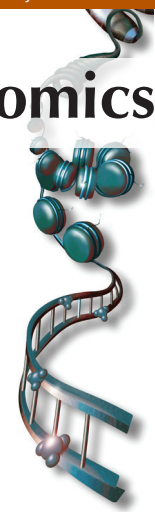
Review

For reprint orders, please contact: reprints@futuremedicine.com

Epigenomics



Epigenome-modifying tools in asthma

Peter O Brook[1], Mark M Perry[1], Ian M Adcock[1] & Andrew L Durham*,[1]

[1]Imperial College London, National Heart & Lung Institute, Dovehouse Street, London, SW3 6LY, UK

*Author for correspondence: a.durham@imperial.ac.uk

Asthma is a chronic disease which causes recurrent breathlessness affecting 300 million people worldwide of whom 250,000 die annually. The epigenome is a set of heritable modifications and tags that affect the genome without changing the intrinsic DNA sequence. These marks include DNA methylation, modifications to histone proteins around which DNA is wrapped and expression of noncoding RNA. Alterations in all of these processes have been reported in patients with asthma. In some cases these differences are linked to disease severity and susceptibility and may account for the limited value of genetic studies in asthma. Animal models of asthma suggest that epigenetic modifications and processes are linked to asthma and may be tractable targets for therapeutic intervention.

Keywords: asthma • DNA methylation • epigenetics • histone modification • miRNA • T cell • therapeutics

Asthma is a chronic disease of the airways that causes reversible difficulty in breathing through bronchoconstriction, mucus hypersecretion and airway remodeling and afflicts over 300 million people worldwide [1,2]. Asthma is a heterogeneous disease that can be atopic or nonatopic, and demonstrates various subclinical phenotypes [3]. The variety in asthma phenotypes provides challenges to treatment as phenotyping asthma is neither easy nor readily affordable. This may account for the failure of many drugs to proceed beyond early Phase II studies as patients are not adequately phenotyped [4]. Until better matching of phenotypes to driver pathways or molecules is achieved, phenotype-specific treatment using expensive biologicals for example will not be cost effective [5].

The adaptive immune response in asthma is regulated by $CD4^+$ T-cell subsets. The principle two subtypes of T helper cells are type 1 (Th1) and type 2 (Th2) which drive the cellular and humoral immune responses respectively [6]. Asthma is characterized at the cellular level by hyper-responsiveness of Th2 in both atopic (allergic) and nonatopic asthmatics [7,8]. In susceptible individuals, the Th2 response is stimulated by environmental effectors including allergens, temperature, humidity and air pollution [6]. This process of activation in allergic asthma begins with the airway epithelium, which upon stimulation releases factors which subsequently activate phagocytic cells and together they enable the activation of Th2 cells [6]. Th2 cells are able to self-propagate by the release of IL-4; drive infiltration of eosinophils by releasing IL-5 and activate B cells, which release antibodies against the allergens, by releasing IL-13 [6]. IL-13 also plays a major modulating role on airway epithelial cells increasing the production of mucins, periostin and other mediators [9].

Other T-cell subtypes also play a role in asthma, including the balance of the IL-17-producing Th17 cells and Treg. Th17 cells are associated with neutrophilic inflammation [10] and have been shown to contribute to severe asthma and relative corticosteroid insensitivity [11]. By contrast, Treg cells are able to repress cytokine release and proliferation from other T-cell subtypes [12], including

Future Medicine part of fsg

Th17 cells. The balance of Th17/Treg cells in peripheral blood of asthmatics is skewed toward Th17 [13,14] which inhibits the resolution of inflammation and increases neutrophilic infiltration.

Generally asthma is well controlled by inhaled corticosteroids and bronchodilators; however, 10% of patients suffer from 'severe' asthma that is poorly controlled even by high doses of inhaled and oral steroids and other treatments such as theophylline, anti-IgE or leukotriene receptor agonists [5]. While asthma can be controlled it cannot be cured, therefore the development of new treatments and identification of novel drug targets are a priority in asthma research.

Asthma has been shown to have a heritable component in large twin studies [15], and by using polygenic heritability estimates [16]. A heritable phenotype can be the product of many different mechanisms; these can be divided into two categories: those that change the DNA sequence and those that do not. To date much of the research into the heritability of asthma has focused on the changes to the DNA sequence, however large-scale genome-wide association studies (GWAS) have only identified a handful of genetic changes or SNPs linked to asthma which are highly significant on a population scale but not predictive at the individual level [17,18]. It is also unknown whether these changes are causative and it is likely that causality will be linked to different environmental exposures in selected subphenotypes of asthma.

