environmental exposures, the epigenetic signature of lung cells gets altered, triggering aberrant gene expression programs that can lead to the development of chronic lung diseases. In addition to driving disease, epigenetic marks can serve as attractive lung disease biomarkers, due to early onset, disease specificity, and stability, warranting the need for more epigenetic research in the lung field.

Despite substantial progress in mapping epigenetic alterations (mostly DNA methylation) in chronic lung diseases, the molecular mechanisms leading to their establishment are largely unknown. This review is meant as a guide for clinicians and lung researchers interested in epigenetic regulation with a focus on DNA methylation. It provides a short introduction to the main epigenetic mechanisms (DNA methylation, histone modifications and non-coding RNA) and the machinery responsible for their establishment and removal. It presents examples of epigenetic dysregulation across a spectrum of chronic lung diseases and discusses the current state of epigenetic therapies. Finally, it introduces the concept of epigenetic editing, an exciting novel approach to dissecting the functional role of epigenetic modifications. The promise of this emerging technology for the functional study of epigenetic mechanisms in cells and its potential future use in the clinic is further discussed.



**Contents**



*Abbreviations:* AHRR, aryl hydrocarbon receptor repressor; AM, alveolar macrophages; ATAC-seq, assay for transposase-accessible chromatin with sequencing; BAL, bronchoalveolar lavage; dCas9, catalytically inactive / dead Cas 9 nuclease (from the CRISPR system); ChIP-seq, chromatin immunoprecipitation with sequencing; COPD, chronic obstructive pulmonary disease; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; CpG site, cytosine–guanine dinucleotide separated by a phosphate; DNMT, DNA methyltransferase; DNMTi, DNA methyltransferase inhibitor; EWAS, epigenome-wide association study; FFPE, formalin-fixed paraffin-embedded; GWAS, genome-wide association study; HDAC, histone deacetylase; IPF, idiopathic pulmonary disease; lncRNAs, long non-coding RNAs; miRNA, micro-RNA; ncRNAs, non-coding RNAs; MBD, methyl binding domain; MTase, methyltransferase; NGS, next-generation sequencing; PTM, posttranslational modification; RRBS, reduced-representation bisulfite sequencing; SAM, S-adenosyl-L-methionine; scRNA-seq, single-cell RNA sequencing; TET proteins, Ten-Eleven-Translocation proteins; TDG, Thymine DNA glycosylase; TF, transcription factor; UHRF1, ubiquitin-like, containing PHD and RING finger domains 1; WGBS, whole genome bisulfite sequencing; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5fC, 5-formylcytosine; 5caC, 5-carboxylcytosine.

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## 1. Epigenetics – an ingenious mechanism for regulating genetic information

The advent of the next-generation sequencing (NGS) technologies revolutionised biomedical research. We deciphered the sequence of the human genome (Lander et al., 2001; Nurk et al., 2022; Venter et al., 2001) and identified genetic drivers of numerous human diseases (including lung diseases), bringing biomedicine into the genomic era (Ashley, 2016). The development of single-cell transcriptomic technologies offered further unprecedented insights into the complexity and cellular identity of the human and mouse lungs (Negretti et al., 2021; Travaglini et al., 2020). It led to the identification of novel cell types (Montoro et al., 2018; Plasschaert et al., 2018) and differentiation states (Ruiz Garcia et al., 2019). It revealed unique aspects of disease processes in the human lung, opening doors to the functional dissection of cellular pathology, and moving the research into the single-cell transcriptomic era (Adams et al., 2023; Van de Sande et al., 2023). However, our understanding of the molecular processes orchestrating gene expression and cellular differentiation programs in the human lung is still very limited. Similarly, the regulatory mechanisms driving lung cell dysfunction in response to environmental stimuli are not well understood. To provide the missing link between the environment, our genes, and their regulation in disease we need to move lung research into the epigenetic era. For further progress and translation of epigenetic research to the clinics, there is an urgent need for functional studies on how epigenetic mechanisms in lung cells are impacted by environmental insults and how their dysregulation drives the development of chronic lung disease.

We begin as a single cell, the zygote, and yet there are more than 400 cell types in the adult human body (Hatton et al., 2023). How can a single genetic sequence give rise to so many different cells, each with unique morphology, gene expression programs and function? We know now that lineage-specific gene expression programmes are initiated by transcription factors and modulated by epigenetic mechanisms that regulate how the genetic information is read and interpreted by the cellular machinery. The development of epigenetic systems was proposed as a fundamental step in the evolution of multicellular organisms because of their need to maintain stable cellular differentiation (Jeltsch, 2013). Epigenetic mechanisms are defined as "heritable changes in gene function that cannot be explained by changes in the DNA sequence" (Riggs & Porter, 1996).

To fit in the cell nucleus, our DNA is tightly packaged into chromatin. The basic building unit of the chromatin, the nucleosome, consists of DNA wrapped around an octamer of histone proteins (two of each of the four core histones H2A, H2B, H3 and H4). Both the DNA and the histone proteins (especially their tails that protrude from the nucleosome surface) are decorated with a variety of chemical groups, called epigenetic modifications. This epigenetic code determines which genes are active and which are silenced. The regulatory function of epigenetic modifications is mediated by controlling the accessibility of the DNA to the cellular machinery and regulating the binding of transcriptional activators and repressors (Allis & Jenuwein, 2016). The main epigenetic mechanisms include DNA methylation, posttranslational modifications (PTM) of histones, chromatin remodelling as well as non-coding RNA (ncRNA) (Fig. 1).

In this review, I will briefly introduce the main epigenetic mechanisms and examine their dysregulation in a variety of chronic lung diseases, with a focus on DNA methylation. I will present the main technologies used for mapping and functional validation of DNA methylation alterations and discuss the promise of epigenetic editing and epigenetic therapies.

## 2. DNA methylation – a small group with a big biological impact

DNA methylation is the best-described example of an epigenetic mechanism and a key epigenetic signal used for the direct control of gene expression [for general reviews on DNA methylation, please refer to (Greenberg & Bourc'his, 2019; Jurkowska, Jurkowski, & Jeltsch, 2011; Schubeler, 2015; Smith et al., 2024; Smith & Meissner, 2013)]. Owing to this vital regulatory function, DNA methylation plays important biological roles in regulating genome stability (through silencing repetitive elements), genomic imprinting, X-chromosome inactivation and regulation of gene expression in development and response to environmental and occupational cues. The importance of DNA methylation is demonstrated by the lethal phenotypes of the genetic knockouts of any of the active DNA methyltransferase enzymes in mice (Li et al., 1992; Okano et al., 1999) and by the ever-growing number of diseases associated with altered DNA methylation signatures, including pulmonary and non-pulmonary disease conditions (Bergman & Cedar, 2013; Liu et al., 2023; Michalak et al., 2019; Zhao et al., 2021).

The methylation of human and mammalian DNA was identified in the early 80s [reviewed in (Jurkowska & Jeltsch, 2022b)]. It arises through the covalent addition of the methyl group (CH3) to the cytosine residues in the DNA, generating 5-methylcytosines (5mC) (Fig. 2). In humans, it occurs predominantly in the context of CpG sites, where a cytosine is followed by a guanine residue. Non-CpG DNA methylation (arising on CA, CC or CT sites) occurs at much lower levels in differentiated tissues and is mostly present in embryonic stem cells and the brain (Schultz et al., 2015).

Depending on the cell type, roughly 60–80 % of all CpG sites in the DNA are methylated, corresponding to 3–8 % of all cytosines in the human genome (Greenberg & Bourc'his, 2019). Importantly, only selected CpG sites are methylated, resulting in the generation of a tissue and cell-type-specific pattern consisting of methylated and non-methylated sites. Hence, the DNA methylation pattern confers the epigenetic cell identity (Loyfer et al., 2023). This has important consequences for studying epigenetic modifications in a complex organ or tissue (like for example lung tissue, epithelium or blood), as different cell types will carry unique patterns of DNA methylation (and other epigenetic modifications). Consequently, the DNA methylation profile (the methylome) from bulk tissues represents an average of different cell types, limiting our understanding of cell-type-specific contributions to disease development. Thus, studies of purified lung cell populations or single-cell approaches are urgently needed to investigate the role of DNA methylation (and other epigenetic modifications) in driving cellular function in healthy and diseased lungs.

Regions of the genome characterised by a high density of CpG sites are called CpG islands. They are found in the promoters (or the first exons) of around two-thirds of human genes, and less commonly in gene bodies. They constitute regulatory units for DNA methylation, as their methylation correlates with the transcriptional activity of their neighbouring genes (Jones, 2012). In healthy somatic tissues, CpG islands in gene promotors are usually unmethylated, whereas CpG

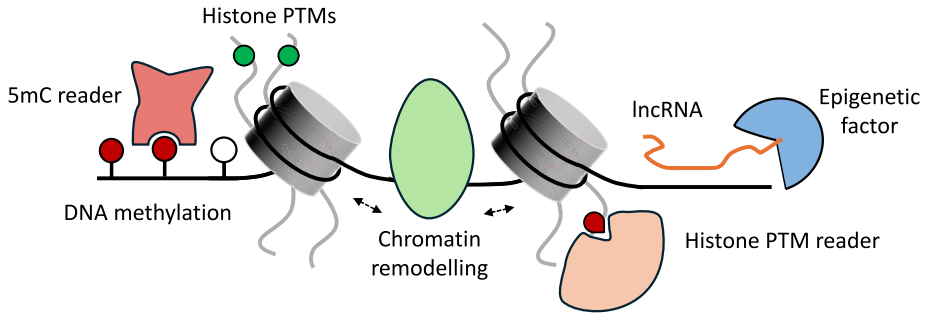


**Fig. 1.** Different layers of epigenetic modifications regulating chromatin structure.
Schematic diagram of the main epigenetic mechanisms, including DNA methylation (displayed as a pattern of methylated – red lollipops and unmethylated – white lollipops CpG sites), posttranslational modifications (PTMs) of the histone tails, long non-coding RNA (lncRNA) and chromatin remodelling. Different epigenetic modifications recruit specific reader proteins that elicit biological responses. Methylated DNA (5mC) reader protein and histone PTM reader are shown as two examples.

islands located in gene bodies tend to become methylated during development depending on the transcriptional activity of their genes (Jeziorska et al., 2017). The aberrant DNA methylation of CpG islands in the promoters of tumour suppressor genes, leading to their inactivation, is a key hallmark of epigenetic dysregulation in cancer cells (Bergman & Cedar, 2013).

How can a small methyl group exert a biological effect? The methyl group of the methylated cytosine is positioned in the major groove of the DNA, where it can be recognised by proteins interacting with DNA, for example, transcription factors. DNA methylation can regulate their binding to the DNA and thereby modulate gene expression (Yin et al., 2017). In addition, DNA methylation can recruit specific methyl reader proteins (Fig. 1) (e.g., so-called methyl-binding domain (MBD) proteins) or additional epigenetic modifiers (e.g. histone-modifying enzymes or chromatin remodelling enzymes), leading to changes in the accessibility of chromatin to the transcriptional machinery. The exact mechanism by which DNA methylation affects chromatin structure is not fully understood, but it is known that methylated DNA is closely associated with a closed, relatively inactive chromatin structure (Keshet et al., 1986). As DNA methylation adds additional information to the genetic code that is not encoded in the DNA sequence itself and is heritable through cell divisions, the 5mC is sometimes referred to as the "5$^{th}$ letter of the genetic alphabet". In addition to the 5-methylcytosine, 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (Fig. 2), which arise in the process of active DNA demethylation, are present in the human DNA, further extending the (epi)genetic alphabet.

In the early 1980s, global loss of DNA methylation (DNA hypomethylation) was reported in cancer cells (Feinberg & Vogelstein, 1983; Gama-Sosa et al., 1983). This finding was followed by the identification of the aberrant gain of methylation (DNA hypermethylation) in the promoters of tumour suppressor genes, leading to their inactivation in cancer (Baylin et al., 1986; Greger et al., 1989). These seminal discoveries exemplified DNA methylation changes characteristic of most cancer cells and demonstrated the fundamental role of DNA methylation in disease development and progression (Bergman & Cedar, 2013). Since these initial discoveries, the number of diseases associated with altered DNA methylation signatures in different organs has been constantly growing (Bergman & Cedar, 2013; R. Liu et al., 2023; Michalak et al., 2019; Zhao et al., 2021).

### *2.1. Molecular machinery setting and erasing DNA methylation*

The methyl groups are added to the DNA by specific enzymes called DNA methyltransferases (MTases or DNMTs) and can be removed by the Ten-Eleven-Translocation (TET) DNA demethylases (Jurkowska & Jeltsch, 2022a; Jurkowska & Jurkowski, 2019; Ravichandran et al., 2018). Three active MTases (DNMT1, DNMT3A and DNMT3B) and three TET enzymes (TET1, TET2 and TET3) exist in humans (and other mammals). DNMT3A and DNMT3B proteins (with the help of a stimulatory factor DNMT3L) introduce DNA methylation patterns during early embryonic development and gametogenesis (Fig. 3). They are highly expressed in undifferentiated cells and germ cells, and present at much lower levels in somatic differentiated cells. After establishment, patterns of DNA methylation are mostly preserved, with only small tissue-specific changes. However, they can get significantly altered in response to environmental exposures and in diseases (as discussed below).

DNA methylation information consisting of methylated and unmethylated CpG sites is preserved over rounds of cell divisions by a well-designed maintenance mechanism, which enables the function of DNA methylation as a key epigenetic mechanism mediating long-term gene repression. As CpG sites are symmetric and usually methylated in both strands of DNA, the methylation signal is present in two DNA

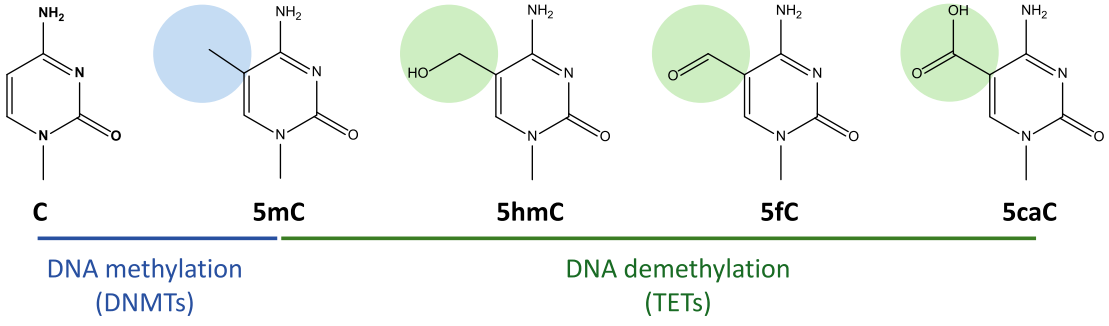


**Fig. 2.** Cytosine modifications present in human DNA.
Schematic representation of the unmethylated cytosine (C), methylated cytosine (5mC), as well as its oxidised forms 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), generated by the TET enzymes. Parts of the figure were adapted from (Ravichandran et al., 2018) with permission from the Royal Society of Chemistry.

strands. The process of DNA replication generates a strand of unmethylated DNA, converting fully methylated CpGs into hemimethylated sites (with one strand unmethylated and one methylated) (Fig. 3). These sites are then re-methylated by a maintenance methyltransferase enzyme (DNMT1), a molecular copy machine, which is highly expressed in proliferating cells, localizes to the replication forks and has a high preference towards hemimethylated DNA (Jeltsch & Jurkowska, 2014; Petryk et al., 2021). The recruitment of DNMT1 to hemimethylated DNA during replication is facilitated by a chromatin factor, UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1), a key component of the DNA maintenance machinery and regulator of the DNMT1 enzyme (Bostick et al., 2007).

DNA methylation was once believed to be an irreversible epigenetic signal. Despite the existence of a specific maintenance mechanism, DNA methylation can be lost by either a passive mechanism, when the maintenance MTase activity is absent over consecutive cycles of cell divisions or via an active DNA demethylation process (Fig. 3). Active DNA demethylation involves stepwise oxidation of 5mC to 5hmC, 5fC and 5caC by TET enzymes (Ito et al., 2011) (Fig. 2). The oxidised bases are then removed by the Thymine DNA glycosylase (TDG) and the base excision repair (BER) mechanism, restoring DNA to the unmethylated state (Jurkowska & Jurkowski, 2019; Ravichandran et al., 2018; X. Wu & Zhang, 2017). TET1 and TET2 are expressed in multiple tissues, including embryonic stem cells, but are generally downregulated during differentiation, whereas TET3 is mostly present in oocytes, the zygote, and neurons [reviewed in (Jurkowska & Jurkowski, 2019; X. Wu & Zhang, 2017)].

Hence, the patterns of DNA methylation in living cells are shaped by the combined action of DNA methyltransferases and TET demethylases and their targeting, localization and activity need to be precisely controlled.

### 2.2. Domain structure of human methyltransferase and demethylases

Human DNA methyltransferases and demethylases are all large, multi-domain proteins. They contain a large regulatory N-terminal part and a smaller C-terminal part involved in catalysis [reviewed in (Jurkowska & Jeltsch, 2022a)]. The N-terminal part contains several domains that mediate the localization of the enzymes to the cell nucleus and regulate their interaction with other proteins, chromatin and DNA. The C-terminal domain of DNMTs is required for the binding of the methylation cofactor (S-adenosyl-L-methionine, SAM), recognition of the DNA and catalysis. The catalytic core of TET enzymes contains a conserved double-stranded β-helix (DSBH) domain, a cysteine-rich domain, and binding sites for the substrate DNA, as well as iron (Fe 2+) and 2-oxoglutarate (2-OG), two key cofactors required for catalysis (Ravichandran et al., 2018).

Notably, the spatial arrangement of the various domains in DNMTs plays a crucial role in the regulation of their function, providing an elegant mechanism for direct control of the enzymes' activity and function in cells [reviewed in (Jeltsch & Jurkowska, 2016)]. Interaction with protein partners (e.g. stimulatory factor DNMT3L), other chromatin modifications (e.g. specific PTMs on histone tails) or modifications of the linkers between various domains can lead to rearrangements of the DNMTs domains leading to their allosteric activation or inhibition (Bashtrykov et al., 2014; Rajavelu et al., 2018).

Unlike most transcription factors, DNMTs and TET enzymes do not display strict sequence specificity beyond the preference for the CpG sites and can modify CpG sites in a variety of sequence contexts. However, they are sensitive to the sequences flanking their target sites (Adam et al., 2022; Handa & Jeltsch, 2005; Jurkowska, Siddique, et al., 2011) and can display up to 250-fold preference for the most favorable sites (Ravichandran et al., 2022). In addition, DNMT3A and DNMT3B form heterotetrameric complexes with a stimulatory factor DNMT3L (Jia et al., 2007; Gao et al., 2020). The arrangement in the complex positions the active sites at a specific distance, allowing simultaneous methylation of the CpGs sites separated by 8–10 bps (Jurkowska et al., 2008, 2011). These intrinsic biochemical properties of DNMTs and TETs may contribute to DNA target selection and the establishment of DNA methylation patterns in development but also in disease. Indeed, the imprints of these properties have been observed in genome-wide DNA methylation studies (Jeltsch & Jurkowska, 2013; Ravichandran et al., 2022).

Despite significant progress in understanding the biochemical properties and the mechanism of the human DNA methyltransferases and demethylases, their genomic targeting as well as their regulation in cells is not well understood. Even less is known about the expression and regulation of DNMTs and TET enzymes in healthy and diseased lungs. This is a significant research gap that should be addressed, as the number of lung diseases with altered methylation signatures is

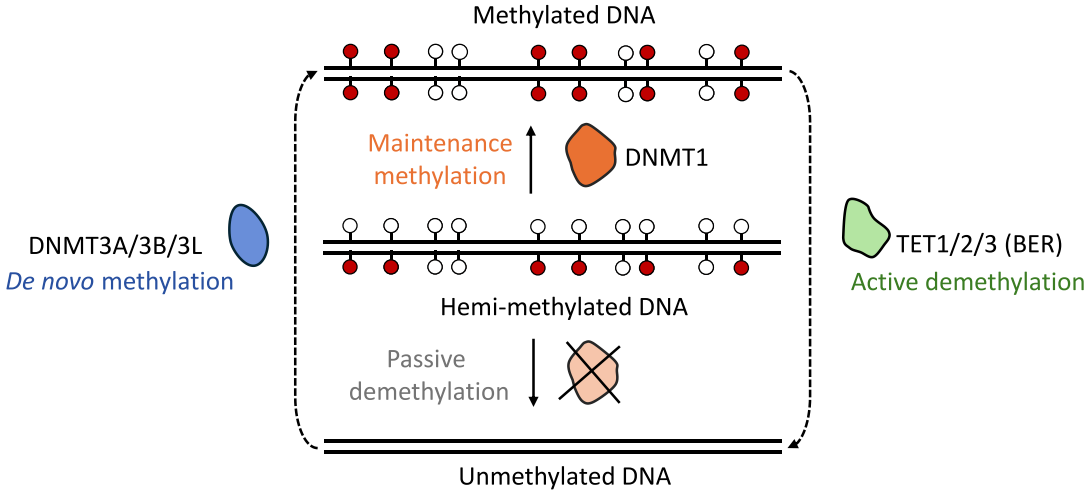


**Fig. 3.** DNA methylation cycle: establishment, maintenance and removal of DNA methylation.
DNA methylation pattern is generated on unmethylated cytosines by DNMT3A and DNMT3B with the help of the stimulatory factor DNMT3L during the development and establishment of germ cells (blue). It is maintained during DNA replication by a maintenance enzyme DNMT1 (orange), which recognises hemimethylated DNA, consisting of a methylated parental strand (red lollipops) and an unmethylated daughter strand (white lollipops) and returns it to its original pattern. DNA methylation can be lost by a passive mechanism when DNMT1 activity is absent. It can also be actively removed by subsequent oxidation of 5mC to 5hmC, 5fC and 5caC by TET enzymes, followed by the removal of 5fC and 5caC by TDG and base excision repair (BER) enzymes, leading to the restoration of unmethylated DNA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

constantly growing and epigenetic enzymes and pathways constitute attractive druggable targets for clinical applications (as described below).

## 3. Histone posttranslational modifications – a sophisticated code for gene regulation

In addition to the DNA, all four core histone proteins in the nucleosomes (H2A, H2B, H3 and H4) and the linker histone H1 can be subject to posttranslational modifications (PTMs), including acetylation, phosphorylation, methylation, ubiquitination and sumoylation, among others. Less-studied examples of histone PTMs include adenosine diphosphate (ADP) ribosylation, deimination, proline isomerization, crotonylation, propionylation, butyrylation, formylation, hydroxylation and O-GlcNAcylation [for a recent comprehensive review of different PTMs, please refer to (Millán-Zambrano et al., 2022)]. These modifications occur on specific amino acid residues of the histones, mostly on their N-terminal flexible tails that protrude from the nucleosome core (Fig. 4). Histone PTMs are introduced and removed by specific classes of enzymes (writers and erasers, respectively), which often reside in large protein complexes carrying multiple enzymatic activities. The pattern of histone PTMs constitutes a sophisticated molecular code that can be specifically recognised and interpreted by a variety of proteins (so-called epigenetic readers) that influence chromatin structure and thus gene expression (Turner, 1993). In addition to recruiting protein readers, PTMs can also directly modify the physical properties of the histone tails and thereby directly impact chromatin accessibility. Histone acetylation and phosphorylation are examples of PTMs that can directly influence nucleosome packaging through the modification of charges.

Specific patterns of histone modifications have been suggested to provide activating ("ON") or repressing ("OFF") signatures for gene expression. For example, histone acetylation often correlates with transcriptionally active, open chromatin, which is devoid of DNA methylation, whereas deacetylated histones are found in repressed, condensed regions, which also contain DNA methylation (Fig. 5) (Cosgrove et al., 2004). However, the same type of modification (e.g., methylation of lysines) may have opposite effects on gene expression depending on the context, localisation and the exact residue that is modified. For example, trimethylation of lysine 9 of histone H3 (H3K9me3) is associated with repressed chromatin, trimethylation of lysine 4 on H3 (H3K4me3) marks active promoters, while monomethylation of lysine 4 on H3 (H3K4me1) is associated with enhancer elements. The complexity of the histone code is further increased by the crosstalk between different adjacent modifications or even between different histone tails (Millán-Zambrano et al., 2022).

Thanks to the multi-institutional efforts of international epigenomic initiatives (like for example, NIH Roadmap Epigenomics (Bernstein et al., 2010), Encyclopaedia of DNA Elements (ENCODE) project (Consortium, 2004), Blueprint project (Martens & Stunnenberg, 2013) or the International Human Epigenome Consortium (IHEC) (Stunnenberg, et al., 2016)), various histone modifications have been mapped at high resolution across numerous human tissues and cells, providing reference epigenomic maps. These efforts led to the identification of chromatin states characterised by specific types of histones PTMs and provided key evidence of the association of chromatin modifications with the regulation of gene expression. They can be used to define different functional elements of the human genome (e.g. promoters, proximal and distal enhancers, repressed regions etc) and enable the integration of multiple omics data.

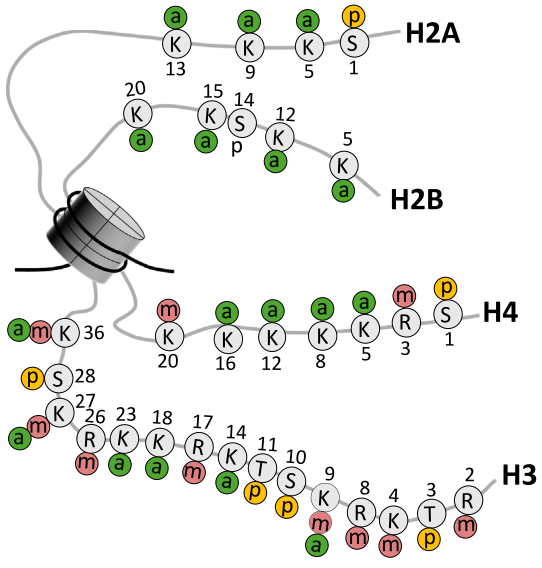


**Fig. 4.** The histone code.
Selected examples of the most common posttranslational modifications of the N-terminal tails of core histones are displayed as green (acetylation), red (methylation) and yellow (phosphorylation) circles. Numerous additional modifications have been described, refer to the text for details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 4. Non-coding RNA

Even though a large fraction of our genetic information is transcribed into RNA, only a low percent of these transcripts encodes actual proteins. The majority of all RNAs are non-coding RNAs (ncRNAs) and their function remains an area of intense research. Non-coding RNAs contribute another key component to epigenetic regulation in living cells [for recent reviews, please refer to (Mattick et al., 2023; Nemeth et al., 2024)]. They are classified by size into long ncRNAs (lncRNAs, >200 bps) and short ncRNAs (<200 bps), and play prominent roles in the regulation of transcription, silencing of transposons, and RNA modification, among others. RNA research is evolving very fast and new classes of ncRNAs, e.g. enhancer RNAs or circular RNAs have been recently identified (Uszczynska-Ratajczak et al., 2018). Long non-coding RNAs play a prominent role in the recruitment of various chromatin factors to their target sites, leading to the establishment of silenced or active chromatin domains (Fig. 1). The most remarkable example, where a single lncRNA can trigger epigenetic silencing of an entire chromosome is the X inactive-specific transcript (XIST) RNA, which is critical for the inactivation of X-chromosome and dosage compensation in females (Loda & Heard, 2019). The best-studied examples of small ncRNAs are micro RNAs (miRNAs), which are endogenous small (between 19 and 22 nucleotides in length) ncRNAs. They play important roles in the regulation of gene expression by controlling mRNA translation (Nemeth et al., 2024).

## 5. Reader domains confer biological functions of epigenetic modifications

One of the interesting questions that has been keeping the epigenetic field busy is how the message encoded in the pattern of modifications of DNA and histones is read by the cellular machinery to exert biological functions. This interest led to the discovery of the so-called epigenetic readers, protein domains that can specifically recognise and bind to defined epigenetic modifications and trigger downstream signalling (Fig. 1). Bromodomains, which recognise acetylated lysines and chromodomains, which bind to methylated lysines are two best-known examples of such reading domains in humans (Franklin et al., 2022). In addition, the methylated cytosines on DNA can also be recognised by the methyl-binding domain (MBDs) proteins that transmit the downstream signalling generally associated with transcriptional repression (Fig. 1). In addition to the epigenetic enzymes that set the

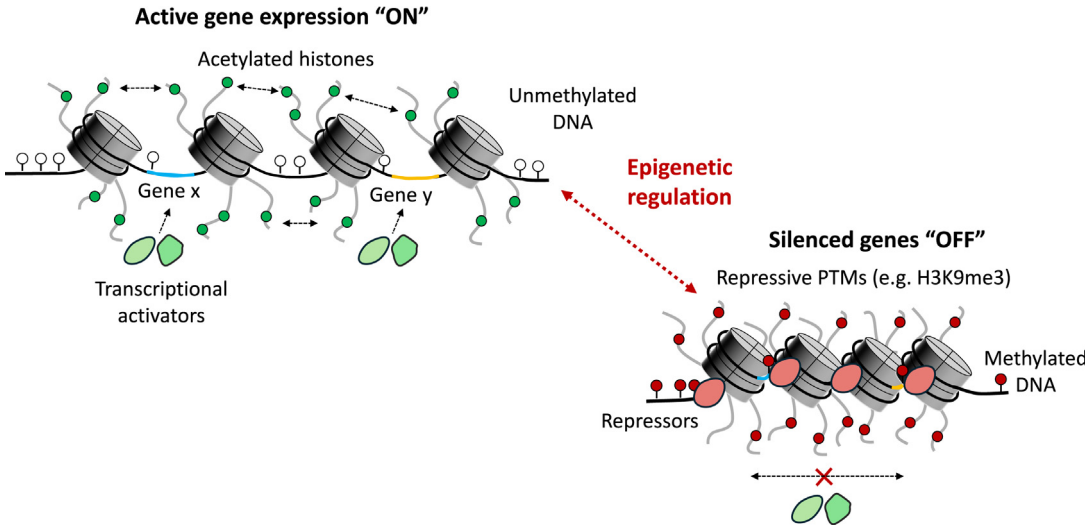


**Fig. 5.** Basic principles of epigenetic regulation of gene expression.
Active chromatin is open, decorated with activating epigenetic marks like acetylated histones, is usually devoid of DNA methylation and is permissive for binding of transcriptional activators that enable active gene expression ("ON"). Silenced chromatin is characterised by deacetylated histones, enriched with repressive histone modifications and DNA methylation, which lead to binding of repressor complexes and chromatin compaction. Compacted chromatin prevents the binding of transcriptional activators to the regulatory elements and contributes to gene repression ("OFF").

epigenetic mark (writers) and remove it (erasers), epigenetic readers are also an attractive class of druggable targets for clinical applications (as discussed below).

## 6. Methods for genome-wide mapping of DNA methylation

Because epigenetic mechanisms, like DNA methylation, provide an interface between environmental factors and the genome, the methylation pattern of a cell or tissue can also be considered a cellular memory of past exposures. This is particularly relevant to the human lung, which is constantly exposed to environmental insults. Hence, mapping DNA methylation (and other epigenetic modification) changes across the course of a disease may lead to the identification of molecular pathways driving its onset and progression. In addition to driving disease development, DNA methylation can serve as attractive disease biomarkers, due to their early onset, disease specificity, and stability (Costa-Pinheiro et al., 2015) and can be used to develop DNA methylation-based predictors of health and disease (Yousefi et al., 2022). With the growing recognition of the importance of DNA methylation in regulating human health, arose a need for the development of robust methods for the detection of DNA methylation in a genome-wide manner.

The most popular use of DNA methylation in epidemiology is in the epigenome-wide association studies (EWAS), which are population-based studies that analyse the association between the levels of DNA methylation (or any other epigenetic modification) and a specific exposure (for example, cigarette smoking or air pollution), disease or phenotype (Birney et al., 2016).

Several methods can be employed to measure DNA methylation at thousands or millions of CpG sites simultaneously [for a general review on methods of DNA methylation, refer to (Laird, 2010)]. Genome-wide interrogation of DNA methylation at single CpG sites can be performed via array technologies or by harnessing the power of next-generation sequencing (NGS) (Fig. 6). The gold standard in the field relies on the chemical treatment of the DNA with sodium bisulfite which enables the differentiation of unmethylated from methylated cytosines. This is possible because, upon bisulfite treatment, unmethylated cytosines are converted to uracils, whereas methylated cytosines are protected (Fig. 6). The bisulfite-based methods offer single-nucleotide resolution and require low input of DNA, making them applicable to small amounts of human material. Of note, classical bisulfite sequencing cannot distinguish between 5mC and 5hmC, therefore, data reported as DNA methylation will likely be a mix of DNA methylation and hydroxymethylation. This is important, as emerging research indicates that 5hmC is not just intermediate in DNA demethylation, but a novel epigenetic modification with regulatory functions on its own (Bachman et al., 2014). Several new techniques have now been developed to differentiate between the two epigenetic states and allow reliable mapping of all four modified cytosine bases (5mC, 5hmC, 5fC and 5caC) in the human DNA (Liu et al., 2021; Liu et al., 2019; Schutsky et al., 2018; Vaisvila et al., 2021; Yu et al., 2012).

### *6.1. DNA methylation arrays*

Due to their robust performance, cost-effectiveness, scalability and user-friendly data analysis, Illumina DNA methylation arrays have been widely used to investigate DNA methylation patterns in large cohorts in disease research and are a method of choice for the EWAS in epidemiology. Different arrays have been developed in the past 15 years, the most well-known are the human BeadChip arrays which are based on Illumina's Infinium technology. They enable the hybridization of DNA to specific probes immobilised on beads (hence the name BeadChip) that distinguish methylated and unmethylated sequenced based on their differential sequence after bisulfite treatment. They have evolved over several generations, starting from 27 K (Bibikova et al., 2009), 450 K (Bibikova et al., 2011), EPIC (Pidsley et al., 2016), to the most recent EPIC version 2 (EPICv2) (D. Kaur et al., 2023). Each generation extended the array's coverage of the human genome and showed improved probe design.

The first one, the 27 K BeadChip array (Bibikova et al., 2009) included around 27,000 probes covering CpG sites located within promoter regions, focusing on genes implicated in cancer. Infinium HumanMethylation450 Beadchip (also known as the 450 K array) which measures over 450,000 CpG sites, included representation of gene bodies (Bibikova et al., 2011), offering greatly improved genomic coverage. The EPIC array (also known as the 850 K or EPICv1), released in 2015, expanded the coverage of cis-regulatory elements identified by the ENCODE (Consortium, 2012) and FANTOM5 (Lizio et al., 2015) projects, enabling the analysis of numerous enhancers. The latest addition, the Infinium BeadChip EPICv2, with further expanded genomic coverage (>935,000 CpG sites), was recently launched by Illumina.

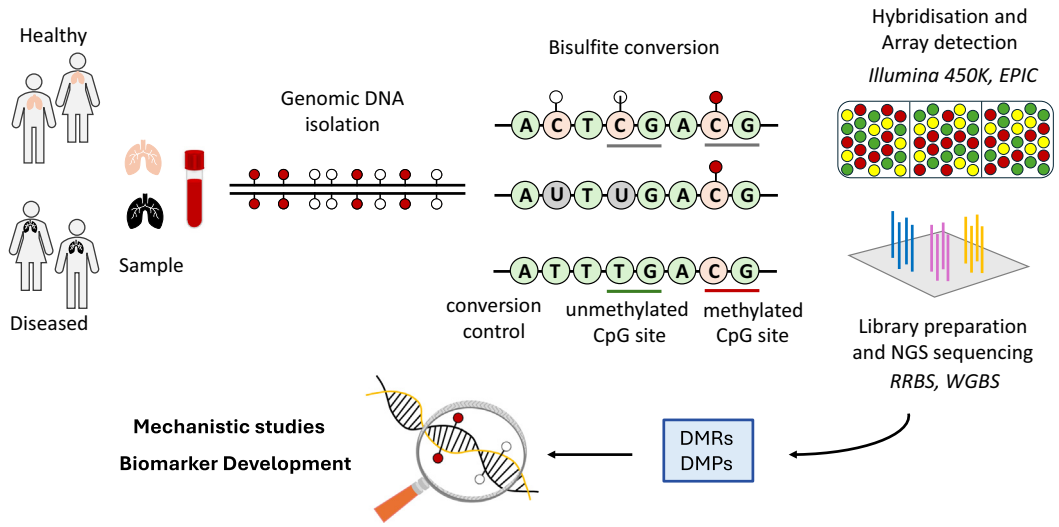


**Fig. 6.** DNA methylation profiling using bisulfite-based methods.
Bisulfite conversion of the isolated DNA from biomaterials enables the analysis of DNA methylation in each CpG site, as it allows differentiation of methylated and unmethylated cytosines based on their differential modification upon treatment. Unmethylated cytosines are deaminated to uracils, which get substituted with thymines in the subsequent PCR step. Methylated cytosines are protected from deamination and remain as cytosines. Converted DNA can then be hybridised to a set of probes on an array (e.g on Illumina Epic array) or used for next-generation sequencing library preparation, followed by sequencing (RRBS, WGBS). As a result, differentially methylated probes (DMPs) or differentially methylated regions (DMRs) can be identified and used in biomarker development or further characterised in mechanistic studies.

The versality of the DNA methylation arrays is demonstrated by their compatibility with formalin-fixed paraffin-embedded (FFPE) samples (Moran et al., 2014), enabling the analysis of DNA methylation patterns from the archived collections. It is further enhanced by the ability to examine other cytosine modifications, like the recently identified 5-hydroxymethylcytosine mark (Stewart et al., 2015). However, despite increased overall coverage, due to the cell type specificity of the distal regulatory elements (like enhancers), their coverage for any cell type on the arrays remains limited. Recently, some concerns have been raised regarding low concordance across 450 K and EPIC platforms in different tissues (Olstad et al., 2022; Sugden et al., 2020; Zhuang et al., 2024), making comparing data and replication challenging and illustrating a need for better cross-platform translatability. Measuring other epigenetic marks, such as histone modifications in hundreds of clinical samples, with a high degree of accuracy is not yet possible and necessitates further technological developments.

### 6.2. Genome-wide approaches

While cost-effective and thus applicable to larger cohorts, DNA methylation arrays only allow interrogation of the selected parts of the genome, covering less than <1 million out of the 28 million of the CpG sites present in the human genome. Whole genome bisulfite sequencing (WGBS) offers the most comprehensive coverage. It harnesses the power of next-generation sequencing, leveraging single-nucleotide resolution with genome-wide coverage (Singer, 2019) (Fig. 6). Its higher sequencing cost makes it more suitable for smaller-scale discovery research, complementing array-based larger-cohort initiatives. Alternatively, reduced-representation bisulfite sequencing (RRBS), which includes enrichment of regulatory regions, combines very good coverage (around 2 million CpG sites) with lower sequencing costs (Meissner et al., 2005). It uses the digestion of genomic DNA with restriction enzymes followed by size selection of the DNA to focus the analysis on the genomic regions containing CpG dinucleotides, which are the main targets of DNA methylation.

Sequencing-based approaches have an additional advantage over arrays, as they provide a binary read-out of the methylation state of individual CpG sites (which can be either methylated or unmethylated) that is easier to interpret than probe signal intensities of the DNA methylation arrays. With the lowering cost of sequencing, they are predicted to replace array technologies in the future. The development of reliable methods for quantification of DNA methylation on a genome-wide scale has enabled fine mapping of DNA methylation dysregulation across various lung diseases, empowering investigation of epigenetic mechanisms and future biomarker development (Fig. 6).

## 7. Epigenetic dysregulation in chronic lung diseases

As chronic lung conditions are largely caused by environmental exposures (in genetically susceptible individuals), which are known to remodel the epigenetic landscape of cells; it is not surprising that genome-wide aberrant DNA methylation changes have been identified in response to smoking, air pollution and across a spectrum of chronic lung diseases.

### 7.1. Cigarette smoking profoundly remodels the epigenome of cells

Cigarette smoking is the best-described example of an environmental exposure with a well-established massive impact on the epigenetic landscape in humans. Selected examples are discussed below, but the reader is encouraged to refer to (Gao et al., 2015; Kaur et al., 2019; Silva & Kamens, 2021; Zong et al., 2019) for recent systematic reviews on the association of smoking exposure with epigenetic changes.