Research is therefore becoming more focused on heritable characteristics that are not due to altered DNA, termed epigenetic modifications, and the sum of these modifications termed the epigenome. These epigenetic processes include modifications to DNA-binding histones, applying methylation marks to cysteine in DNA and noncoding RNAs such as miRNA [19].

To date studies of the epigenome in asthmatics have demonstrated changes in monocytic DNA methylation [20] and histone modification [21], blood leukocyte [22] and eosinophil [23] methylation, $CD4^+$ T-cell histone modifications [24–26] and smooth muscle and T-cell miRNA expression [27,28] and histone acetylation at distinct residues [29] compared with their healthy controls. What these data are unable to show is whether these changes cause, or are a result of, asthma although animal models may help resolve this issue.

Various techniques have been developed to investigate epigenetic regulation of gene expression. These include using methods such as chromatin immunoprecipitation (ChIP) to confirm specific histone modifications at single gene promoters or at a genome-wide level, or to map the location of specific histone modifications with chromatin structure (using ChIP-Seq [24] and DNase 1 hypersensitivity [30]), measuring DNA methylation (bisulfite sequencing using array or next-generation sequencing [31]) and expression profiles of noncoding RNA (gene array or PCR-based analysis [32]). There is growing number of available tools which can alter the extent to which cells modify histones and methylate DNA which means we can begin to investigate the role of epigenetic modifications in asthma and identify potential new therapeutics. It is important to note that each cell type has a distinct epigenome and it is important to examine changes with disease in single cell types [33] or to use bioinformatic tools to deconvolute data to allocate to single cells [23].

The encyclopedia of DNA elements (ENCODE [34]) a project to catalog the regulatory elements in human cells and its follow-up of the Epigenome Roadmap [35] are two projects that have built reference epigenomes for 127 tissue and cell types [36,37]. The Epigenome Roadmap has been able to gather reference epigenomes for a variety of T-cell subtypes and B cells and this information is ripe for analysis if compared with more asthmatic samples.

Histone modifications

Nucleosomes are octomeric complexes of histone proteins which bind to DNA. Each octomer comprises two pairs of the histones H2A and H2B and two pairs of the histones H3 and H4 [38]. 146bp of DNA is wrapped and stored around each nucleosome which are tethered together by histone H1 [39].

Histones serve multiple functions in normally functioning cells: they store DNA safely [38], they can stop transcription by tightly coiling DNA [40] and they can encourage transcription by forming a suitable local structure to enable accessibility of transcription factors [41]. When the DNA is loosely bound to the histones in what is known as the euchromatin or 'open' state, transcription may readily take place [42]. However, when histones are tightly bound to DNA, known as the heterochromatin or 'closed' state, DNA polymerase II and transcriptional activators are unable to readily access DNA and transcription is limited. The strength of binding between histones and DNA can be controlled by post-transcriptional modifications to the N-terminal tail of the histone proteins, usually pertaining to the addition of acetyl or methyl groups to amino acids such as lysine and arginine [43].

These modifications can change the charge of the histones which either repels DNA if more negatively charged, or attract DNA if more positively charged [44]. Alternatively, these modifications on histones residues form epitopes that enable other proteins including transcription factors and transcriptional co-factors to be recruited to the site of original driver modification in a temporal manner. The site-specific and time-

dependent formation of these histone modifications, including acetylation and methylation, is termed the histone code or language. The histone code is 'read' by proteins termed readers which in turn help recruit the transcription factors. Although the histone code is a useful concept, the myriad numbers of different combinations of modifications that can occur means that this has not been proven. In addition, phylogenetic analysis of histone deacetylases (HDACs) and histone acetyltransferases (HATs) suggests that these existed before histones implicating that acetylation of nonhistone proteins will play an important role in regulating cellular function [45]. Indeed, it is now clear that many cytoplasmic and nuclear proteins including transcription factors, signaling molecules and structural proteins are acetylated.

Histone acetylation

The role of histone acetylation

Histone acetylation is primarily associated with the euchromatin state (loosely packed DNA) and increased transcription as acetylation is associated with a positive electrostatic charges of the histones, repelling the DNA and increasing the DNA's accessibility and ability to be transcribed [44,45]. Multiple lysine residues on a single histone subunit can be acetylated. For example acetylation on histone 3 lysine 9 (H3K9) and lysine 27 (H3K27) have been shown to increase transcription [45,46].