Smoking is a major cause of premature deaths worldwide (Ezzati & Lopez, 2003) and a risk factor for the development of several human diseases, including chronic obstructive pulmonary disease (COPD), idiopathic pulmonary disease (IPF), several cancers (including lung cancer), cardiovascular disease, osteoporosis, and others (Prevention, 2014; Wiklund et al., 2019). Notably, even after smoking cessation, the risk for some diseases persists, indicating the existence of a cellular memory of smoking exposure. Due to its biochemical stability and heritability through cellular divisions, DNA methylation emerged as one of the mechanisms that may confer this long-term risk. Correspondingly, several studies indicated that DNA methylation changes from prenatal exposure to maternal smoking may persist into adulthood (Hoang et al., 2024; Richmond et al., 2015) and confer future risk of disease (Wiklund et al., 2019).

Multiple studies consistently demonstrated genome-wide alterations in DNA methylation in smokers (Table 1), indicating a broad remodelling of the epigenome in response to cigarette smoke exposure in the blood (Chen et al., 2013; Shenker et al., 2013; Zeilinger et al., 2013), lung tissue (Bosse et al., 2012; Seiler et al., 2020), sputum (Belinsky et al., 2002), buccal mucosa (Wan et al., 2015) and airway epithelium (Buro-Auriemma et al., 2013). In addition, statistically robust CpG sites associated with various smoking-related parameters, comprising smoking status (current, never, or former) (Joehanes et al., 2016), time since smoking cessation (Zeilinger et al., 2013), as well as cumulative exposures (Guida et al., 2015; McCartney, Stevenson, et al., 2018) were identified. These studies led to the development of DNA methylation-based predictors for smoking behaviours (Bollepalli et al., 2019; Maas et al., 2019; McCartney, Hillary, et al., 2018; McCartney, Stevenson, et al., 2018), which may prove an attractive alternative to self-reported smoking or current smoking biomarkers (e.g., serum cotinine measurements) in clinical practice (Bojesen et al., 2017). These studies exemplify the usefulness of DNA methylation as a biomarker of exposure.

One of the largest EWAS in adults on cigarette smoking to date included 15,907 participants from 16 different cohorts. Using Illumina 450 K BeadChip array, the authors identified differential DNA methylation between current and non-smokers at more than 2500 CpG sites mapped to 1405 genes (Joehanes et al., 2016), highlighting the profound impact of smoking on the epigenetic landscape of blood cells. Notably, genes with smoke-induced DNA methylation changes were also enriched for genes associated with smoking-related diseases, like COPD or cardiovascular disease in the genome-wide association studies (GWAS). The enrichment of sites with altered DNA methylation in gene regulatory regions, like island shores, gene bodies, and enhancers suggested that smoking-associated changes may impact gene expression. However, this association was not functionally investigated (Joehanes et al., 2016).

A very recent large (5 cohorts, 15,014 participants) meta-analysis of smoking using a more comprehensive Illumina EPIC array replicated many of the previously reported associations and identified an additional 1405 genes with methylation changes in CpG sites not covered by the 450 K array (Hoang et al., 2024).

#### *7.1.1. The curious case of the AHRR gene*

The most prominent and best-characterised example of smoking-associated DNA methylation alterations occurs in the human AHRR (aryl hydrocarbon receptor (AHR) repressor) gene. AHRR is a transcription factor repressing the aryl hydrocarbon receptor-dependent gene expression. The AHR signalling cascade, which mediates dioxin toxicity, is involved in the detoxification of compounds from tobacco smoke (like polyaromatic hydrocarbons) and is involved in the regulation of cell growth, apoptosis and differentiation and the modulation of the immune system (Vogel & Haarmann-Stemmann, 2017).

In the AHRR gene, more than 100 differentially methylated sites associated with various smoking-related parameters were identified (Silva & Kamens, 2021; Zeilinger et al., 2013). Several were robustly replicated in multiple studies (Bojesen et al., 2017; Philibert et al., 2012), making AHRR the most popular biomarker of smoking habits (Maas et al., 2019). Among the most studied sites, the CpG cg05575921 (annotation on the 450 K array) located in an intronic enhancer of the gene displayed the highest methylation change (Joehanes et al., 2016; Zeilinger et al., 2013). Decrease in methylation (hypomethylation) of this site was associated with higher levels of smoking in several studies across European, African and Asian ancestries, both sexes and a range of ages (Dogan et al., 2014; Philibert et al., 2013; Shenker et al., 2013; Zeilinger et al., 2013), as well as maternal smoking during pregnancy

**Table 1**
Examples of genome-wide methylation studies associated with cigarette smoking.

| Study | Material | Sample number | Sample type | Technology | Gene Expression | PMID |
|---|---|---|---|---|---|---|
| Zeilinger et al., 2013 | whole blood | discovery 1793, replication 479 | current, former and never smokers | Illumina 450 K Array | no | 23691101 |
| Guida et al., 2015 | peripheral white blood cells (buffy coats) | 745 | current, former and never smokers women, two independent European populations | Illumina 450 K Array | integrated with published data | 25556184 |
| Joubert et al., 2012 | cord blood | 1062 | newborns (maternal smoking) | Illumina 450 K Array | no | 22851337 |
| Shenker et al., 2013 | peripheral white blood cells, with some targets validated in lung tissue | 554 | current, former and never smokers, healthy individuals who subsequently developed breast or colon cancer and matched controls | Illumina 450 K Array | qRT-PCR for some target genes | 23175441 |
| Keshawarz et al., 2022 | whole blood | 421 | current smokers, interim quitters, former and never smokers, longitudinal | Illumina 450 K Array | Affymetrix Human Exon 1.0 ST microarrays | 34570667 |
| Joehanes et al., 2016 | whole blood, CD4+ T cells, monocytes | 15,907 (16 cohorts) | current, former and never smokers | Illumina 450 K Array | integrated with published data | 27651444 |
| Hoang et al., 2024 | whole blood | 15,014 (5 cohorts) | current, former and never smokers | Illumina EPIC Array | integrated with published data | 38199042 |
| Monick et al., 2012 | lymphoblast cell lines, alveolar macrophages from BAL | 119 (lymphoblasts), 19 (alveolar macrophages) | current and never smokers | Illumina 450 K Array | qRT-PCR for AHRR | 22232023 |
| Ringh et al., 2019 | BAL | 49 | smokers and never smokers | Illumina EPIC Array | RNA sequencing | 31303497 |

| column | description |
|---|---|
| Study | reference to the original publication |
| Material | type of material for DNA methylation analysis; BAL: bronchoalveolar lavage |
| Sample number | total number of samples used for the analysis |
| Donor type | characteristics of donors used in the analysisis |
| Technology | methodology used for DNA methylation analysis |
| Gene expression | indicates if the study associated DNA methylation with gene expression changes |
| PMID | PMID reference for the original publication |

(Joubert et al., 2012). It was suggested that AHRR (cg05575921) hypomethylation may also provide a clinically relevant prediction of future smoking-related morbidity and mortality (Bojesen et al., 2017). The exact mechanism of how changes in AHRR DNA methylation regulate its expression is not known, but the decreased DNA methylation may mediate upregulation of the gene, reported in current smokers compared to non-smokers (Chatziioannou et al., 2017; Parker et al., 2017).

One of the most fascinating aspects of smoking-induced DNA methylation changes is their varied reversibility upon smoking cessation. While the great majority of DNA methylation signatures in the blood seem to revert to the levels observed in non-smokers within 5 years of smoking cessation, some changes persist for more than 30 years (Guida et al., 2015; Hoang et al., 2024; Joehanes et al., 2016). For example, hypomethylation of the AHRR (cg05575921) reverts slowly after smoking cessation, with 22 years needed to reach the non-smoking levels (Ambatipudi et al., 2016). Across different EWAS studies, 4267 unique CpGs were significantly associated with smoking cessation [see (Fang et al., 2023) for a recent systematic review of epigenetic biomarkers of smoking cessation]. Examples of genes linked to CpG sites with persisting smoke-related changes include AHRR, TIAM2, PRRT1, F2RL3, GNG12, LRRN3 and APBA2 (Fang et al., 2023; Hoang et al., 2024; Joehanes et al., 2016). The mechanisms behind the persistence of methylation changes are not fully clear, smoking-induced alterations in haematopoietic stem cells of the bone marrow were suggested as a potential explanation (Guida et al., 2015).

Of note, it is not fully clear whether the "reversibility" of specific sites is caused by the loss of an acquired DNA methylation change, the depletion of a specific cell type (and its methylation signature) from the investigated tissue or both. As most of the smoking EWAS were performed in mixed material (blood or tissue), there are two possible explanations for the observed results: 1) the smoke-related DNA methylation change is a true epigenetic remodelling event, where exposure to cigarette smoke leads to an acquired change in the DNA methylation pattern in the exposed cells or 2) the detected DNA methylation change is a reflection of the changed cellular composition of the studied tissue upon cigarette smoke exposure (e.g., reflecting the influx of inflammatory cells). Consequently, the reversibility of DNA methylation may be a true loss of an acquired methylation change or may reflect the depletion of a cellular component with a characteristic methylation at a given locus (e.g., less inflammatory cells present in blood or lung tissue upon smoking cessation). Hence, adjusting for changes in cell-type composition in complex tissues is critical when analysing and interpreting findings from EWAS, as DNA methylation change in bulk tissues often manifests as an inflated signal due to a shift in cell-type proportions between cases and controls (Houseman et al., 2015; Teschendorff & Zheng, 2017).

Analysis of primary bronchial epithelial cells from never, current, and former smokers revealed that most tobacco smoke-driven gene expression changes were rapidly reversible (Beane et al., 2007). Interestingly, a subset of genes displayed only slow or even persistent changes, mirroring the varied reversibility patterns in DNA methylation observed in earlier population studies. In vitro models of cigarette smoke exposure may help provide mechanistic insights into the kinetics and mechanisms of the reversibility of smoke-related epigenetic and transcriptional changes.

The key limitation of the EWAS studies is that they provide only correlative data, and do not allow the establishment of the causal link between smoke exposure, altered DNA methylation and changed gene expression. For this, functional studies are needed. In addition, longitudinal data quantitatively measuring DNA methylation after smoking cessation over time are still very limited, as most studies performed to date are cross-cohort studies associating DNA methylation with time since quitting. Carefully designed longitudinal studies are needed to validate these results and uncover the details of the different reversal kinetics among smoking-associated sites.

### *7.2. Remodelling of DNA methylation in chronic obstructive pulmonary disease (COPD)*

Chronic obstructive pulmonary disease (COPD) is a prevalent chronic lung disease, affecting more than 250 million people worldwide, caused by exposure to cigarette smoke and other toxic gases. It is characterised by a progressive airflow obstruction caused by the remodelling of small airways and destruction of the lung parenchyma, known as emphysema (Barnes et al., 2015; GOLD, 2023). Numerous genetic loci have been associated with COPD and lung function (Cho et al., 2014; Hancock et al., 2010; Heinbockel et al., 2018; Hobbs et al., 2017; Sakornsakolpat et al., 2019; Wain et al., 2015; Wyss et al., 2018), however, they explain only a small fraction of the COPD risk. Current studies estimate that the genetic component of COPD is <3 % (for monogenic risk) and combined polygenic risk scores may explain up to 38 % of COPD susceptibility (Ragland et al., 2019). Notably, the environmental cause of COPD (cigarette smoke) and the disease phenotypes, including aberrant cell differentiation (e.g., Goblet cell metaplasia) indicate the involvement of epigenetic mechanisms in COPD development. Indeed, numerous studies provided strong evidence for the association of dysregulated DNA methylation and COPD (Table 2) in the blood (Bermingham et al., 2019; Busch et al., 2016; Carmona et al., 2018; Qiu et al., 2012), sputum (Sood et al., 2010), oral mucosa (Wan et al., 2015), lung tissue (Morrow et al., 2016; Sood et al., 2010; Sundar et al., 2017; Yoo et al., 2015), bronchial brushings (Vucic et al., 2014) and isolated lung fibroblasts (Clifford et al., 2018; Schwartz et al., 2023). Importantly, DNA methylation changes were associated with altered expression of genes and pathways important to COPD pathology, indicating their potential role in driving aberrant gene expression programs in COPD cells (Llamazares Prada et al., 2023; Schwartz et al., 2023). In addition, a recent study suggested that DNA methylation changes may originate in early life (Kachroo et al., 2020), further emphasising the potential role of DNA methylation in conferring long-term risk for chronic lung disease development.

Most studies profiled DNA methylation using complex material with mixed cell populations and thus could not identify the cellular origin contributing to the observed DNA methylation and expression changes in COPD. Hence, the precise epigenetic changes in COPD driving cell populations and their contribution to altered transcriptional patterns in COPD are still not well understood. Only three studies to date investigated DNA methylation changes in COPD patients in isolated lung cells (Clifford et al., 2018; Llamazares Prada et al., 2023; Schwartz et al., 2023). Using Illumina 450 K BeadChip Array, Clifford et al. identified 887 and 44 differentially methylated regions in parenchymal and airway fibroblasts of COPD patients, respectively (Clifford et al., 2018). Our group has pioneered the use of high-resolution profiling to examine the DNA methylation landscape in COPD in purified cells. Using whole genome bisulfite sequencing, a genome-wide approach, we identified widespread DNA methylation changes in primary lung fibroblasts (Schwartz et al., 2023) and alveolar type 2 cells (Llamazares Prada et al., 2023) isolated from a small cohort of COPD patients. DNA methylation changes were associated with dysregulated expression of key pathways involved in COPD pathology, like proliferation, inflammation, transforming growth factor β (TGFβ) and Wnt signalling, indicating that they may contribute to disease development. Using epigenetic editing, we provided the first evidence of the functional role of aberrant DNA methylation in driving interferon signalling in COPD (Llamazares Prada et al., 2023), highlighting the potential of cell-type resolved epigenetic profiling in identifying epigenetic drivers of disease phenotypes.

Little is known about the correlation of DNA methylation with disease severity, trajectories, or progression. DNA methylation changes in 13 genes have been identified in the lung tissue of COPD GOLD I and II patients compared to controls (Casas-Recasens et al., 2021). WGBS data from our group revealed that genome-wide DNA methylation changes are present in lung fibroblasts from COPD GOLD I patients (mild COPD) compared to controls with matched smoking status and

**Table 2**
Examples of genome-wide methylation studies associated with COPD.

| Study | Material | Sample number | Sample type | Technology | Gene Expression | PMID |
|---|---|---|---|---|---|---|
| Bermingham et al., 2019 | blood | 3193 | COPD and controls | Illumina EPIC Array | no | 30935889 |
| Wan et al., 2015 | oral mucosa/buccal brushes | 82 | COPD and controls | Illumina 450 K Array | no | 25517428 |
| Morrow et al., 2016 | lung tissue | 157 | former smokers with COPD and controls | Illumina 450 K Array | HumanHT-12 BeadChips | 27564456 |
| Sundar et al., 2017 | lung tissue | 24 | non-smokers, smokers, smokers with COPD | Illumina 450 K Array | no | 32733890 |
| Casas-Recasens et al., 2021 | lung tissue | 189 | non-smokers, current smokers, current/former smokers | Illumina EPIC Array | no | 32822219 |
| Hobbs et al., 2023 | lung tissue | 78 | ex-smokers, ex-smokers with severe | Illumina 450 K Array | Illumina HT-12 Array | 37041558 |
| Clifford et al., 2018 | airway and parenchymal fibroblasts | 15 (airway), 46 (parenchymal) | COPD and controls | Illumina 450 K Array | RT-qPCR on selected genes | 29527240 |
| Schwartz et al., 2023 | parenchymal fibroblasts | 11 | ex-smokers, ex-smokers with COPD I and COPDII-IV | WGBS | RNA sequencing | 37143403 |
| Llamazares Prada et al., 2023 | alveolar type II cells | 11 | ex-smokers, ex-smokers with COPD I and COPDII-IV | WGBS | RNA sequencing | bioRxiv |

| column | description |
|---|---|
| Study | reference to the original publication |
| Material | type of material for DNA methylation analysis; BAL: bronchoalveolar lavage |
| Sample number | total number of samples used for the analysis |
| Sample type | characteristics of donors used in the analysisis |
| Technology | methodology used for DNA methylation analysis |
| Gene expression | indicates if the study associated DNA methylation with gene expression changes |
| PMID | PMID reference for the original publication |

history (Schwartz et al., 2023), demonstrating that epigenetic changes occur already in mild disease. Some of these changes were also present in severe COPD. However, another study found distinct DNA methylation signatures between mild and severe COPD patients, indicating a possible non-linear relation between DNA methylation and disease development (Casas-Recasens et al., 2021).

These initial studies demonstrate that DNA methylation may provide a sensitive biomarker for COPD detection and patient stratification. Carefully designed longitudinal studies in larger cohorts are needed to validate these results obtained with the small number of samples and uncover the details of the epigenetic changes associated with different COPD subtypes and disease trajectories. The combination of epigenetic profiling with other NGS-based omics approaches (e.g. proteomics, metabolomics, transcriptomics, radiomics) holds promise for the identification of COPD subtypes driven by common pathological mechanisms (endotypes) that are key to the development of curative therapies for COPD (Hobbs et al., 2023; Olvera et al., 2024; Polverino & Kalhan, 2023).

### *7.3. Remodelling of DNA methylation patterns in pulmonary fibrosis*

Lung fibrosis is caused by an aberrant lung tissue repair process characterised by excessive deposition of extracellular matrix and proliferation of fibroblasts (Lederer & Martinez, 2018). Among fibrotic lung disease, idiopathic pulmonary disease (IPF) is an incurable lung disease, affecting 5 million people worldwide, with increasing prevalence and healthcare burden (Martinez et al., 2017). It is a devastating disease leading to irreversible scarring of the lung, progressing breathing difficulties, and ultimately death 3–5 years after diagnosis (Lederer & Martinez, 2018; Raghu et al., 2018). Despite the discovery of genes predisposing to IPF (Allen et al., 2017), the molecular mechanisms involved in the initiation, development, and progression of IPF are unknown. Considering that most known risk factors for IPF, including age, cigarette smoke exposure, and male sex are associated with DNA methylation changes, DNA methylation is likely to play a role in IPF development. Changes in DNA methylation have been identified in lung tissue (Rabinovich et al., 2012; Sanders et al., 2012; Yang et al., 2014) and fibroblasts (Huang et al., 2014; Lee et al., 2019) from IPF patients, providing the first evidence of dysregulated epigenetic signalling in IPF. Yet, comprehensive profiling of epigenetic dysregulation in IPF has not been carried out.

Previous gene-specific studies using cellular models or lung tissues of IPF patients identified altered DNA methylation and expression of several genes important to IPF pathology. These include fibrogenic or anti-fibrotic genes, like Thy-1 antigen (THY1), Prostaglandin E receptor 2 (PTGER2), Caveolin 1 (CAV1), α-smooth muscle actin (α-SMA), Phosphatase and tensin homolog (PTEN), chemokine IP-10 and cyclin-dependent kinase 4 inhibitor B (CDKN2B), where a change of DNA methylation was associated with altered gene expression (Huang et al., 2010; Sanders et al., 2008; Sanders et al., 2017; Scruggs et al., 2018).

Several studies investigated DNA methylation changes using more comprehensive approaches (Table 3). Using low-coverage arrays (focusing on promoters), three pioneering studies provided evidence of widespread dysregulation of DNA methylation in IPF lung tissue and its association with gene expression changes (Rabinovich et al., 2012; Sanders et al., 2012; Yang et al., 2014). The most comprehensive of the three examined 4.6 million CpG sites and identified 2130 significant DMRs, 870 of which were associated with differentially expressed genes. (Yang et al., 2014). DNA methylation changes were predominantly located in gene bodies and CpG island shores, with only 10 % located in gene promoters (and outside CpG islands). IPF methylomes also did not exhibit hypomethylation of retrotransposons (Rabinovich et al., 2012), suggesting that methylome features in IPF may differ from typical changes characteristic of cancer cells.

These studies profiled whole lung tissue and hence did not account for epigenetic differences in individual cell types. This was addressed by subsequent studies that identified alterations in DNA methylation in IPF using purified lung cells, including parenchymal fibroblasts (Huang et al., 2014; Lee et al., 2019) and alveolar macrophages (AM) (McErlean et al., 2021). The most recent one profiled AM isolated from bronchioalveolar lavage (BAL) of IPF patients and controls using Illumina EPIC array (McErlean et al., 2021). The authors identified epigenetic heterogeneity as a key feature of AM in IPF, mirroring transcriptional heterogeneity of AM identified in single-cell RNA sequencing studies and confirming earlier results from IPF fibroblasts (Huang et al., 2014). One challenge of DNA methylation studies in complex

**Table 3**
Examples of genome-wide methylation studies associated with lung fibrosis.

| Study | Material | Sample number | Sampl type | Technology | Gene Expression | PMID |
|---|---|---|---|---|---|---|
| Yang et al., 2014 | lung tissue | 158 | IPF and control lungs (adjacent to cancer) | Nimblegen CHARM Array | Agilent expression array | 25333685 |
| Sanders et al., 2012 | lung tissue | 19 | IPF and control lungs (adjacent to cancer) | Illumina 27 K Array | Illumina HT-12 Array | 22700861 |
| Rabinovich et al., 2012 | lung tissue | 32 | 12 IPF lungs, 10 lung adenocarcinomas, 10 normal histology lungs | Agilent human CpG Islands Microarray after immunoprecipitation of methylated DNA | RT-qPCR of selcted genes | 22506007 |
| Huang et al., 2014 | fibroblasts | 12 | 6 IPF, 3 controls, 3 commercial cell lines | Illumina 27 Array | RT-qPCR of selcted genes | 25215577 |
| Lee et al., 2017 (expression) Lee et al., 2019 (methylation) | fibroblasts | 12 | 8 IPF, 4 controls from cancer resections | Illumina 450 K Array | Illumina HT-12 Array | 31305135 28057004 |
| McErlean et al., 2021 | BAL AM | 44 | control and IPF | Illumina EPIC Array | integrated with published data | 34280322 |

| column | description |
|---|---|
| Study | reference to the original publication |
| Material | type of material for DNA methylation analysis; BAL: bronchoalveolar lavage |
| Sample number | total number of samples used for the analysis |
| Sample type | characteristics of donors used in the analysisis |
| Technology | methodology used for DNA methylation analysis |
| Gene expression | indicates if the study associated DNA methylation with gene expression changes |
| PMID | PMID reference for the original publication |

diseases like IPF is their heterogeneous nature, as different stages of the disease might have different epigenetic landscapes and even regions with active disease may differ from the areas with established fibrosis within the same lung. Indeed, hypermethylation of the THY1 promoter was observed in fibroblasts within active fibroblastic foci, but not in dense, fibrotic areas, indicating the temporal and spatial regulation of DNA methylation in IPF (Sanders et al., 2008). In addition, rejected donor lungs are often used as controls for IPF studies as obtaining an age-matched normal cohort is challenging. As these tend to be younger; some of the identified changes may be due to ageing as the DNA methylation landscape changes with age and the chronological age can be quantified by DNA methylation patterns (Horvath & Raj, 2018).

Overall, DNA methylation profiling studies provide strong evidence that widespread alterations in DNA methylation are associated with the dysregulation of genes important in the pathogenesis of IPF in the lung. However, the enrichment of DNA methylation changes outside promoters and CpG islands makes inferring their functional relevance more difficult, as complex interactions exist between epigenetic mechanisms, chromatin structure, nuclear architecture, and gene expression. Functional studies are needed to evaluate the role of identified alterations in disease pathology and to understand the interaction between genetic predisposition and epigenetic regulation in IPF.

### *7.4. Alterations of DNA methylation patterns in asthma*

Asthma is the most common chronic lung disease, affecting around 300 million people worldwide (Porsbjerg et al., 2023). It is characterised by variable respiratory symptoms and reversible airway obstruction. In contrast to COPD and IPF, for which age is a significant risk factor, asthma can affect people of all ages. There is strong evidence indicating that the aetiology of asthma, as well as its clinical course results from complex interactions between host genotype and environmental exposures (Bonnelykke & Ober, 2016; Melen et al., 2022). Early-life exposures, including intrauterine exposure to cigarette smoke, viral infections or maternal diet are known risk factors, highlighting the important role of environmental exposures in asthma pathology. Among chronic lung diseases, asthma is the one with the best-established association with epigenetic dysregulation, as altered DNA methylation patterns were associated with allergy, and atopy in both children and adults.

A few selected examples illustrating the contribution of dysregulated DNA methylation to asthma are discussed below (Table 4), but the reader is encouraged to refer to (Edris et al., 2019; Legaki et al., 2022; Sheikhpour et al., 2021) for recent systematic reviews of asthma-associated DNA methylation changes in a variety of relevant clinical samples. Several genes and CpGs with altered DNA methylation have been suggested as potential asthma biomarkers. Among the most stably replicated were: interleukin 5 receptor subunit alpha (IL5RA), eosinophil peroxidase (EPX), SMAD3 family member 3 (SMAD3) and RUNX family transcription factor 3 (RUNX3) (Cardenas et al., 2019), key genes involved in T cell maturation, Th2 immunity and asthma pathology.

Two large-scale meta-analyses investigated the association of DNA methylation changes in blood to childhood asthma (Reese et al., 2019; Xu et al., 2018). Using data from more than 5000 children from six European cohorts, Xu et al. (2018) reported reduced DNA methylation at 14 specific CpG sites, strongly associated with childhood asthma (Xu et al., 2018). The effects were strongly driven by lower DNA methylation within eosinophils, demonstrating the key role of this cell population as an epigenetic contributor to childhood asthma. In turn, Reese et al. (2019) identified 9 CpGs and 35 regions differentially methylated in newborn blood that may be potential biomarkers of risk for asthma development (Reese et al., 2019).

DNA methylation profiling of airway epithelial cells in adult asthmatics was used to identify epigenetic signatures of distinct disease endotypes, reflecting key components of asthma pathogenesis: airway remodelling, eosinophilia and nitride oxide (NO) response (Nicodemus-Johnson et al., 2016). The authors concluded that DNA methylation profiles constitute a more stable disease biomarker than transcriptional signatures (Nicodemus-Johnson et al., 2016). A recent study in whole blood characterised the complete genome-wide DNA-methylation patterns associated with childhood asthma using WGBS (Thurmann et al., 2023). It reported a prominent loss of DNA methylation (hypomethylation) affecting predominantly enhancer elements, associated with key immune genes involved in asthma pathology, demonstrating the value of unbiased, high-resolution DNA methylation profiling.

The nasal epithelium is a promising tissue for studying DNA methylation changes related to asthma, as demonstrated in several genome-wide studies (Table 4) (Cardenas et al., 2019; Forno et al., 2019; Zhang

**Table 4**
Examples of genome-wide methylation studies associated with asthma.

| Study | Material | Sample number | Sample type | Technology | Gene Expression | PMID |
|---|---|---|---|---|---|---|
| Xu et al., 2018 | blood, validation in nasal epithelium and eosinophils | 1548 (discovery) 3196 (validation) 6 cohorts | asthmatic and control children | Illumina 450 K Array | RNA sequencing | 29496485 |
| Reese et al., 2019 | blood | 1299 (17 cohorts) | newborns and children | Illumina 450 K Array | integrated with published data | 30579849 |
| Nicodemus-Johnson et al., 2016 | endobronchial airway epithelial cell brushings | 115 | asthmatic and non asthmatic adults | Illumina 450 K Array | RNA sequencing | 27942592 |
| Yang et al., 2017 | nasal epithelium | 72 | children | Illumina 450 K Array | Agilent Human Gene Expression arrays | 27745942 |
| Forno et al., 2019 | nasal epithelium | 483 (discovery) 504 (reeplication) | children | Illumina 450 K Array | RNA sequencing | 30584054 |
| Cardenas et al., 2019 | nasal swab cells replication in epithelial nasal cells | 547 | children | Illumina EPIC Array | no | 31300640 |
| Popovic et al., 2019 | saliva | 136 | infants | Illumina 450 K Array | no | 30681197 |

| column | description |
|---|---|
| Study | reference to the original publication |
| Material | type of material for DNA methylation analysis; BAL: bronchoalveolar lavage |
| Sample number | total number of samples used for the analysis |
| Sample type | characteristics of donors used in the analysisis |
| Technology | methodology used for DNA methylation analysis |
| Gene expression | indicates if the study associated DNA methylation with gene expression changes |
| PMID | PMID reference for the original publication |

et al., 2018). Interestingly, many asthma-associated CpG sites discovered in whole blood replicated in nasal epithelial cells, probably reflecting common inflammatory processes (Cardenas et al., 2019). While genes associated with DNA methylation changes in blood samples included inflammatory mediators, these identified in nasal cells also included many proteins of extracellular matrix and membrane proteins. Nasal epithelia are easier to access than blood samples and might be a better proxy for pathological changes in airway cells [reviewed in (Solazzo et al., 2020)]. However, the nasal epithelium is still a complex material containing several cell types, hence some of the obtained profiles may reflect differences in cell type composition of diseased and not diseased samples. Indeed, after cell-type adjustment, many effects were markedly reduced (Cardenas et al., 2019).

## 8. Occupational exposures modify DNA methylation and the risk of diseases

The association of DNA methylation changes with adverse health effects caused by exposure to environmental and occupational toxicants (e.g., particulate matter, cigarette smoke, diesel exhaust fumes, ozone, silica, coal dust, asbestos and a variety of other toxic chemicals and metals, among others) is well documented in experimental and epidemiological studies [see (Martin & Fry, 2018; Nwanaji-Enwerem & Colicino, 2020) for comprehensive general reviews]. Animal models provide emerging evidence that occupational exposures may affect not only the lungs but also other organs, including the brain and are associated with epigenetic dysregulation and neurodegeneration (Shoeb et al., 2020). Notably, some environmentally induced epigenetic changes may even be heritable across generations (Anway et al., 2005; Greger et al., 1989), potentially contributing to familial aggregation of chronic diseases. Hence, it is not surprising that occupational exposures not only increase the risk of developing chronic lung diseases, including COPD, IPF and asthma but also lead to disease exacerbations, as documented in population association studies as well as animal models [see (Dao & Bernstein, 2018; Gandhi et al., 2024; Murgia & Gambelunghe, 2022; Walters, 2020) for reviews].

Due to the strong association between epigenetic changes and exposures, DNA methylation has the potential to serve as a measurable biomarker of exposure to occupational or toxic agents and a possible mediator of exposure effects, leading to the development of the concept of "toxicomethylomics"(Szyf, 2011) [or "toxicoepigenetics" in general]. However, several limitations need to be considered before alterations in DNA methylation can be used as biomarkers for environmental or occupational exposures (Svoboda et al., 2022).

One of the main challenges in environmental epigenetic toxicology is the complex interaction between various environmental factors, diet and hormone signalling, which are all associated with DNA methylation changes. For example, a high-fat diet may change the susceptibility of the lung response in individuals exposed to specific occupational exposures, as documented in animal models (Antonini et al., 2019). Another important consideration is the time and duration of exposure. Because changes in DNA methylation can persist for years, as highlighted above for cigarette smoke, it is difficult to establish a causal relationship between the initial exposure and the development of a given disease, as there could be a substantial lag between the two. In addition, due to the unavailability of the disease-relevant tissue, most methylation-based biomarkers of environmental/occupational exposures were derived using blood, often with limited representation of different ethnicities in the studied populations. Therefore, there may be limited translatability of blood biomarkers to other populations or to target organs.

The establishment of prospective and ethnically diverse cohorts, with longitudinal analysis of different sample types is required to advance epigenetic toxicology research. There is an urgent need for the inclusion of detailed nutritional and environmental assessments in the studies to better characterise the samples and account for complex exposures (the exposome) and interactions. Sex is a well-known factor in the development of chronic lung diseases, hence sex-based differences in DNA methylation should also be investigated because they may influence the response to environmental exposure (Huen et al., 2014) and mediate disease risk.

A key research priority is to determine whether environmentally induced epigenetic alterations may have a causative effect on disease development. For this, the functional implication of the identified changes needs to be investigated in experimental models using innovative technologies, like epigenetic editing described below. Mechanistic understanding of how environmental/occupational exposures impact the epigenome to cause/exacerbate chronic lung diseases may enable the development of better screening assays and inform policies for the safety assessment of agents and occupational hazards, as well as open new avenues for non-invasive biomonitoring and prevention strategies.

## 9. Evolving concepts in DNA methylation

Genome-wide DNA profiling studies provided strong evidence of dysregulated DNA methylation in response to smoke exposure and in chronic lung diseases, including COPD, IPF, asthma and others. Interestingly, they found that most differentially methylated regions/sites are located outside gene promoters and are not enriched in CpG islands, as previously reported in cancer (Jones, 2012). Instead, an enrichment in gene bodies, CpG islands shores, intergenic regions and distal enhancers have been observed (Hoang et al., 2024; Llamazares Prada et al., 2023; Rabinovich et al., 2012; Schwartz et al., 2023; Thurmann et al., 2023). Hypomethylation of enhancer regions emerges as a key feature in COPD (Llamazares Prada et al., 2023; Schwartz et al., 2023) and asthma (Thurmann et al., 2023), indicating that DNA methylation at enhancers may play a pivotal role in the pathogenesis of chronic lung diseases.

DNA methylomes from chronic lung diseases also did not exhibit hypomethylation of retrotransposons typically observed in cancer cells (Rabinovich et al., 2012). These observations highlight the differences in the DNA methylation landscape between cancer and chronic lung diseases, indicating disease specificity despite shared risk factors. They also evidence that genome-wide methods (like WGBS) with more comprehensive coverage are urgently needed to characterise the DNA methylation landscape of chronic lung diseases.

Another important observation came from the investigations of the correlation between DNA methylation and gene expression. As DNA methylation is a repressive epigenetic signal, methylation changes are often expected to be inversely correlated with gene expression (hypomethylation with increased expression and hypermethylation with repression, respectively). Hence, a positive correlation between gene expression and DNA methylation was previously considered contradictory. With the recent high-resolution mapping of DNA methylation and gene expression across multiple tissues and cells, it has become increasingly clear that DNA methylation has complex and context-dependent roles. The canonical negative correlation is mostly observed in gene promoters (Weber et al., 2007), although examples of positive correlation were also observed (Llamazares Prada et al., 2023; Spainhour et al., 2019). The mechanism of repression can be explained by the recruitment and binding of transcriptional repressors that show higher affinity to methylated DNA (so-called methyl Plus TF) or by DNA methylation inhibiting the binding of activating complexes [reviewed in (Greenberg & Bourc'his, 2019)]. In turn, DNA methylation in gene bodies is associated with high gene expression (Lister et al., 2009; Varley et al., 2013). There, it prevents spurious transcription activation from alternative promoters, contributing to higher gene expression efficiency. The varied role of DNA methylation across different genomic regions has important implications for understanding the role of altered DNA methylation in disease, as its effects will be context and location dependent.

Finally, with the availability of genome-wide methylome across tissues and disease states, it became clear that methylome studies from complex tissues need to be interpreted cautiously, as cellular heterogeneity is an important confounder in DNA methylation (also in gene expression studies) (Houseman et al., 2015; Teschendorff & Zheng, 2017). For example, whole blood comprises at least 7–8 main cell types, while human lung tissue contains 58 different cell types (Adams et al., 2023), each with a unique DNA methylation pattern. Hence, cellular composition variations between tested sample groups (e.g., due to disease process) can confound DNA methylation analyses. Indeed, it has been demonstrated that the observed DNA methylation effects (e.g. positive associations in EWAS) can often result from an inflated signal due to a shift in cell type proportions between the cases and controls (Jaffe & Irizarry, 2014). Similarly, a recent study provided strong evidence that differences in the proportion of naïve and differentiated subsets of CD4+ and CD8+ T cells is a key factor contributing to DNA methylation variation in the blood that may mediate associations between DNA methylation and environmental exposures (Bergstedt et al., 2022). Therefore, validation in purified cell populations or deconvolution of DNA methylation data to adjust for cell type proportions should be a prerequisite for epigenetic association analysis. A variety of deconvolution strategies and statistical methods have been developed and systematically evaluated (Jeong et al., 2022; Song & Kuan, 2022) [refer to (Teschendorff & Zheng, 2017; Titus et al., 2017) for a critical review of available approaches]. In general, deconvolution can be performed reference-free, using reference methylomes from the cell types of interest (Teschendorff & Zheng, 2017) or more recently using single-cell RNA seq data (Teschendorff et al., 2020; Zhu et al., 2022). To enable further progress, we need to generate high-resolution genome-wide reference epigenetic profiles of healthy and diseased lung cells isolated from well-clinically characterised biomaterials. The recent development of novel workflows based on tissue cryopreservation could enable the biobanking of viable lung tissue for future cell isolation and profiling (Llamazares-Prada et al., 2021; Pohl et al., 2023). In addition, several novel technologies have recently been developed to map DNA methylation and other epigenetic modifications with single-cell and even spatial resolution (Ahn et al., 2021; Baysoy et al., 2023). Although not mature yet and applied to selected contexts only, further development of such technologies will enable the investigation of epigenetic modifications with single-cell and spatial resolution, as is already the case for transcriptomic research.

## 10. Mechanism of epigenetic changes in chronic lung diseases

Despite substantial progress in mapping epigenetic alterations in chronic lung diseases, the molecular mechanisms leading to their establishment are largely unknown. Due to the vital role of DNMTs and TET enzymes, their recruitment and activity in cells need to be tightly controlled. Several models have been proposed to explain how specific DNA methylation patterns are established and edited in cells [reviewed in (Jurkowska & Jurkowski, 2019)]. They highlight the key role of protein partners and chromatin modifications as well as the contribution of the inherent properties of the enzymes themselves.

Changes in DNA methylation at specific genomic regions can result from the altered expression, localisation or activity of the enzymes that set (DNA methyltransferases) or remove (TET enzymes) the methylation marks. In addition, DNA methylation cross-talks to other epigenetic layers, therefore, changes in histone modifications and chromatin structure will affect DNA methylation and vice versa. Numerous examples of all these mechanisms have been documented across different tissues and disease states, but little is known about them in the context of chronic lung diseases.

Overexpression of DNA methyltransferases (or their splicing isoforms) has been observed in multiple cancers, including lung cancer. Altered expression of DNMT1, DNMT3A, DNMT3B and MeCP2 (methylated DNA reader) were reported in IPF lung tissue and fibrotic lungs of bleomycin-treated mice (Sanders et al., 2012; Wei et al., 2022) and in response to cigarette smoke condensate in cells (Liu et al., 2010). These selected examples indicate that transcriptional dysregulation of the writers, readers and erasers of DNA methylation occurs also in chronic lung diseases, providing a first mechanistic link for the observed global alterations in the DNA methylation landscape reported in profiling studies. However, the molecular mechanism of how altered expression of epigenetic factors is established by environmental exposures remains unknown.

### *10.1. How are DNA methyltransferases and TET recruited?*

In addition, DNMTs and TETs get recruited via interactions with specific chromatin modifications, transcription factors or other protein partners [reviewed in (Jurkowska & Jeltsch, 2022a)].

Chromatin modifications emerged as one of the key mechanisms for recruiting DNA methyltransferases to specific genomic regions. All

human MTases possess specific reading domains in their N-terminal domains that can directly recognise and bind specific histone modifications, bringing the MTase activity to specific genomic regions. For example, DNMT3A and DNMT3B methyltransferases interact via their ATRX-DNMT3-DNMT3L (ADD) domains with histone H3 tails unmodified at Lysine 4 (Otani et al., 2009; Zhang et al., 2010). Notably, H3 binding allosterically activates the enzyme, stimulating methylation of the neighbouring DNA (Guo et al., 2015). This is a beautiful example of the cooperation between different epigenetic signals, whereby one epigenetic modification can lead to the establishment of another mark in the same region. Conversely, H3K4me4, which marks active gene promoters, blocks the binding of DNMT3 proteins, preventing DNA methylation of these active regions and keeping them open to transcriptional machinery. These are just two selected examples illustrating the important role of chromatin modifications in the recruitment and regulation of DNA methyltransferase and demethylase machinery, for more details about the chromatin interaction of DNMTs, the reader is referred to a recent review (Jurkowska & Jeltsch, 2022a).

Numerous protein partners that can recruit epigenetic enzymes to specific genomic regions and directly modulate the activity have been identified (Jurkowska & Jeltsch, 2022a). For example, MeCP2, a methyl-binding protein that can recognise methylated CpG sites, directly interacts with and inhibits the activity of DNMT3A methyltransferase via an allosteric mechanism (Rajavelu et al., 2018). Similarly, UHRF1, which is required for efficient maintenance of DNA methylation during DNA replication, was shown to both recruit DNMT1 maintenance MTase to the replicating chromatin and at the same time to stimulate the activity of the enzyme (Bashtrykov et al., 2014), providing another example of the multifaceted regulatory mechanism controlling the activity and localisation of these important enzymes.