Histone acetylation in asthma

HAT activity is increased in asthmatic biopsies in both adults [47] and in children [48] and the HAT/HDAC ratio alters according to asthma severity. Histone 3 lysine 27 (H3K27) acetylation (H3K27Ac) is associated with the enhancer regions of genes that are actively being expressed [49]. H3K27 is acetylated by the HAT Gcn5. This tag has been shown to be an important component of the histone code and reanalysis of asthma GWAS achieved greater predictive power by only using SNPs in coding or regulatory regions and linking this to H3K27Ac and H3K4me1 marks in different cell types [25]. These two marks are critical for TH2 cell accumulation and differentiation in asthma although the H3K4me2 mark was the most highly enriched at sites encoding transcription factors and microRNAs [24].

Th2 cells and follicular helper T cells (Tfh cells) are the major producers of IL-4 but require distinct and overlapping, molecular mechanisms [50]. The DNA methylltransferase Dnmt3a also regulates Th2, particularly IL-13, expression. Loss of Dnmt3a leads to decreased DNA methylation and changes in the H3K27Ac status at the IL-13 locus and is associated with increased lung inflammation in a mouse model of asthma [51].

The deposition of acetyl tags appears less selective than the removal of these marks by HDACs as determined in a genome-wide acetylome analysis [52] HDACs remove acetylation marks from both histones and from nonhistone proteins such as the glucocorticoid receptor (GR) and other transcription factors. A combination of GR deacetylation and changes in histone acetylation status are involved in the mechanisms by which corticosteroids reduce the expression of NF-κB-activated inflammatory genes [53]. Defective HDAC2 expression and activity is found in some corticosteroid-insensitive disease cells/tissues and models and corticosteroid sensitivity can be restored by increasing HDAC2 expression [40,54]. This is highly relevant to severe asthma, the form of asthma that remains steroid insensitive.

HDAC1 function is vital to the repair and remodeling of the airway epithelium and epithelial cell growth stops upon HDAC1 inhibition [55]. Epithelial cells form the principle barrier between the lung and the environment and induce the first stages of the immune response during an asthma attack. Increased airway epithelium and remodeling is associated with asthma severity [56] and correspondingly increased HDAC1 is found in patients with severe asthma compared with normal patients and may represent a biomarker to distinguish between severe and nonsevere asthma [57].

In addition to enabling general switching between the eu- and hetero-chromatin states, site-specific histone acetylation also promotes transcription factor binding. One key example for asthma is the binding of the pro-inflammatory transcription factor NF-κB. Acetylation of histones at H3K9 and K27 is recognized by histone code readers including the bromodomain containing family of proteins. One such protein, Brd4, contains two bromodomain regions and is able to bind acetylated histones and acetylated p65 (part of the NF-κB complex). NF-κB p65 activation is controlled by its acetylation status, for example, on K310 and binding of Brd proteins is important for controlling full NF-κB activity in cell-dependent manner [58]. Thus, Brd proteins can bring together nucleosomes and nuclear transcription factors to increase proliferation and inflammation [59,60]. Acetylated histone's ability to bring together DNA and transcription factors positions it as vital pivot for the expression of specific inflammatory genes regulated by NF-κB.

Potential asthma therapies targeting histone acetylation

As histone (de)acetylation plays a role in the activation of inflammation and its resolution, drugs that target histone acetylases and deacetylases have been

investigated in asthma (Table 1). Pan-histone deacetylase inhibitors, such as Trichostatin A (TSA) and Vorinostat, have been used as cancer treatments and have been investigated in asthma although their efficacy at reducing inflammation remains controversial probably due to their lack of selectivity [61]. TSA reduces inflammation in human precision cut lung slices and in *in vivo* mouse models [61]. In two recent studies TSA treatment reduced inflammation, IL-17 and T-helper cell number while increasing Treg-cell activation [62]. Furthermore, the expression of TGF-β in the bronchoalveolar lavage fluid was increased following HDAC inhibition in a mouse model of asthma [62,63]. These changes were associated with increased acetylation at the TGF-β promoter. It is also of interest to note that this study also showed that severity of asthma was linked to HDAC9 [62].

Vorinostat was the first HDAC inhibitor approved by the US FDA for cutaneous T-cell lymphoma. It was demonstrated to be beneficial in graft-versus-host disease (GVHD) as it increased activation of Tregs [63]. HDAC inhibition reduced plasma cytokine levels without inhibiting T-cell responses to nonspecific stimuli allowing the immune system to continue to function.

While pan-HDAC inhibitors show anti-inflammatory effects in some studies, other studies are unable to replicate these effects [78–81]. There is also evidence that HDAC inhibitors can in fact enhance inflammation through NF-κB-driven inflammatory gene transcription [60–62]. The use of HDAC inhibitors as a potential treatment for asthma is further complicated by the role of HDAC proteins in the anti-inflammatory response to glucocorticoids [61]. As reduced HDAC2 activity is associated with steroid insensitivity, the activation, rather than inhibition of HDAC2, would be beneficial. The drug theophylline has been associated with improved steroid sensitivity in several studies and has a putative mechanism of action which involves restoring HDAC2 activation [82]. It has been shown to improve steroid sensitivity in COPD which may be similar in mechanism to asthma [83].