Finally, environmental exposures may influence the activity of epigenetic enzymes by regulating the availability of their required cofactors. For example, TET demethylases and the histone lysine demethylating (Jumanji) proteins are oxygen-dependent enzymes. Thus, oxygen levels can directly influence their catalytic activity, which is particularly relevant in chronic lung diseases often associated with hypoxia. Reduced TET activity due to hypoxia led to DNA hypermethylation of gene promoters in cancer (Thienpont et al., 2016). Hence, hypoxia emerges as one of the regulators of DNA methylation patterns in disease. Similarly, levels of methyl cofactors for DNA and histone methylation (SAM) can be regulated by the availability of folate, coupling metabolism and diet to epigenetic regulation.

### *10.2. How do altered DNA methylation patterns translate into biological effects?*

How altered DNA methylation patterns translate into biological effects in lung cells is currently unclear. DNA methylation in regulatory regions (e.g. promoters, enhancers, insulators) can modulate the binding of transcriptional activators and repressors to DNA (Stadler et al., 2011). Indeed, DNA methylation within the binding sequence of a transcription factor (TF) can directly prevent their binding (these are so-called Methyl minus TF), leading to the loss of their function at the methylated regions. Conversely, methylation of DNA may enhance the binding of selected TFs (Methyl plus TF), providing a potential gain of function mechanism for downstream signalling (Yin et al., 2017). In addition, DNA methylation (together with repressing histone modifications) usually leads to chromatin compaction, making DNA inaccessible to transcriptional machinery. An elegant example of the prominent role of DNA methylation in contributing to lung disease risk was recently provided by Helling and colleagues (Helling et al., 2017), who investigated the molecular mechanism behind the mucin 5B (MUC5B) promoter variant (rs35705950), the largest genetic risk factor for IPF development. They revealed that the variant is associated with regional changes in DNA methylation of an enhancer element and MUC5B expression, which may be mediated by the transcription factor FOXA2 (Helling et al., 2017).

Of note, whole-genome DNA methylation or chromatin accessibility profiling (e.g. by assay for transposase-accessible chromatin with sequencing (ATAC-seq)) allows for inferring transcriptional regulators potentially mediating (or responding to) the epigenetic alterations in regulatory regions (Stadler et al., 2011). Our recent WGBS methylation study in fibroblasts reported a significant enrichment of binding sites for TCF21 and FOSL2/FRA2 transcription factors in the differentially methylated regions overlapping with strong enhancers in COPD lung fibroblasts, identifying them as potential disease regulators and mediators of epigenetic changes in cells (Schwartz et al., 2023). However, follow-up studies directly footprinting the binding of TF in healthy and diseased cells (e.g. by chromatin immunoprecipitation with sequencing (ChIP-seq)) combined with their genetic manipulation (e.g knockdown or overexpression) are needed to unravel the mechanisms of epigenetic regulation in response to DNA methylation alteration.

## 11. Epigenetic mechanisms as potential therapeutic targets – promises and challenges

Unlike disease-associated genetic mutations, epigenetic alterations are reversible and hence constitute attractive intervention targets. Epigenetic-based therapies aim to restore the normal epigenetic state and reverse aberrantly activated or silenced genes. The main promise of this approach is that it targets the epigenetic regulatory layer itself, hence correcting gene expression dysregulation at its source without making changes to the underlying genetic sequence. With the fine mapping of the specific epigenetic alterations in human diseases and the advancement in mechanistic understanding of epigenetic enzymes, came the idea that epigenetic factors may provide useful targets for clinical applications. All three types of epigenetic proteins—writers, readers, and erasers—are in principle druggable and can be targeted through small-molecule inhibitors.

Cancer is the best-known example of a disease with a demonstrated benefit of epigenetic modulation as a therapeutic approach. Several small-molecule inhibitors targeting histone- and DNA-modifying enzymes (e.g., DNA methyltransferases, histone deacetylases and histone methyltransferases) have been developed. Eight have been approved by the Food and Drug Administration (FDA). Seven are currently used in clinics, five for the treatment of hematologic malignancies and two for solid tumors (Feehley et al., 2023) (Table 5). Many others are widespread in clinical trials for malignancies and other diseases (Feehley et al., 2023; Ganesan et al., 2019; Yu et al., 2024).

### *11.1. Targeting DNA methyltransferases*

DNA methyltransferases can be inhibited by targeting different parts of the catalytic pocket (e.g. DNA binding site, SAM cofactor binding sites or both) or by targeting the allosteric regulatory regions outside the enzymatic domain. The field of epigenetic-based therapy was pioneered by Peter Jones with the development of 5-azacytidine and 5-aza-deoxycytidine (decitabine) (Jones & Taylor, 1980), the first two DNA methyltransferase inhibitors (DNMTi) approved for the treatment of haematological cancers. 5-azacytidine (and its analogues) get incorporated into the DNA and form an irreversible covalent complex with DNA methyltransferases, often referred to as a suicidal complex. This leads to the degradation of DNMTs in cells, loss of DNA methylation and re-expression of tumour suppressor genes previously silenced by DNA methylation. However, despite their efficacy, both drugs have poor stability, low bioavailability, lack selectivity and are associated with relatively high toxicity, limiting their clinical utility (Ganesan et al., 2019; Ma & Ge, 2021). Later, other modalities, for example, the methyl donor cofactor (SAM) analogues were also introduced to inhibit

**Table 5**
FDA-approved epigenetic compounds.

| Compound | Epigenetic Target | Clinical indication | FDA approval |
|---|---|---|---|
| Azacytidine | DNMTs | Myelodysplastic syndrome, Acute myeloid leukemia | 2004 |
| Decitabine | DNMTs | Myelodysplastic syndrome, Acute myeloid leukemia | 2006 |
| Vorinostat/SAHA | Pan-HDACs | Cutaneous T-cell lymphoma | 2006 |
| Romidepsin | Class I HDACs | Cutaneous T-cell lymphoma | 2009 |
| Belinostat | Pan-HDACs | Peripheral T-cell lymphoma | 2014 |
| Panobinostat | HDACs | Multiple myeloma | accelerated FDA approval in 2015, withdrawn in 2022 |
| Tucidinostat | HDACs | Peripheral T-cell lymphoma, adult T-cell leukemia/lymphoma (ATLL) | Chinese FDA-approved, 2015 PMDA-approved, (Sun et al., 2022) |
| Tazemetostat | Histone methyltransferase inhibitors | relapsed/refractory follicular lymphoma and epithelioid sarcoma | 2020 |

| column | description |
|---|---|
| Compound | name of the compound/drug |
| Epigenetic target | epigenetic enzyme targeted by the drug; DNMTs: DNA Methyltranferases, HDACs: histone deacetylases |
| Clinical indication | disease for which the compound is used in the clinics |
| FDA approval | year of the FDA approval; PMDA: Japanese Pharmaceuticals and Medical Devices Agency |

DNA methyltransferases without inducing DNA damage. Today, many derivatives of these initial compounds with improved stability and pharmacokinetics have been developed (e.g. Guadecitabine, SGI-110) and are tested in clinical trials but are not yet in clinical practice (Feehley et al., 2023; Ganesan et al., 2019; Yu et al., 2024). Further development led to the idea of combination treatments, where DNMT inhibitors could be combined with additional epigenetic or antitumor drugs, resulting in higher efficacy (Hu et al., 2021).

### *11.2. Targeting histone-modifying enzymes*

Another class of epigenetic drugs used in oncology targets histone-modifying enzymes, with the most prominent example of histone deacetylase inhibitors (HDACis). HDACi block histone deacetylases (HDAC), enzymes that remove acetyl groups from lysine residues on histones, leading to chromatin compaction, and gene silencing. HDACis counteract the abnormal acetylation exhibited by cancer cells, leading to gene reactivation (Falkenberg & Johnstone, 2014). One of the mechanisms of HDACis is the activation of apoptosis pathways that impede the growth and survival of tumour cells (Matthews et al., 2012). To date, several generations of HDAC inhibitors with various chemistries have been developed, most of which act by binding the zinc cation in the HDAC active site. Five have been approved by the FDA (vorinostat, belinostat, romidepsin, tucidinostat and panobinostat) for the treatment of various indications, mostly haematological neoplasms (Bondarev et al., 2021). Among them, vorinostat and romidepsin were the first approved epigenetic drugs that targeted modifications of histones (Ganesan et al., 2019).

Interestingly, the utility of epigenetic therapy is also actively investigated in the context of viral infections aiming to reactivate epigenetically silenced latent viruses (e.g., human immunodeficiency virus (HIV1) or human cytomegalovirus (HCMV)) to enable their eradication by immune cells or antiviral therapy. Several Phase I/II clinical trials have been conducted to evaluate the effect of HDACi (and other epigenetic drugs) in the reactivation of HIV1 viral latency (Nehme et al., 2019).

Like DNMTi, the pharmacokinetic profile of HDACi is not optimal, and they display off-target effects due to non-selective metal binding, hence the search for more specific next-generation HDACi continues. As epigenetic processes are interconnected, combining several epi-drugs might be a promising approach to epigenetic therapy. A novel strategy addressing the low selectivity and efficacy of epigenetic inhibitors is the use of bifunctional compounds. This approach involves the design of inhibitors with dual specificity which can be achieved by linking two (or more) active moieties in a single drug.

### *11.3. Targeting epigenetic readers*

Epigenetic writers and erasers are not the only classes of druggable epigenetic factors. The third group of potential targets contains the epigenetic reading domains, which bind epigenetic modifications and translate them into biological signals. Unlike inhibiting the catalytic activity of epigenetic enzymes, inhibitors of epigenetic readers (e.g., histone binding modules) typically disrupt protein-protein interactions. Two seminal studies in 2010, provided an elegant proof of concept for this approach with the development of bromodomain inhibitors JQ1 (Filippakopoulos et al., 2010) and the synthetic compound I-BET (Nicodeme et al., 2010). Bromodomains are widespread epigenetic reading modules present in more than 40 human chromatin proteins that specifically recognise acetylated lysines in histones (and other proteins). I-BET showed potent anti-inflammatory effects by inhibiting the binding of bromodomain-containing Bromodomain and Extra Terminal domain (BET) proteins to acetylated histones, thereby disrupting chromatin complexes essential for the expression of key inflammatory genes (Nicodeme et al., 2010).

In addition, epigenetic drugs have been successfully tested in combination with chemotherapy, radiotherapy, hormone therapy, anti-angiogenic therapy, or immunotherapy [reviewed in (Morel et al., 2020)], indicating that beyond their potential as monotherapies, they could have significant synergistic roles with other anticancer therapies.

In the context of lung diseases, lung cancer is not the only therapeutic area that could benefit from such epigenetic interventions, as many chronic lung diseases, including COPD, asthma, and lung fibrosis, have been associated with epigenetic and transcriptional dysregulation (as described above). Several studies provided initial evidence supporting the potential benefit of targeting epigenetic processes in lung diseases. A few examples are discussed below.

Treatment with a combination of DNA methyltransferase inhibitor and trichostatin A, an HDAC inhibitor, reduced the mortality rate, lung inflammation and lung injury in a lipopolysaccharide (LPS)-induced mouse model of acute lung injury (ALI) (Thangavel et al., 2014). Targeting DNMT1/DNMT3a and the peroxisome proliferator-activated receptor-γ (PPAR-γ) axis with 5-aza led to demethylation of the PPAR-γ promoter, restored PPAR-γ expression, and alleviated lung fibrosis (Wei et al., 2022).

Although epigenetic therapy is a promising strategy for the treatment of cancers and other lung diseases in the future, important challenges remain to be solved. Despite their use in clinics, epigenetic drugs demonstrate poor pharmacokinetics and low tolerability, mainly due to their lack of genomic and cell-type specificity. Hence, novel approaches with increased precision are needed to advance the epigenetic therapy field.

### 11.4. Epigenetic editing

Multiple EWAS studies identified DNA methylation alterations associated with lung function, smoke exposure or chronic lung diseases, suggesting that epigenetic signalling may play a pathogenic role in lung disease development. However, the key limitation of the EWAS studies (and most current epigenetic studies in basic research) is that they provide only correlative data, and do not allow the establishment of the causal link between environmental exposure (e.g., smoking), altered DNA methylation and the observed phenotype. Thus, currently, we do not know which epigenetic alterations are the cause and which are the consequence of the disease process. Therefore, it remains unclear whether epigenetic mechanisms can be targets for novel therapeutic approaches for lung regeneration. With the advent of designer epigenetic editing technologies, we may finally be able to address that challenge and provide direct evidence for the pathogenic role of DNA methylation in disease development.

Epigenetic editing is an exciting new technology to revert epigenetic modifications at a genomic region of interest [reviewed in (Jeltsch et al., 2007; Jurkowski et al., 2015; Nakamura et al., 2021)]. It employs a programable DNA targeting domain fused to an epigenetic effector domain, which can be specifically targeted to a desired gene to change its epigenetic state, and consequently its expression (Fig. 7). Upon delivery into target cells, the epigenetic programmer is recruited to the target region (e.g., gene promoter or enhancer) via sequence-specific interaction of the DNA-binding domain, enabling the co-delivery of the fused epigenetic domain (e.g., DNA methyltransferase, demethylase or transcriptional regulator). The epigenetic effector domain will then exert its enzymatic (or transcriptional) activity, leading to gene activation or silencing.

Several different DNA binding platforms have been engineered to enable locus-specific targeting of epigenetic domains. These include the modular zinc-finger (ZF) transcription factors (Wolfe et al., 2000), transcription activator-like effectors (TALEs) (Boch et al., 2009) and more recently the catalytically inactive Cas9 (dCas9) nuclease (Qi et al., 2013) from the RNA-directed clustered regulatory interspaced palindromic repeats (CRISPR) system [reviewed in (Jurkowski et al., 2015)]. dCas9 is the most exciting addition to the epigenetic targeting toolbox due to the simplicity of its target design, as directing Cas9 to a specific genomic location only requires designing a guide RNA (gRNA) specific for the desired target (Hsu et al., 2014).

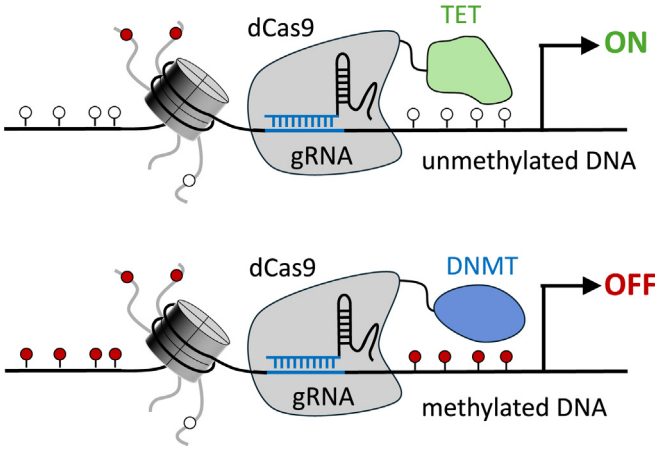


**Fig. 7.** Targeted DNA methylation and demethylation using CRISPR-based epigenetic editing.
Principle of epigenetic editing for gene activation (top) and gene repression (bottom). A programable DNA targeting domain consisting of the catalytically dead CRISPR/Cas9 (dCas9) is fused to an epigenetic effector domain, like TET demethylase or DNA methyltransferase (DNMT). The fusion domain can be targeted by specific guide RNAs (gRNAs) to a desired genomic region (e.g., gene promoter) via the recognition of the target sequence. Once targeted, the epigenetic effector domain can remove (top) or introduce (bottom) DNA methylation modification, leading to subsequent gene activation (top) or silencing (bottom). gRNA sequences can be designed to target any desired gene/region and a combination of different epigenetic effector domains can be used to target different layers of epigenetic regulation.

A variety of epigenetic effector domains for efficient engineering of transcriptional states in living cells or organisms have been designed and validated [reviewed in (Lau & Suh, 2018; Nakamura et al., 2021)]. These range from transcriptional activators (e.g. VP64 (Perez-Pinera et al., 2013) or tripartite VPR (Chavez et al., 2015)) or repressors (e.g. KRAB), through epigenetic enzymes or their isolated domains (e.g., DNA methyltransferases, demethylases or histone modifying enzymes) or even more complex fusions consisting of multiple functional domains joined by a linker (e.g. engineered Dnmt3a-Dnmt3L (Stepper et al., 2017) or Dnmt3a-KRAB fusion proteins).

Targeting DNA methyltransferases (or demethylases) to specific genomic loci provides unique tools to investigate the causal role of DNA methylation of gene expression and explore the potential role of epigenetic editing for epigenetic therapy. The first proof of concept for this approach was provided by a pioneering study by Xu and Bestor in 1997 (Xu & Bestor, 1997). Since then, epigenetic editing has been successfully used to study program epigenetic states in different cells (Chavez et al., 2015; Liu et al., 2016; Saunderson et al., 2017; Song et al., 2017; Dordevic et al., 2023) and pre-clinical mouse models (Horii et al., 2020; Liao et al., 2017; Matharu et al., 2019; Zhou et al., 2018), offering exciting prospects for future therapeutic interventions, including lung diseases (Wu et al., 2018).

Epigenetic editing can also further our understanding of the interplay between genetic and epigenetic disease contributions. Most of the identified disease-associated genetic variations lie outside protein-coding regions, therefore, they likely affect gene regulation by interplaying with epigenetic mechanisms.

While currently used mostly as a research tool to investigate the functional relevance of epigenetic alterations on gene expression and disease phenotypes, precision epigenetic editing approaches may not be that far from clinical application. Following the historic approval of the first CRISPR-based therapeutics for gene therapy, epigenetic editing is gaining momentum as well. Several epigenomic modulators are currently in preclinical development, with the first one already in clinical trials (Table 6). The appealing aspect of epigenetic regulation is that once the altered epigenetic signal is corrected, cellular epigenetic mechanisms will maintain the new state of the locus, such that it can be inherited through cell divisions. Hence, theoretically, epigenetic editing could be used as a single, "hit-and-run" intervention.

The FDA has recently granted an orphan drug designation to OTX-2002, a first-in-class "epigenomic controller", for the treatment of hepatocellular carcinoma (HCC) (Table 6). OTX-2002 is a messenger RNA (mRNA) molecule encoding ZF-DNMT and ZF-KRAB proteins, encapsulated in a lipid nanoparticle. It aims to epigenetically inactivate the expression of MYC oncogene, a master transcription factor regulating cell proliferation, differentiation, and apoptosis of cancer cells. OTX-2002 is currently in Phase I/II clinical trial (NCT05497453) that evaluates the safety, tolerability, and preliminary antitumor activity in patients with HCC (ClinicalTrials.gov, 2022; Rodriguez-Rivera et al., 2023). Although the trial is ongoing and the results have not been published yet, Omega Therapeutics reported that treatment with OTX-2002 in the first eight patients resulted in intended epigenetic state change and c-MYC downregulation.

Another precision epigenomic programmer EPI-321, based on catalytically inactive dCas9, is being developed by Epic Bio. It recently obtained the FDA orphan drug designation as a treatment for facioscapulohumeral muscular dystrophy (FSHD), the most common form of adult muscular dystrophy. The company plans to start a Phase 1/2 clinical trial of EPI-321 to assess its safety, activity, and preliminary efficacy in people with FSHD in 2024. Several additional programs at Epic Bio are in the pipeline (https://epic-bio.com/). Excitingly for the lung field, one seeks to address alpha-1 antitrypsin deficiency, a genetic cause of COPD/emphysema. An epigenetic programmer (EPIC-341) is

**Table 6**
Examples of epigenetic modulators in preclinical or clinical development.

| Modulator | Company | Principle | Target | Clinical indication | Status | Link |
|---|---|---|---|---|---|---|
| OTX-2002 | Omega Therapeutics | Bicistronic mRNA molecule encoding for ZF-DNMT and ZF-KRAB proteins | donwregulation of MYC oncogene | Hepatocellular Carcinoma | IND cleared by FDA, In Phase 1/2, MYCHELANGELO I trial as monotherapy and NCT05497453 trial in combination with Atezolizumab (anti-PD-L1 inhibitor) | https://prn.to/3NwgygD |
| ST-501 | Sangamo Therapeutics | Zinc finger (ZNF) protein technology delivered via AAV to modulate the expression of key genes involved in neurological diseases | tau-targeted ZNF-TF | Alzheimer's disease | Preclinical development | https://www.sangamo.com/programs/ |
| ST-502 | Sangamo Therapeutics | Zinc finger (ZNF) protein technology delivered via AAV to modulate the expression of key genes involved in neurological diseases | alpha synuclein-targeted ZNF-TF | Parkinson's disease | Preclinical development | https://www.sangamo.com/programs/ |
| EPIC-321 | Epic Bio | Restores methylation to the DNA region containing the *DUX4* gene to block the toxic production of the DUX4 protein in muscle cells; packaged in AAV clinically validated to be delivered to muscle | halting abnormal toxic expression of the DUX4 gene | facioscapulohumeral muscular dystrophy (FSHD) | IND cleared by FDA, clinical trial to begin in 2024 | Pipeline - Epic Bio (epic-bio.com) |
| EPIC-341 | EpicBio | Suppresses the endogenous mutated gene and replace it with exogenous wildtype version | alpha-1 antitrypsin | alpha-1 antitrypsin deficiency | Preclinical development, EpicBio pipeline | Pipeline - Epic Bio (epic-bio.com) |
| TUNE-401 | Tune Therapeutics | Inactivates viral DNA integrated into host chromosomes while simultaneously silencing the extra-chromosomal, closed circular DNAs (cccDNAs) necessary for sustained HBV infection via targeted methylation, mRNA sequence encoding the epigenetic repressors and a guide RNA sequence targeting HBVdelivered via Lipid nanoparticle technology | Hepatitis B | Hepatitis B | Preclinical development, Tune Therapeutics pipeline | https://tunetx.com/ |

| column | description |
|---|---|
| Modulator | name of the modulator |
| Company | name of the company developing the modulator |
| Principle | additional information about the modulator (e.g., type of the epigenetic modulator used, principle behind its mode of action); ZF: zinc-finger, DNMT: DNA methyltranferase, AAV: adeno-associated virus, HVB: Hepatitis B virus |
| Target | name of the gene/protein/target targeted by the epigenetic modulator |
| Clinical Indication | disease for which the epigenetic modulator is being developed |
| Status | indicates the status of the epigenetic modulator; IND: Investigational New Drug, |
| Link | link to the company website where the modulator is described |

being developed to epigenetically suppress the endogenous mutated gene and replace it with an exogenous functional version of the gene.

## 12. Future directions

Despite the growing recognition of the contribution of DNA methylation (and other epigenetic mechanisms) to the pathology of lung diseases and substantial progress in mapping DNA methylation changes across a spectrum of lung conditions, many basic questions still await answers and future challenges emerge from our current knowledge. How is the aberrant DNA methylation (and other epigenetic modifications) established in response to environmental insults? How are DNA MTases and TET enzymes targeted and regulated in lung diseases? Which biological functions play the oxidised forms of 5-methylcytosine (5hmeC, 5fC and 5caC) in the pathology of lung diseases? Can we use epigenetic editing to repair disease-causing epigenetic states and target chronic lung diseases? These questions cannot be answered now as lung epigenetic research is still in its infancy, but they will inspire new generations of scientists to further explore epigenetic regulation in the context of chronic lung disease.

## CRediT authorship contribution statement

**Renata Z. Jurkowska:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The author has no conflicts of interest related to this publication.

## Data statement

No new data was created during this study.

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## References

Adam, S., Bracker, J., Klingel, V., Osteresch, B., Radde, N. E., Brockmeyer, J., et al. (2022). Flanking sequences influence the activity of TET1 and TET2 methylcytosine dioxygenases and affect genomic 5hmC patterns. *Communications Biology 5*, 92.

Adams, T. S., Marlier, A., & Kaminski, N. (2023). Lung cell atlases in health and disease. *Annual Review of Physiology 85*, 47–69.

Ahn, J., Heo, S., Lee, J., & Bang, D. (2021). Introduction to single-cell DNA methylation profiling methods. *Biomolecules 11*.

Allen, R. J., Porte, J., Braybrooke, R., Flores, C., Fingerlin, T. E., Oldham, J. M., et al. (2017). Genetic variants associated with susceptibility to idiopathic pulmonary fibrosis in people of European ancestry: a genome-wide association study. *The Lancet Respiratory Medicine 5*, 869–880.

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews. Genetics 17*, 487–500.
Ambatipudi, S., Cuenin, C., Hernandez-Vargas, H., Ghantous, A., Le Calvez-Kelm, F., Kaaks, R., et al. (2016). Tobacco smoking-associated genome-wide DNA methylation changes in the EPIC study. *Epigenomics 8*, 599–618.
Antonini, J. M., Kodali, V., Meighan, T. G., Roach, K. A., Roberts, J. R., Salmen, R., et al. (2019). Effect of age, high-fat diet, and rat strain on serum biomarkers and telomere length and global DNA methylation in peripheral blood mononuclear cells. *Scientific Reports 9*, 1996.
Anway, M. D., Cupp, A. S., Uzumcu, M., & Skinner, M. K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science 308*, 1466–1469.
Ashley, E. A. (2016). Towards precision medicine. *Nature Reviews. Genetics 17*, 507–522.
Bachman, M., Uribe-Lewis, S., Yang, X., Williams, M., Murrell, A., & Balasubramanian, S. (2014). 5-Hydroxymethylcytosine is a predominantly stable DNA modification. *Nature Chemistry 6*, 1049–1055.
Barnes, P. J., Burney, P. G., Silverman, E. K., Celli, B. R., Vestbo, J., Wedzicha, J. A., et al. (2015). Chronic obstructive pulmonary disease. *Nature Reviews. Disease Primers 1*, 15076.
Bashtrykov, P., Jankevicius, G., Jurkowska, R. Z., Ragozin, S., & Jeltsch, A. (2014). The UHRF1 protein stimulates the activity and specificity of the maintenance DNA methyltransferase DNMT1 by an allosteric mechanism. *The Journal of Biological Chemistry 289*, 4106–4115.
Baylin, S. B., Hoppener, J. W., de Bustros, A., Steenbergh, P. H., Lips, C. J., & Nelkin, B. D. (1986). DNA methylation patterns of the calcitonin gene in human lung cancers and lymphomas. *Cancer Research 46*, 2917–2922.
Baysoy, A., Bai, Z., Satija, R., & Fan, R. (2023). The technological landscape and applications of single-cell multi-omics. *Nature Reviews. Molecular Cell Biology 24*, 695–713.
Beane, J., Sebastiani, P., Liu, G., Brody, J. S., Lenburg, M. E., & Spira, A. (2007). Reversible and permanent effects of tobacco smoke exposure on airway epithelial gene expression. *Genome Biology 8*, R201.
Belinsky, S. A., Palmisano, W. A., Gilliland, F. D., Crooks, L. A., Divine, K. K., Winters, S. A., et al. (2002). Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. *Cancer Research 62*, 2370–2377.
Bergman, Y., & Cedar, H. (2013). DNA methylation dynamics in health and disease. *Nature Structural & Molecular Biology 20*, 274–281.
Bergstedt, J., Azzou, S. A. K., Tsuo, K., Jaquaniello, A., Urrutia, A., Rotival, M., et al. (2022). The immune factors driving DNA methylation variation in human blood. *Nature Communications 13*, 5895.
Bermingham, M. L., Walker, R. M., Marioni, R. E., Morris, S. W., Rawlik, K., Zeng, Y., et al. (2019). Identification of novel differentially methylated sites with potential as clinical predictors of impaired respiratory function and COPD. *EBioMedicine 43*, 576–586.
Bernstein, B. E., Stamatoyannopoulos, J. A., Costello, J. F., Ren, B., Milosavljevic, A., Meissner, A., et al. (2010). The NIH roadmap epigenomics mapping consortium. *Nature Biotechnology 28*, 1045–1048.
Bibikova, M., Barnes, B., Tsan, C., Ho, V., Klotzle, B., Le, J. M., et al. (2011). High density DNA methylation array with single CpG site resolution. *Genomics 98*, 288–295.
Bibikova, M., Le, J., Barnes, B., Saedinia-Melnyk, S., Zhou, L., Shen, R., et al. (2009). Genome-wide DNA methylation profiling using Infinium(R) assay. *Epigenomics 1*, 177–200.
Birney, E., Smith, G. D., & Greally, J. M. (2016). Epigenome-wide association studies and the interpretation of disease -omics. *PLoS Genetics 12*, Article e1006105.
Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., et al. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science 326*, 1509–1512.
Bojesen, S. E., Timpson, N., Relton, C., Davey Smith, G., & Nordestgaard, B. G. (2017). AHRR (cg05575921) hypomethylation marks smoking behaviour, morbidity and mortality. *Thorax 72*, 646–653.
Bollepalli, S., Korhonen, T., Kaprio, J., Anders, S., & Ollikainen, M. (2019). EpiSmokEr: a robust classifier to determine smoking status from DNA methylation data. *Epigenomics 11*, 1469–1486.
Bondarev, A. D., Attwood, M. M., Jonsson, J., Chubarev, V. N., Tarasov, V. V., & Schioth, H. B. (2021). Recent developments of HDAC inhibitors: Emerging indications and novel molecules. *British Journal of Clinical Pharmacology 87*, 4577–4597.
Bonnelykke, K., & Ober, C. (2016). Leveraging gene-environment interactions and endotypes for asthma gene discovery. *The Journal of Allergy and Clinical Immunology 137*, 667–679.
Bosse, Y., Postma, D. S., Sin, D. D., Lamontagne, M., Couture, C., Gaudreault, N., et al. (2012). Molecular signature of smoking in human lung tissues. *Cancer Research 72*, 3753–3763.
Bostick, M., Kim, J. K., Esteve, P. O., Clark, A., Pradhan, S., & Jacobsen, S. E. (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science 317*, 1760–1764.
Buro-Auriemma, L. J., Salit, J., Hackett, N. R., Walters, M. S., Strulovici-Barel, Y., Staudt, M. R., et al. (2013). Cigarette smoking induces small airway epithelial epigenetic changes with corresponding modulation of gene expression. *Human Molecular Genetics 22*, 4726–4738.
Busch, R., Qiu, W., Lasky-Su, J., Morrow, J., Criner, G., & DeMeo, D. (2016). Differential DNA methylation marks and gene comethylation of COPD in African-Americans with COPD exacerbations. *Respiratory Research 17*, 143.
Cardenas, A., Sordillo, J. E., Rifas-Shiman, S. L., Chung, W., Liang, L., Coull, B. A., et al. (2019). The nasal methylome as a biomarker of asthma and airway inflammation in children. *Nature Communications 10*, 3095.
Carmona, J. J., Barfield, R. T., Panni, T., Nwanaji-Enwerem, J. C., Just, A. C., Hutchinson, J. N., et al. (2018). Metastable DNA methylation sites associated with longitudinal lung function decline and aging in humans: An epigenome-wide study in the NAS and KORA cohorts. *Epigenetics 13*, 1039–1055.
Casas-Recasens, S., Noell, G., Mendoza, N., Lopez-Giraldo, A., Garcia, T., Guirao, A., et al. (2021). Lung DNA methylation in chronic obstructive pulmonary disease: Relationship with smoking status and airflow limitation severity. *American Journal of Respiratory and Critical Care Medicine 203*, 129–134.
Chatziioannou, A., Georgiadis, P., Hebels, D. G., Liampa, I., Valavanis, I., Bergdahl, I. A., et al. (2017). Blood-based omic profiling supports female susceptibility to tobacco smoke-induced cardiovascular diseases. *Scientific Reports 7*, 42870.
Chavez, A., Scheiman, J., Vora, S., Pruitt, B. W., Tuttle, M., et al. (2015). Highly efficient Cas9-mediated transcriptional programming. *Nature Methods 12*, 326–328.
Chen, A., Zeilinger, S., Kühnel, B., Klopp, N., Baurecht, H., Kleinschmidt, A., et al. (2013). Tobacco smoking leads to extensive genome-wide changes in DNA methylation. *PLoS One 8*, Article e63812.
Cho, M. H., McDonald, M. L., Zhou, X., Mattheisen, M., Castaldi, P. J., Hersh, C. P., et al. (2014). Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *The Lancet Respiratory Medicine 2*, 214–225.
Clifford, R. L., Fishbane, N., Patel, J., MacIsaac, J. L., McEwen, L. M., Fisher, A. J., et al. (2018). Altered DNA methylation is associated with aberrant gene expression in parenchymal but not airway fibroblasts isolated from individuals with COPD. *Clinical Epigenetics 10*, 32.
ClinicalTrials.gov (2022). *A phase 1/2 study to evaluate OTX-2022 in patients with hepatocellular carcinoma and other solid tumor types known for association with the MYC oncogene (MYCHELANGELO I)vol. 2024..*
Consortium, E. P. (2004). The ENCODE (ENCyclopedia of DNA elements) project. *Science 306*, 636–640.
Consortium, E. P. (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature 489*, 57–74.
Cosgrove, M. S., Boeke, J. D., & Wolberger, C. (2004). Regulated nucleosome mobility and the histone code. *Nature Structural & Molecular Biology 11*, 1037–1043.
Costa-Pinheiro, P., Montezuma, D., Henrique, R., & Jeronimo, C. (2015). Diagnostic and prognostic epigenetic biomarkers in cancer. *Epigenomics 7*, 1003–1015.
Dao, A., & Bernstein, D. I. (2018). Occupational exposure and asthma. *Annals of Allergy, Asthma & Immunology 120*, 468–475.
Dogan, M. V., Shields, B., Cutrona, C., Gao, L., Gibbons, F. X., Simons, R., et al. (2014). The effect of smoking on DNA methylation of peripheral blood mononuclear cells from African American women. *BMC Genomics 15*, 151.
Dordevic, M., Stepper, P., Feuerstein-Akgoz, C., Gerhauser, C., Paunović, V., Tolić, A., et al. (2023). EpiCRISPR targeted methylation of Arx gene initiates transient switch of mouse pancreatic alpha to insulin-producing cells. *Frontiers in Endocrinology 14*, Article 1134478.
Edris, A., den Dekker, H. T., Melen, E., & Lahousse, L. (2019). Epigenome-wide association studies in asthma: A systematic review. *Clinical and Experimental Allergy 49*, 953–968.
Ezzati, M., & Lopez, A. D. (2003). Estimates of global mortality attributable to smoking in 2000. *Lancet 362*, 847–852.
Falkenberg, K. J., & Johnstone, R. W. (2014). Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. *Nature Reviews. Drug Discovery 13*, 673–691.
Fang, F., Andersen, A. M., Philibert, R., & Hancock, D. B. (2023). Epigenetic biomarkers for smoking cessation. *Addiction Neuroscience 6*.
Feehley, T., O'Donnell, C. W., Mendlein, J., Karande, M., & McCauley, T. (2023). Drugging the epigenome in the age of precision medicine. *Clinical Epigenetics 15*, 6.
Feinberg, A. P., & Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature 301*, 89–92.
Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W. B., Fedorov, O., et al. (2010). Selective inhibition of BET bromodomains. *Nature 468*, 1067–1073.
Forno, E., Wang, T., Qi, C., Yan, Q., Xu, C. J., Boutaoui, N., et al. (2019). DNA methylation in nasal epithelium, atopy, and atopic asthma in children: a genome-wide study. *The Lancet Respiratory Medicine 7*, 336–346.
Franklin, K. A., Shields, C. E., & Haynes, K. A. (2022). Beyond the marks: Reader-effectors as drivers of epigenetics and chromatin engineering. *Trends in Biochemical Sciences 47*, 417–432.
Gama-Sosa, M. A., Slagel, V. A., Trewyn, R. W., Oxenhandler, R., Kuo, K. C., Gehrke, C. W., et al. (1983). The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Research 11*, 6883–6894.
Gandhi, S. A., Min, B., Fazio, J. C., Johannson, K. A., Steinmaus, C., Reynolds, C. J., et al. (2024). The impact of occupational exposures on the risk of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. *Annals of the American Thoracic Society 21*, 486–498.
Ganesan, A., Arimondo, P. B., Rots, M. G., Jeronimo, C., & Berdasco, M. (2019). The timeline of epigenetic drug discovery: From reality to dreams. *Clinical Epigenetics 11*, 174.
Gao, L., Emperle, M., Guo, Y., et al. (2020). Comprehensive structure-function characterization of DNMT3B and DNMT3A reveals distinctive de novo DNA methylation mechanisms. *Nat Commun 11*, 3355. https://doi.org/10.1038/s41467-020-17109-4.
Gao, X., Jia, M., Zhang, Y., Breitling, L. P., & Brenner, H. (2015). DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies. *Clinical Epigenetics 7*, 113.
GOLD (2023). *The global strategy for diagnosis, management and prevention of chronic obstructive pulmonary disease (updated 2023).*
Greenberg, M. V. C., & Bourc'his, D. (2019). The diverse roles of DNA methylation in mammalian development and disease. *Nature Reviews. Molecular Cell Biology 20*, 590–607.
Greger, V., Passarge, E., Hopping, W., Messmer, E., & Horsthemke, B. (1989). Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Human Genetics 83*, 155–158.