The multiple roles of histone acetylation in regulating cell function and cell division [84] mean that pan-HDAC inhibitors or activators will almost certainly have large negative side effects if used clinically. More recently, a link between HDAC inhibition of STAT5-activated gene expression and Brd functions has been reported [85]. The consensus is that more work is needed to explore how epigenome modifying drugs will effect acetylation mechanisms in airways disease [86,87], in order to overcome the principle issue of targeting the change in acetylation status to the relevant genes.

Targeted modifications to histone acetylation have been undertaken in neurones using modified transcription factors to increase acetylation at the Fosb gene [88]. If targeted transcription factors can be used to increase acetylation then it is possible that they can be used to decrease acetylation as tools and potentially therapies. Many histone modifications including H3K9 acetylation H3K27me3, H3K9me1 and H4K8ac have been regulated *in vitro* by using light-inducible transcriptional effectors (LITEs). These are a set of blue-light activated restriction enzymes that have been developed for high level temporal and spatial control of target gene expression and may be used to target specific histone effectors and thereby modify the epigenome. For example, H3K9 acetylation was reduced twofold at the target gene Grm2 using this method resulting in repression of Grm2 [89]. This approach may pave the way for epigenome modification *in vivo*.

The bromo and extraterminal (BET) family of proteins recognise lysine acetylation on many proteins including histones [90], which aids the recruitment of transcription factors and the RNA polymerase transcription complex to enable gene transcription [91]. The BET protein Brd4 binds to acetylated histones in the euchromatin state [92] but can also interact with other nonhistone proteins [60] such as NF-κB [93]. Mimics of BET proteins such as JQ1 [94] reduce asthma relevant processes including IL-1β-induced inflammation [95] and proliferation [96]. JQ1 and similar compounds are currently being investigated as anticancer treatments in man [64] but the broad action of the BET family inhibitors may require the development of novel compounds with different selectivity. Alternatively, the use of inhaled delivery to the airways, linked to improved lung retention and rapid systemic breakdown, may reduce the side effect profile to enable thier use in asthma.

Histone methylation

Lysine residues in histone tails can be modified to have between one and three methyl groups: mono, di or trimethylation. The functional effect of the modification depends upon the residue targeted and the number of methyl groups added.

The role of H3K4 methylation

Genes have pretranscriptional regions before transcriptional start site and by modifying this region control can be exerted over gene expression. Histone modifications in this region can either inhibit or activate transcription; known as a bivalent gene or effect. When two contradictory acting histone modifications are bound at the same promoter the inhibitory marker takes precedence [97], leading

Table 1. List of epigenetic modifying tool compounds.

Epigenetic mark	Target	Tool compound	Affected process
Histone acetylation	Pan histone deacetylase inhibitors	Trichostatin A	Cancer treatment reduced IL-17 and T helper cell number, reduced TGF-β in mouse BAL [62,63]
		Vorinostat	Cancer treatment and graft vs host disease [63]
	BET domain mimics – block readers of acetylation	JQ1	Prevent cell cycle progression and investigated as an anticancer treatment [64,65]
Histone methylation:			
– Histone 3 lysine 4 (H3K4)	SETD7	PFI-2	H3K4 is linked to activation of inflammatory responses [26,66]
– Histone 3 lysine 9 (H3K9)	G9a (H3K9 methyltransferase)	UNC0642	Macrophages and dendritic cells of asthmatics undergoing allergen exposure may benefit from the inhibition of H3K9 methyltransferases, to prevent activation of the inflammation
	JMJD2 H3K9 demethylase	ML324	Decrease the effects of Herpes virus in mice [67]
– Histone 3 lysine 27 (H3K27)	JMJ3D (H3K27 demethylase)	GSK-J1 + GSK-J4	Inhibits LPS-induced macrophage inflammation [68]
	Lysine-specific demethylases	Compound 12d	Additive effects with HDAC inhibitors on inhibiting cell proliferation and may inhibit inflammatory cytokines [69]
DNA methylation	DNMT1 complexes		DNMT inhibitors may return T cells to a Th1 phenotype in asthma [70]
	DNMT1/PCNA	Peptide inhibitor - 163–174	Target DNA methylation at specific regions [71]
	DNMT1/USP7	Peptide inhibitor - 561–567	Target DNA methylation at specific regions [71]
	DNMT1/STAT3	Peptide inhibitor - 683–174	Target DNA methylation at specific regions [71]
	DNMT1/CFP1	Peptide inhibitor - 1081–1097	Target DNA methylation at specific regions [71]
miRNAs	miR-34 mimic	MRX34	Inhibits tumor growth [72]
	miR-122		Hepatitis C is supressed by the inhibition of miR-122 [73]
	miR-150	Nanovesicles containing miR-150	Enter effector T cells and suppressing allergic contact dermatitis and promoting antigen-specific tolerance in mice [74]
	miR-9	miR-9 antagamirs	Inhibition of miR-9 increased PP2A activity and GR nuclear translocation in macrophages and restored steroid sensitivity in multiple mouse models of steroid-resistant AHR [75]
	miR-145	miR-145 antagamir	miR-145 antagamir inhibited eosinophilic inflammation, mucus hypersecretion, Th2 cytokine production and AHR in murine model of asthma [76]