Guida, F., Sandanger, T. M., Castagne, R., Campanella, G., Polidoro, S., Palli, D., et al. (2015). Dynamics of smoking-induced genome-wide methylation changes with time since smoking cessation. *Human Molecular Genetics 24*, 2349–2359.
Guo, X., Wang, L., Li, J., Ding, Z., Xiao, J., Yin, X., et al. (2015). Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature 517*, 640–644.
Hancock, D. B., Eijgelsheim, M., Wilk, J. B., Gharib, S. A., Loehr, L. R., Marciante, K. D., et al. (2010). Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nature Genetics 42*, 45–52.
Handa, V., & Jeltsch, A. (2005). Profound flanking sequence preference of Dnmt3a and Dnmt3b mammalian DNA methyltransferases shape the human epigenome. *Journal of Molecular Biology 348*, 1103–1112.
Hatton, I. A., Galbraith, E. D., Merleau, N. S. C., Miettinen, T. P., Smith, B. M., & Shander, J. A. (2023). The human cell count and size distribution. *Proceedings of the National Academy of Sciences of the United States of America 120*, Article e2303077120.
Heinbockel, L., Marwitz, S., Schromm, A. B., Watz, H., Kugler, C., Ammerpohl, O., et al. (2018). Identification of novel target genes in human lung tissue involved in chronic obstructive pulmonary disease. *International Journal of Chronic Obstructive Pulmonary Disease 13*, 2255–2259.
Helling, B. A., Gerber, A. N., Kadiyala, V., Sasse, S. K., Pedersen, B. S., Sparks, L., et al. (2017). Regulation of MUC5B expression in idiopathic pulmonary fibrosis. *American Journal of Respiratory Cell and Molecular Biology 57*, 91–99.
Hoang, T. T., Lee, Y., McCartney, D. L., Kersten, E. T. G., Page, C. M., Hulls, P. M., et al. (2024). Comprehensive evaluation of smoking exposures and their interactions on DNA methylation. *EBioMedicine 100*, Article 104956.
Hobbs, B. D., de Jong, K., Lamontagne, M., Bosse, Y., Shrine, N., Artigas, M. S., et al. (2017). Genetic loci associated with chronic obstructive pulmonary disease overlap with loci for lung function and pulmonary fibrosis. *Nature Genetics 49*, 426–432.
Hobbs, B. D., Morrow, J. D., Wang, X. -W., Liu, Y. -Y., DeMeo, D. L., Hersh, C. P., et al. (2023). Identifying chronic obstructive pulmonary disease from integrative omics and clustering in lung tissue. *BMC Pulmonary Medicine 23*, 115.
Horii, T., Morita, S., Hino, S., Kimura, M., Hino, Y., Kogo, H., et al. (2020). Successful generation of epigenetic disease model mice by targeted demethylation of the epigenome. *Genome Biology 21*, 77.
Horvath, S., & Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nature Reviews. Genetics 19*, 371–384.
Houseman, E. A., Kelsey, K. T., Wiencke, J. K., & Marsit, C. J. (2015). Cell-composition effects in the analysis of DNA methylation array data: a mathematical perspective. *BMC Bioinformatics 16*, 95.
Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell 157*, 1262–1278.
Hu, C., Liu, X., Zeng, Y., Liu, J., & Wu, F. (2021). DNA methyltransferase inhibitors combination therapy for the treatment of solid tumor: Mechanism and clinical application. *Clinical Epigenetics 13*, 166.
Huang, S. K., Fisher, A. S., Scruggs, A. M., White, E. S., Hogaboam, C. M., Richardson, B. C., et al. (2010). Hypermethylation of PTGER2 confers prostaglandin E2 resistance in fibrotic fibroblasts from humans and mice. *The American Journal of Pathology 177*, 2245–2255.
Huang, S. K., Scruggs, A. M., McEachin, R. C., White, E. S., & Peters-Golden, M. (2014). Lung fibroblasts from patients with idiopathic pulmonary fibrosis exhibit genome-wide differences in DNA methylation compared to fibroblasts from nonfibrotic lung. *PLoS One 9*, Article e107055.
Huen, K., Yousefi, P., Bradman, A., Yan, L., Harley, K. G., Kogut, K., et al. (2014). Effects of age, sex, and persistent organic pollutants on DNA methylation in children. *Environmental and Molecular Mutagenesis 55*, 209–222.
Ito, S., Shen, L., Dai, Q., Wu, S. C., Collins, L. B., Swenberg, J. A., et al. (2011). Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science 333*, 1300–1303.
Jaffe, A. E., & Irizarry, R. A. (2014). Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biology 15*, R31.
Jeltsch, A. (2013). Oxygen, epigenetic signaling, and the evolution of early life. *Trends in Biochemical Sciences 38*, 172–176.
Jeltsch, A., & Jurkowska, R. Z. (2013). Multimerization of the dnmt3a DNA methyltransferase and its functional implications. *Progress in Molecular Biology and Translational Science 117*, 445–464.
Jeltsch, A., & Jurkowska, R. Z. (2014). New concepts in DNA methylation. *Trends in Biochemical Sciences 39*, 310–318.
Jeltsch, A., & Jurkowska, R. Z. (2016). Allosteric control of mammalian DNA methyltransferases - a new regulatory paradigm. *Nucleic Acids Research 44*, 8556–8575.
Jeltsch, A., Jurkowska, R. Z., Jurkowski, T. P., Liebert, K., Rathert, P., & Schlickenrieder, M. (2007). Application of DNA methyltransferases in targeted DNA methylation. *Applied Microbiology and Biotechnology 75*, 1233–1240.
Jeong, Y., de Andrade, E. S. L. B., Thalmeier, D., Toth, R., Ganslmeier, M., Breuer, K., et al. (2022). Systematic evaluation of cell-type deconvolution pipelines for sequencing-based bulk DNA methylomes. *Briefings in Bioinformatics 23*.
Jeziorska, D. M., Murray, R. J. S., De Gobbi, M., Gaentzsch, R., Garrick, D., Ayyub, H., et al. (2017). DNA methylation of intragenic CpG islands depends on their transcriptional activity during differentiation and disease. *Proceedings of the National Academy of Sciences of the United States of America 114*, E7526–E7535.
Jia, D., Jurkowska, R. Z., Zhang, X., Jeltsch, A., & Cheng, X. (2007). Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature 449*, 248–251.
Joehanes, R., Just, A. C., Marioni, R. E., Pilling, L. C., Reynolds, L. M., Mandaviya, P. R., et al. (2016). Epigenetic signatures of cigarette smoking. *Circulation. Cardiovascular Genetics 9*, 436–447.
Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nature Reviews. Genetics 13*, 484–492.
Jones, P. A., & Taylor, S. M. (1980). Cellular differentiation, cytidine analogs and DNA methylation. *Cell 20*, 85–93.
Joubert, B. R., Haberg, S. E., Nilsen, R. M., Wang, X., Vollset, S. E., Murphy, S. K., et al. (2012). 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environmental Health Perspectives 120*, 1425–1431.
Jurkowska, R. Z., Anspach, N., Urbanke, C., Jia, D., Reinhardt, R., Nellen, W., et al. (2008). Formation of nucleoprotein filaments by mammalian DNA methyltransferase Dnmt3a in complex with regulator Dnmt3L. *Nucleic Acids Research 36*(21), 6656–6663.
Jurkowska, R. Z., & Jeltsch, A. (2022a). Enzymology of mammalian DNA methyltransferases. *Advances in Experimental Medicine and Biology 1389*, 69–110.
Jurkowska, R. Z., & Jeltsch, A. (2022b). Mechanisms and biological roles of DNA methyltransferases and DNA methylation: From past achievements to future challenges. *Advances in Experimental Medicine and Biology 1389*, 1–19.
Jurkowska, R. Z., & Jurkowski, T. P. (2019). Establishment, erasure and synthetic reprogramming of DNA methylation in mammalian cells. In S. Jurga, & J. Barciszewski (Eds.), *The DNA, RNA, and histone Methylomes* (pp. 1–26). Cham: Springer International Publishing.
Jurkowska, R. Z., Jurkowski, T. P., & Jeltsch, A. (2011). Structure and function of mammalian DNA methyltransferases. *Chembiochem 12*, 206–222.
Jurkowska, R. Z., Rajavelu, A., Anspach, N., Urbanke, C., Jankevicius, G., Ragozin, S., et al. (2011). Oligomerization and binding of the Dnmt3a DNA methyltransferase to parallel DNA molecules: Heterochromatic localization and role of Dnmt3L. *The Journal of Biological Chemistry 286*, 24200–24207.
Jurkowska, R. Z., Siddique, A. N., Jurkowski, T. P., & Jeltsch, A. (2011). Approaches to enzyme and substrate design of the murine Dnmt3a DNA methyltransferase. *Chembiochem 12*, 1589–1594.
Jurkowski, T. P., Ravichandran, M., & Stepper, P. (2015). Synthetic epigenetics-towards intelligent control of epigenetic states and cell identity. *Clinical Epigenetics 7*, 18.
Kachroo, P., Morrow, J. D., Kho, A. T., Vyhlidal, C. A., Silverman, E. K., Weiss, S. T., et al. (2020). Co-methylation analysis in lung tissue identifies pathways for fetal origins of COPD. *European Respiratory Journal 56*.
Kaur, D., Lee, S. M., Goldberg, D., Spix, N. J., Hinoue, T., Li, H. -T., et al. (2023). Comprehensive evaluation of the Infinium human MethylationEPIC v2 BeadChip. *Epigenetics Communications 3*, 6.
Kaur, G., Begum, R., Thota, S., & Batra, S. (2019). A systematic review of smoking-related epigenetic alterations. *Archives of Toxicology 93*, 2715–2740.
Keshawarz, A, Joehanes, R, Guan, W, Huan, T, DeMeo, DL, et al. (2022). Longitudinal change in blood DNA epigenetic signature after smoking cessation. *Epigenetics 10*, 1098–1109.
Keshet, I., Lieman-Hurwitz, J., & Cedar, H. (1986). DNA methylation affects the formation of active chromatin. *Cell 44*, 535–543.
Laird, P. W. (2010). Principles and challenges of genomewide DNA methylation analysis. *Nature Reviews. Genetics 11*, 191–203.
Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., et al. (2001). Initial sequencing and analysis of the human genome. *Nature 409*, 860–921.
Lau, C. H., & Suh, Y. (2018). In vivo epigenome editing and transcriptional modulation using CRISPR technology. *Transgenic Research 27*, 489–509.
Lederer, D. J., & Martinez, F. J. (2018). Idiopathic pulmonary fibrosis. *The New England Journal of Medicine 378*, 1811–1823.
Lee, JU, Cheong, HS, Shim, EY, Bae, DJ, Chang, HS, Uh, ST, et al. (2017). Gene profile of fibroblasts identify relation of CCL8 with idiopathic pulmonary fibrosis. *Respir Res 18* (1), 3.
Lee, J. U., Son, J. H., Shim, E. Y., Cheong, H. S., Shin, S. W., Shin, H. D., et al. (2019). Global DNA methylation pattern of fibroblasts in idiopathic pulmonary fibrosis. *DNA and Cell Biology 38*, 905–914.
Legaki, E., Arsenis, C., Taka, S., & Papadopoulos, N. G. (2022). DNA methylation biomarkers in asthma and rhinitis: Are we there yet? *Clinical and Translational Allergy 12*, Article e12131.
Li, E., Bestor, T. H., & Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell 69*, 915–926.
Liao, H. K., Hatanaka, F., Araoka, T., Reddy, P., Wu, M. Z., Sui, Y., et al. (2017). In vivo target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation. *Cell 171* (1495–1507), Article e1415.
Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., et al. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature 462*, 315–322.
Liu, F., Killian, J. K., Yang, M., Walker, R. L., Hong, J. A., Zhang, M., et al. (2010). Epigenomic alterations and gene expression profiles in respiratory epithelia exposed to cigarette smoke condensate. *Oncogene 29*, 3650–3664.
Liu, R., Zhao, E., Yu, H., Yuan, C., Abbas, M. N., & Cui, H. (2023). Methylation across the central dogma in health and diseases: New therapeutic strategies. *Signal Transduction and Targeted Therapy 8*, 310.
Liu, X. S., Wu, H., Ji, X., Stelzer, Y., Wu, X., Czauderna, S., et al. (2016). Editing DNA methylation in the mammalian genome. *Cell 167*(233–247), Article e217.
Liu, Y., Hu, Z., Cheng, J., Siejka-Zielinska, P., Chen, J., Inoue, M., et al. (2021). Subtraction-free and bisulfite-free specific sequencing of 5-methylcytosine and its oxidized derivatives at base resolution. *Nature Communications 12*, 618.
Liu, Y., Siejka-Zielinska, P., Velikova, G., Bi, Y., Yuan, F., Tomkova, M., et al. (2019). Bisulfite-free direct detection of 5-methylcytosine and 5-hydroxymethylcytosine at base resolution. *Nature Biotechnology 37*, 424–429.
Lizio, M., Harshbarger, J., Shimoji, H., Severin, J., Kasukawa, T., Sahin, S., et al. (2015). Gateways to the FANTOM5 promoter level mammalian expression atlas. *Genome Biology 16*, 22.

Llamazares-Prada, M., Espinet, E., Mijosek, V., Schwartz, U., Lutsik, P., Tamas, R., et al. (2021). Versatile workflow for cell type-resolved transcriptional and epigenetic profiles from cryopreserved human lung. *JCI Insight 6*.
Loda, A., & Heard, E. (2019). Xist RNA in action: Past, present, and future. *PLoS Genetics 15*, Article e1008333.
Llamazares Prada, M. L., Schwartz, U., Pease, D. F., Pohl, S. T., Ackesson, D., Li, R., et al. (2023). *Epigenetic deregulation of IFN and WNT pathways in AT2 cells impairs alveolar regeneration (in COPD)*. bioRxiv, 2023.2010.2022.563483.
Loyfer, N., Magenheim, J., Peretz, A., Cann, G., Bredno, J., Klochendler, A., et al. (2023). A DNA methylation atlas of normal human cell types. *Nature 613*, 355–364.
Ma, J., & Ge, Z. (2021). Comparison between decitabine and azacitidine for patients with acute myeloid leukemia and higher-risk myelodysplastic syndrome: A systematic review and network meta-analysis. *Frontiers in Pharmacology 12*, Article 701690.
Maas, S. C. E., Vidaki, A., Wilson, R., Teumer, A., Liu, F., van Meurs, J. B. J., et al. (2019). Validated inference of smoking habits from blood with a finite DNA methylation marker set. *European Journal of Epidemiology 34*, 1055–1074.
Martens, J. H., & Stunnenberg, H. G. (2013). BLUEPRINT: Mapping human blood cell epigenomes. *Haematologica 98*, 1487–1489.
Martin, E. M., & Fry, R. C. (2018). Environmental influences on the epigenome: Exposure-associated DNA methylation in human populations. *Annual Review of Public Health 39*, 309–333.
Martinez, F. J., Chisholm, A., Collard, H. R., Flaherty, K. R., Myers, J., Raghu, G., et al. (2017). The diagnosis of idiopathic pulmonary fibrosis: Current and future approaches. *The Lancet Respiratory Medicine 5*, 61–71.
Matharu, N., Rattanasopha, S., Tamura, S., Maliskova, L., Wang, Y., Bernard, A., et al. (2019). CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science 363*.
Matthews, G. M., Newbold, A., & Johnstone, R. W. (2012). Intrinsic and extrinsic apoptotic pathway signaling as determinants of histone deacetylase inhibitor antitumor activity. *Advances in Cancer Research 116*, 165–197.
Mattick, J. S., Amaral, P. P., Carninci, P., Carpenter, S., Chang, H. Y., Chen, L. -L., et al. (2023). Long non-coding RNAs: Definitions, functions, challenges and recommendations. *Nature Reviews Molecular Cell Biology 24*, 430–447.
McCartney, D. L., Hillary, R. F., Stevenson, A. J., Ritchie, S. J., Walker, R. M., Zhang, Q., et al. (2018). Epigenetic prediction of complex traits and death. *Genome Biology 19*, 136.
McCartney, D. L., Stevenson, A. J., Hillary, R. F., Walker, R. M., Bermingham, M. L., Morris, S. W., et al. (2018). Epigenetic signatures of starting and stopping smoking. *EBioMedicine 37*, 214–220.
McErlean, P., Bell, C. G., Hewitt, R. J., Busharat, Z., Ogger, P. P., Ghai, P., et al. (2021). DNA methylome alterations are associated with airway macrophage differentiation and phenotype during lung fibrosis. *American Journal of Respiratory and Critical Care Medicine 204*, 954–966.
Meissner, A., Gnirke, A., Bell, G. W., Ramsahoye, B., Lander, E. S., & Jaenisch, R. (2005). Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis. *Nucleic Acids Research 33*, 5868–5877.
Melen, E., Koppelman, G. H., Vicedo-Cabrera, A. M., Andersen, Z. J., & Bunyavanich, S. (2022). Allergies to food and airborne allergens in children and adolescents: Role of epigenetics in a changing environment. *Lancet Child Adolesc Health 6*, 810–819.
Michalak, E. M., Burr, M. L., Bannister, A. J., & Dawson, M. A. (2019). The roles of DNA, RNA and histone methylation in ageing and cancer. *Nature Reviews. Molecular Cell Biology 20*, 573–589.
Millán-Zambrano, G., Burton, A., Bannister, A. J., & Schneider, R. (2022). Histone post-translational modifications — Cause and consequence of genome function. *Nature Reviews Genetics 23*, 563–580.
Monick, M. M., Beach, S. R., Plume, J., Sears, R., Gerrard, M., Brody, G. H., et al. (2012). Coordinated changes in AHRR methylation in lymphoblasts and pulmonary macrophages from smokers. *Am J Med Genet B Neuropsychiatr Genet. 159B*(2), 141–151.
Montoro, D. T., Haber, A. L., Biton, M., Vinarsky, V., Lin, B., Birket, S. E., et al. (2018). A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature 560*, 319–324.
Moran, S., Vizoso, M., Martinez-Cardus, A., Gomez, A., Matias-Guiu, X., Chiavenna, S. M., et al. (2014). Validation of DNA methylation profiling in formalin-fixed paraffin-embedded samples using the Infinium HumanMethylation450 Microarray. *Epigenetics 9*, 829–833.
Morel, D., Jeffery, D., Aspeslagh, S., Almouzni, G., & Postel-Vinay, S. (2020). Combining epigenetic drugs with other therapies for solid tumours - past lessons and future promise. *Nature Reviews. Clinical Oncology 17*, 91–107.
Morrow, J. D., Cho, M. H., Hersh, C. P., Pinto-Plata, V., Celli, B., Marchetti, N., et al. (2016). DNA methylation profiling in human lung tissue identifies genes associated with COPD. *Epigenetics 11*, 730–739.
Murgia, N., & Gambelunghe, A. (2022). Occupational COPD-The most under-recognized occupational lung disease? *Respirology 27*, 399–410.
Nakamura, M., Gao, Y., Dominguez, A. A., & Qi, L. S. (2021). CRISPR technologies for precise epigenome editing. *Nature Cell Biology 23*, 11–22.
Negretti, N. M., Plosa, E. J., Benjamin, J. T., Schuler, B. A., Habermann, A. C., Jetter, C. S., et al. (2021). A single-cell atlas of mouse lung development. *Development 148*.
Nehme, Z., Pasquereau, S., & Herbein, G. (2019). Control of viral infections by epigenetic-targeted therapy. *Clinical Epigenetics 11*, 55.
Nemeth, K., Bayraktar, R., Ferracin, M., & Calin, G. A. (2024). Non-coding RNAs in disease: From mechanisms to therapeutics. *Nature Reviews Genetics 25*, 211–232.
Nicodeme, E., Jeffrey, K. L., Schaefer, U., Beinke, S., Dewell, S., Chung, C. W., et al. (2010). Suppression of inflammation by a synthetic histone mimic. *Nature 468*, 1119–1123.
Nicodemus-Johnson, J., Myers, R. A., Sakabe, N. J., Sobreira, D. R., Hogarth, D. K., Naureckas, E. T., et al. (2016). DNA methylation in lung cells is associated with asthma endotypes and genetic risk. *JCI Insight 1*, Article e90151.
Nurk, S., Koren, S., Rhie, A., Rautiainen, M., Bzikadze, A. V., Mikheenko, A., et al. (2022). The complete sequence of a human genome. *Science 376*, 44–53.
Nwanaji-Enwerem, J. C., & Colicino, E. (2020). DNA methylation-based biomarkers of environmental exposures for human population studies. *Current Environmental Health Reports 7*, 121–128.
Okano, M., Bell, D. W., Haber, D. A., & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell 99*, 247–257.
Olstad, E. W., Nordeng, H. M. E., Sandve, G. K., Lyle, R., & Gervin, K. (2022). Low reliability of DNA methylation across Illumina Infinium platforms in cord blood: Implications for replication studies and meta-analyses of prenatal exposures. *Clinical Epigenetics 14*, 80.
Olvera, N., Sanchez-Valle, J., Nunez-Carpintero, I., Rojas-Quintero, J., Noell, G., Casas-Recasens, S., et al. (2024). Lung tissue multi-layer network analysis uncovers the molecular heterogeneity of COPD. *American Journal of Respiratory and Critical Care Medicine*. https://doi.org/10.1164/rccm.202303-0500OC.
Otani, J., Nankumo, T., Arita, K., Inamoto, S., Ariyoshi, M., & Shirakawa, M. (2009). Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Reports 10*, 1235–1241.
Parker, M. M., Chase, R. P., Lamb, A., Reyes, A., Saferali, A., Yun, J. H., et al. (2017). RNA sequencing identifies novel non-coding RNA and exon-specific effects associated with cigarette smoking. *BMC Medical Genomics 10*, 58.
Perez-Pinera, P., Kocak, D. D., Vockley, C. M., Adler, A. F., Kabadi, A. M., Polstein, L. R., et al. (2013). RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nature Methods 10*, 973–976.
Petryk, N., Bultmann, S., Bartke, T., & Defossez, P. A. (2021). Staying true to yourself: Mechanisms of DNA methylation maintenance in mammals. *Nucleic Acids Research 49*, 3020–3032.
Philibert, R., Monick, M. M., Beach, S. R. H., Plume, J., Sears, R., Gerrard, M., et al. (2012). Smoking is associated with coordinated changes in aryl hydrocarbon receptor repressor methylation in genome wide analyses of lymphoblast and pulmonary macrophage DNA. *Biological Psychiatry 71*, 163s.
Philibert, R. A., Beach, S. R., Lei, M. K., & Brody, G. H. (2013). Changes in DNA methylation at the aryl hydrocarbon receptor repressor may be a new biomarker for smoking. *Clinical Epigenetics 5*, 19.
Pidsley, R., Zotenko, E., Peters, T. J., Lawrence, M. G., Risbridger, G. P., Molloy, P., et al. (2016). Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biology 17*, 208.
Plasschaert, L. W., Zilionis, R., Choo-Wing, R., Savova, V., Knehr, J., Roma, G., et al. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature 560*, 377–381.
Pohl, S. T., Prada, M. L., Espinet, E., & Jurkowska, R. (2023). Practical considerations for complex tissue dissociation for single-cell transcriptomics. *Methods in Molecular Biology 2584*, 371–387.
Polverino, F., & Kalhan, R. (2023). Leveraging omics to predict chronic obstructive pulmonary disease exacerbations: The "Immunome". *American Journal of Respiratory and Critical Care Medicine 208*, 220–222.
Popovic, M, Fiano, V, Fasanelli, F, Trevisan, M, Grasso, C, Assumma, MB, et al. (2019). Differentially methylated DNA regions in early childhood wheezing: An epigenome-wide study using saliva. *Pediatr Allergy Immunol 30*(3), 305–314.
Porsbjerg, C., Melén, E., Lehtimäki, L., & Shaw, D. (2023). Asthma. *The Lancet 401*, 858–873.
Prevention, C. F. D. C. A. (2014). *National Center for Chronic Disease Prevention and Health Promotion (US) office on smoking and health. The health consequences of smoking—50 years of progress: A report of the surgeon general.* Atlanta (GA).
Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., et al. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell 152*, 1173–1183.
Qiu, W., Baccarelli, A., Carey, V. J., Boutaoui, N., Bacherman, H., Klanderman, B., et al. (2012). Variable DNA methylation is associated with chronic obstructive pulmonary disease and lung function. *American Journal of Respiratory and Critical Care Medicine 185*, 373–381.
Rabinovich, E. I., Kapetanaki, M. G., Steinfeld, I., Gibson, K. F., Pandit, K. V., Yu, G., et al. (2012). Global methylation patterns in idiopathic pulmonary fibrosis. *PLoS One 7*, Article e33770.
Raghu, G., Remy-Jardin, M., Myers, J. L., Richeldi, L., Ryerson, C. J., Lederer, D. J., et al. (2018). Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *American Journal of Respiratory and Critical Care Medicine 198*, e44–e68.
Ragland, M. F., Benway, C. J., Lutz, S. M., Bowler, R. P., Hecker, J., Hokanson, J. E., et al. (2019). Genetic advances in chronic obstructive pulmonary disease. Insights from COPDGene. *American Journal of Respiratory and Critical Care Medicine 200*, 677–690.
Rajavelu, A., Lungu, C., Emperle, M., Dukatz, M., Brohm, A., Broche, J., et al. (2018). Chromatin-dependent allosteric regulation of DNMT3A activity by MeCP2. *Nucleic Acids Research 46*, 9044–9056.
Ravichandran, M., Jurkowska, R. Z., & Jurkowski, T. P. (2018). Target specificity of mammalian DNA methylation and demethylation machinery. *Organic & Biomolecular Chemistry 16*, 1419–1435.
Ravichandran, M., Rafalski, D., Davies, C. I., Ortega-Recalde, O., Nan, X., Glanfield, C. R., et al. (2022). Pronounced sequence specificity of the TET enzyme catalytic domain guides its cellular function. *Science Advances 8*, eabm2427.
Reese, S. E., Xu, C. J., den Dekker, H. T., Lee, M. K., Sikdar, S., Ruiz-Arenas, C., et al. (2019). Epigenome-wide meta-analysis of DNA methylation and childhood asthma. *The Journal of Allergy and Clinical Immunology 143*, 2062–2074.
Richmond, R. C., Simpkin, A. J., Woodward, G., Gaunt, T. R., Lyttleton, O., McArdle, W. L., et al. (2015). Prenatal exposure to maternal smoking and offspring DNA methylation

across the lifecourse: Findings from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Human Molecular Genetics 24*, 2201–2217.
Ringh, MV, Hagemann-Jensen, M, Needhamsen, M, Kular, L, Breeze, CE, Sjöholm, LK, ... Ekström, TJ (2019). Tobacco smoking induces changes in true DNA methylation, hydroxymethylation and gene expression in bronchoalveolar lavage cells. *EBioMedicine 46*, 290–304.
Riggs, A., & Porter, T. N. (1996). Overview of epgenetic mechanisms. In C. S. H. Cold Spring Harbor Laboratory Press, NY (Ed.), *Epigenetic mechanisms of gene regulation* (pp. 29–45).
Rodriguez-Rivera, I., Wu, T., Ciotti, R., Senapedis, W., Sullivan, K., Gao, J. Z., et al. (2023). A phase 1/2 open-label study to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of OTX-2002 as a single agent and in combination with standard of care in patients with hepatocellular carcinoma and other solid tumor types known for association with the MYC oncogene (MYCHELANGELO I). *Journal of Clinical Oncology 41*.
Ruiz Garcia, S., Deprez, M., Lebrigand, K., Cavard, A., Paquet, A., Arguel, M. J., et al. (2019). Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. *Development 146*.
Sakornsakolpat, P., Prokopenko, D., Lamontagne, M., Reeve, N. F., Guyatt, A. L., Jackson, V. E., et al. (2019). Genetic landscape of chronic obstructive pulmonary disease identifies heterogeneous cell-type and phenotype associations. *Nature Genetics 51*, 494–505.
Sanders, Y. Y., Ambalavanan, N., Halloran, B., Zhang, X., Liu, H., Crossman, D. K., et al. (2012). Altered DNA methylation profile in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine 186*, 525–535.
Sanders, Y. Y., Liu, H., Scruggs, A. M., Duncan, S. R., Huang, S. K., & Thannickal, V. J. (2017). Epigenetic regulation of Caveolin-1 gene expression in lung fibroblasts. *American Journal of Respiratory Cell and Molecular Biology 56*, 50–61.
Sanders, Y. Y., Pardo, A., Selman, M., Nuovo, G. J., Tollefsbol, T. O., Siegal, G. P., et al. (2008). Thy-1 promoter hypermethylation: a novel epigenetic pathogenic mechanism in pulmonary fibrosis. *American Journal of Respiratory Cell and Molecular Biology 39*, 610–618.
Saunderson, E. A., Stepper, P., Gomm, J. J., Hoa, L., Morgan, A., Allen, M. D., et al. (2017). Hit-and-run epigenetic editing prevents senescence entry in primary breast cells from healthy donors. *Nature Communications 8*, 1450.
Schubeler, D. (2015). Function and information content of DNA methylation. *Nature 517*, 321–326.
Schultz, M. D., He, Y., Whitaker, J. W., Hariharan, M., Mukamel, E. A., Leung, D., et al. (2015). Human body epigenome maps reveal noncanonical DNA methylation variation. *Nature 523*, 212–216.
Schutsky, E. K., DeNizio, J. E., Hu, P., Liu, M. Y., Nabel, C. S., Fabyanic, E. B., et al. (2018). Nondestructive, base-resolution sequencing of 5-hydroxymethylcytosine using a DNA deaminase. *Nature Biotechnology 36*, 1083–1090.
Schwartz, U., Llamazares Prada, M., Pohl, S. T., Richter, M., Tamas, R., Schuler, M., et al. (2023). High-resolution transcriptomic and epigenetic profiling identifies novel regulators of COPD. *The EMBO Journal 42*, Article e111272.
Scruggs, A. M., Koh, H. B., Tripathi, P., Leeper, N. J., White, E. S., & Huang, S. K. (2018). Loss of CDKN2B promotes fibrosis via increased fibroblast differentiation rather than proliferation. *American Journal of Respiratory Cell and Molecular Biology 59*, 200–214.
Seiler, C. L., Song, J. U. M., Kotandeniya, D., Chen, J. J., Kono, T. J. Y., Han, Q. Y., et al. (2020). Inhalation exposure to cigarette smoke and inflammatory agents induces epigenetic changes in the lung. *Scientific Reports 10*.
Sheikhpour, M., Maleki, M., Ebrahimi Vargoorani, M., & Amiri, V. (2021). A review of epigenetic changes in asthma: Methylation and acetylation. *Clinical Epigenetics 13*, 65.
Shenker, N. S., Polidoro, S., van Veldhoven, K., Sacerdote, C., Ricceri, F., Birrell, M. A., et al. (2013a). Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking. *Human Molecular Genetics 22*, 843–851.
Shoeb, M., Mustafa, G. M., Kodali, V. K., Smith, K., Roach, K. A., Boyce, G., et al. (2020). A possible relationship between telomere length and markers of neurodegeneration in rat brain after welding fume inhalation exposure. *Environmental Research 180*, Article 108900.
Silva, C. P., & Kamens, H. M. (2021). Cigarette smoke-induced alterations in blood: A review of research on DNA methylation and gene expression. *Experimental and Clinical Psychopharmacology 29*, 116–135.
Singer, B. D. (2019). A practical guide to the measurement and analysis of DNA methylation. *American Journal of Respiratory Cell and Molecular Biology 61*, 417–428.
Smith, Z. D., Hetzel, S., & Meissner, A. (2024). DNA methylation in mammalian development and disease. *Nature Reviews. Genetics*. https://doi.org/10.1038/s41576-024-00760-8.
Smith, Z. D., & Meissner, A. (2013). DNA methylation: Roles in mammalian development. *Nature Reviews. Genetics 14*, 204–220.
Solazzo, G., Ferrante, G., & La Grutta, S. (2020). DNA methylation in nasal epithelium: Strengths and limitations of an emergent biomarker for childhood asthma. *Frontiers in Pediatrics 8*, 256.
Song, J., Cano-Rodriquez, D., Winkle, M., Gjaltema, R. A., Goubert, D., Jurkowski, T. P., et al. (2017). Targeted epigenetic editing of SPDEF reduces mucus production in lung epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology 312*, L334–L347.
Song, J., & Kuan, P. F. (2022). A systematic assessment of cell type deconvolution algorithms for DNA methylation data. *Briefings in Bioinformatics 23*.
Sood, A., Petersen, H., Blanchette, C. M., Meek, P., Picchi, M. A., Belinsky, S. A., et al. (2010). Wood smoke exposure and gene promoter methylation are associated with increased risk for COPD in smokers. *American Journal of Respiratory and Critical Care Medicine 182*, 1098–1104.
Spainhour, J. C., Lim, H. S., Yi, S. V., & Qiu, P. (2019). Correlation patterns between DNA methylation and gene expression in the cancer genome atlas. *Cancer Informatics 18* 1176935119828776.
Stadler, M. B., Murr, R., Burger, L., Ivanek, R., Lienert, F., Schöler, A., et al. (2011). DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature 480*, 490–495.
Stepper, P., Kungulovski, G., Jurkowska, R. Z., Chandra, T., Krueger, F., Reinhardt, R., et al. (2017). Efficient targeted DNA methylation with chimeric dCas9-Dnmt3a-Dnmt3L methyltransferase. *Nucleic Acids Research 45*, 1703–1713.
Stewart, S. K., Morris, T. J., Guilhamon, P., Bulstrode, H., Bachman, M., Balasubramanian, S., et al. (2015). oxBS-450K: a method for analysing hydroxymethylation using 450K BeadChips. *Methods 72*, 9–15.
Stunnenberg, H. G., International Human Epigenome, C, & Hirst, M. (2016). The international human epigenome consortium: A blueprint for scientific collaboration and discovery. *Cell 167*, 1145–1149.
Sugden, K., Hannon, E. J., Arseneault, L., Belsky, D. W., Corcoran, D. L., Fisher, H. L., et al. (2020). Patterns of reliability: Assessing the reproducibility and integrity of DNA methylation measurement. *Patterns (N Y)*, 1.
Sun, Y, Hong, JH, Ning, Z, Pan, D, Fu, X, Lu, X, & Tan, J (2022). Therapeutic potential of tucidinostat, a subtype-selective HDAC inhibitor, in cancer treatment. *Front Pharmacol 13*, 932914.
Sundar, I. K., Yin, Q., Baier, B. S., Yan, L., Mazur, W., Li, D., et al. (2017). DNA methylation profiling in peripheral lung tissues of smokers and patients with COPD. *Clinical Epigenetics 9*, 38.
Svoboda, L. K., Perera, B. P. U., Morgan, R. K., Polemi, K. M., Pan, J., & Dolinoy, D. C. (2022). Toxicoepigenetics and environmental health: Challenges and opportunities. *Chemical Research in Toxicology 35*, 1293–1311.
Szyf, M. (2011). The implications of DNA methylation for toxicology: Toward toxicomethylomics, the toxicology of DNA methylation. *Toxicological Sciences 120*, 235–255.
Teschendorff, A. E., & Zheng, S. C. (2017). Cell-type deconvolution in epigenome-wide association studies: a review and recommendations. *Epigenomics 9*, 757–768.
Teschendorff, A. E., Zhu, T., Breeze, C. E., & Beck, S. (2020). EPISCORE: Cell type deconvolution of bulk tissue DNA methylomes from single-cell RNA-Seq data. *Genome Biology 21*, 221.
Thangavel, J., Malik, A. B., Elias, H. K., Rajasingh, S., Simpson, A. D., Sundivakkam, P. K., et al. (2014). Combinatorial therapy with acetylation and methylation modifiers attenuates lung vascular hyperpermeability in endotoxemia-induced mouse inflammatory lung injury. *The American Journal of Pathology 184*, 2237–2249.
Thienpont, B., Steinbacher, J., Zhao, H., D'Anna, F., Kuchnio, A., Ploumakis, A., et al. (2016). Tumour hypoxia causes DNA hypermethylation by reducing TET activity. *Nature 537*, 63–68.
Thurmann, L., Klos, M., Mackowiak, S. D., Bieg, M., Bauer, T., Ishaque, N., et al. (2023). Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing preferentially affects enhancer regions. *Allergy 78*, 1489–1506.
Titus, A. J., Gallimore, R. M., Salas, L. A., & Christensen, B. C. (2017). Cell-type deconvolution from DNA methylation: a review of recent applications. *Human Molecular Genetics 26*, R216–R224.
Travaglini, K. J., Nabhan, A. N., Penland, L., Sinha, R., Gillich, A., Sit, R. V., et al. (2020). A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature 587*, 619–625.
Turner, B. M. (1993). Decoding the nucleosome. *Cell 75*, 5–8.
Uszczynska-Ratajczak, B., Lagarde, J., Frankish, A., Guigo, R., & Johnson, R. (2018). Towards a complete map of the human long non-coding RNA transcriptome. *Nature Reviews. Genetics 19*, 535–548.
Vaisvila, R., Ponnaluri, V. K. C., Sun, Z., Langhorst, B. W., Saleh, L., Guan, S., et al. (2021). Enzymatic methyl sequencing detects DNA methylation at single-base resolution from picograms of DNA. *Genome Research 31*, 1280–1289.
Van de Sande, B., Lee, J. S., Mutasa-Gottgens, E., Naughton, B., Bacon, W., Manning, J., et al. (2023). Applications of single-cell RNA sequencing in drug discovery and development. *Nature Reviews. Drug Discovery 22*, 496–520.
Varley, K. E., Gertz, J., Bowling, K. M., Parker, S. L., Reddy, T. E., Pauli-Behn, F., et al. (2013). Dynamic DNA methylation across diverse human cell lines and tissues. *Genome Research 23*, 555–567.
Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., et al. (2001). The sequence of the human genome. *Science 291*, 1304–1351.
Vogel, C. F. A., & Haarmann-Stemmann, T. (2017). The aryl hydrocarbon receptor repressor - more than a simple feedback inhibitor of AhR signaling: Clues for its role in inflammation and cancer. *Current Opinion in Toxicology 2*, 109–119.
Vucic, E. A., Chari, R., Thu, K. L., Wilson, I. M., Cotton, A. M., Kennett, J. Y., et al. (2014). DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. *American Journal of Respiratory Cell and Molecular Biology 50*, 912–922.
Wain, L. V., Shrine, N., Miller, S., Jackson, V. E., Ntalla, I., Soler Artigas, M., et al. (2015). Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. *The Lancet Respiratory Medicine 3*, 769–781.
Walters, G. I. (2020). Occupational exposures and idiopathic pulmonary fibrosis. *Current Opinion in Allergy and Clinical Immunology 20*, 103–111.
Wan, E. S., Qiu, W., Carey, V. J., Morrow, J., Bacherman, H., Foreman, M. G., et al. (2015). Smoking-associated site-specific differential methylation in buccal mucosa in the COPDGene study. *American Journal of Respiratory Cell and Molecular Biology 53*, 246–254.
Weber, M., Hellmann, I., Stadler, M. B., Ramos, L., Paabo, S., Rebhan, M., et al. (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nature Genetics 39*, 457–466.

Wei, A., Gao, Q., Chen, F., Zhu, X., Chen, X., Zhang, L., et al. (2022). Inhibition of DNA methylation de-represses peroxisome proliferator-activated receptor-gamma and attenuates pulmonary fibrosis. *British Journal of Pharmacology 179*, 1304–1318.
Wiklund, P., Karhunen, V., Richmond, R. C., Parmar, P., Rodriguez, A., De Silva, M., et al. (2019). DNA methylation links prenatal smoking exposure to later life health outcomes in offspring. *Clinical Epigenetics 11*, 97.
Wolfe, S. A., Nekludova, L., & Pabo, C. O. (2000). DNA recognition by Cys2His2 zinc finger proteins. *Annual Review of Biophysics and Biomolecular Structure 29*, 183–212.
Wu, D. -D., Song, J., Bartel, S., Krauss-Etschmann, S., Rots, M. G., & Hylkema, M. N. (2018). The potential for targeted rewriting of epigenetic marks in COPD as a new therapeutic approach. *Pharmacology & Therapeutics 182*, 1–14.
Wu, X., & Zhang, Y. (2017). TET-mediated active DNA demethylation: Mechanism, function and beyond. *Nature Reviews. Genetics 18*, 517–534.
Wyss, A. B., Sofer, T., Lee, M. K., Terzikhan, N., Nguyen, J. N., Lahousse, L., et al. (2018). Multiethnic meta-analysis identifies ancestry-specific and cross-ancestry loci for pulmonary function. *Nature Communications 9*, 2976.
Xu, C. J., Soderhall, C., Bustamante, M., Baiz, N., Gruzieva, O., Gehring, U., et al. (2018). DNA methylation in childhood asthma: An epigenome-wide meta-analysis. *The Lancet Respiratory Medicine 6*, 379–388.
Xu, G. L., & Bestor, T. H. (1997). Cytosine methylation targetted to pre-determined sequences. *Nature Genetics 17*, 376–378.
Yang, IV, Pedersen, BS, Liu, AH, O'Connor, GT, Pillai, D, Kattan, M, et al. (2017). The nasal methylome and childhood atopic asthma. *J Allergy Clin Immunol 139*(5), 1478–1488.
Yang, I. V., Pedersen, B. S., Rabinovich, E., Hennessy, C. E., Davidson, E. J., Murphy, E., et al. (2014). Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine 190*, 1263–1272.
Yin, Y., Morgunova, E., Jolma, A., Kaasinen, E., Sahu, B., Khund-Sayeed, S., et al. (2017). Impact of cytosine methylation on DNA binding specificities of human transcription factors. *Science 356*, Article eaaj2239.
Yoo, S., Takikawa, S., Geraghty, P., Argmann, C., Campbell, J., Lin, L., et al. (2015). Integrative analysis of DNA methylation and gene expression data identifies EPAS1 as a key regulator of COPD. *PLoS Genetics 11*, Article e1004898.
Yousefi, P. D., Suderman, M., Langdon, R., Whitehurst, O., Davey Smith, G., & Relton, C. L. (2022). DNA methylation-based predictors of health: Applications and statistical considerations. *Nature Reviews. Genetics 23*, 369–383.
Yu, M., Hon, G. C., Szulwach, K. E., Song, C. X., Zhang, L., Kim, A., et al. (2012). Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome. *Cell 149*, 1368–1380.
Yu, X., Zhao, H., Wang, R., Chen, Y., Ouyang, X., Li, W., et al. (2024). Cancer epigenetics: From laboratory studies and clinical trials to precision medicine. *Cell Death Discovery 10*, 28.
Zeilinger, S., Kuhnel, B., Klopp, N., Baurecht, H., Kleinschmidt, A., Gieger, C., et al. (2013). Tobacco smoking leads to extensive genome-wide changes in DNA methylation. *PLoS One 8*, Article e63812.
Zhang, X., Biagini Myers, J. M., Burleson, J. D., Ulm, A., Bryan, K. S., Chen, X., et al. (2018). Nasal DNA methylation is associated with childhood asthma. *Epigenomics 10*, 629–641.
Zhang, Y., Jurkowska, R., Soeroes, S., Rajavelu, A., Dhayalan, A., Bock, I., et al. (2010). Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic Acids Research 38*, 4246–4253.
Zhao, S., Allis, C. D., & Wang, G. G. (2021). The language of chromatin modification in human cancers. *Nature Reviews. Cancer 21*, 413–430.
Zhou, H., Liu, J., Zhou, C., Gao, N., Rao, Z., Li, H., et al. (2018). In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. *Nature Neuroscience 21*, 440–446.
Zhu, T., Liu, J., Beck, S., Pan, S., Capper, D., Lechner, M., et al. (2022). A pan-tissue DNA methylation atlas enables in silico decomposition of human tissue methylomes at cell-type resolution. *Nature Methods 19*, 296–306.
Zhuang, B. C., Jude, M. S., Konwar, C., Ryan, C. P., Whitehead, J., Engelbrecht, H. -R., et al. (2024). *Comparison of Infinium MethylationEPIC v2.0 to v1.0 for human population epigenetics: Considerations for addressing EPIC version differences in DNA methylation-based tools.* bioRxiv, 2024.2007.2002.600461.
Zong, D., Liu, X., Li, J., Ouyang, R., & Chen, P. (2019). The role of cigarette smoke-induced epigenetic alterations in inflammation. *Epigenetics & Chromatin 12*, 65.