AHR: Airways hyper-responsiveness; BET: Bromo and extraterminal domain; GR: Glucocorticoid receptor; LPS: Lipopolysaccharide.

Table 1. List of epigenetic modifying tool compounds (cont.).

Epigenetic mark	Target	Tool compound	Affected process
miRNAs (cont.)	miR-126	miR-126 antagamir	Blockade of miR-126 suppressed Th2 responses, inflammation, AHR, eosinophil recruitment and mucus hypersecretion in mouse model of asthma via suppression of GATA3 expression [77]

AHR: Airways hyper-responsiveness; BET: Bromo and extraterminal domain; GR: Glucocorticoid receptor; LPS: Lipopolysaccharide.

to transcriptional pause where the transcriptional complex is bound to the promoter but is halted and unable to generate RNA. Elongation will begin as soon as the inhibitory mark is removed and the RNA polymerase II is able to proceed. Di-methylated histone 3 lysine 4 (H3K4me2) marks are commonly found at poised and active enhancer regions [98].

H3K4 methylation in asthma

Histone methylation has been linked to both T-cell differentiation and function, particularly for the control of $CD4^+$ T-cell differentiation [26]. T-cell fate is principally controlled by the transcription factors T-BET (Th1 cells) and GATA3 (Th2). The ability of these transcription factors to regulate their target genes is dependent on their associated methyltransferase activity [66].

IL-4, IL-5 and IL-13 are all coded for in a single stretch of DNA on chromosome 5 and are separated by the *RAD50* gene. *RAD50* encodes a DNA repair protein [99] and has four conserved enhancer regions in its introns. H3K4me1 modifications at these enhancer regions, are increased in T cells from asthmatic patients [24] and are associated with transcriptional pause. Further environmental signals, such as antigen recognition, trigger other transcription factors to resolve the pause and enable transcription [100]. H3K4me3 is linked to increased transcription of both *IFNG* and *IL-4* [26].

Combining H3K4me2 ChIP-Seq with GWAS in subsets of human peripheral blood T cells (naive, T_H1 and Th2) has shown that the differentiation of Th2 cells is marked by an increased enrichment of H3K4me2 at SNPs within the promoters and cis-regulatory regions of asthma-associated genes including *CCR4* and *CCL5* [24]. CCR4 receptors have shown to be vital in the recruitment of Th2 cells to the lung [101] and CCL5 is chemotactic for T cells [102]. T-cell studies have found patterns of H3K4 dimethlyation at enhancers during Th2-cell differentiation that support a pathogenic role in asthma [24]. Using gene ontology software, it was shown that genes associated with mitosis and regulation of apoptosis were most differentially enriched in asthmatics.

Potential asthma therapies targeting H3K4 methylation

At present no therapeutically licenced drugs exist that target histone methylation, although new compounds, such as PFI-2 that target histone methylation have been developed [103]. PFI-2 competitively inhibits the SET domain containing (lysine methyltransferases) 7 (SETD7), a methyltransferase for H3K4 [104], which may play a role in cell stress and inflammation as SETD7 is able to activate expression at NF-κB binding sites.

As H3K4 methylation is associated with the activation of inflammatory and proasthmatic cytokine production preventing histone methyl-transferase activity may be of future benefit to patients. However, as with histone acetylation the ability to target histone modifications at specific sites, such as the asthma SNPs would be the ultimate goal of therapeutic research [24].

The role of H3K9 methylation

The presence of H3K9me3 at gene promoters is associated with gene repression including that of inflammatory genes [105]. H3K9me3 acts by preventing RNA Pol II binding to target gene promoters.

H3K9 in asthma

Airway remodeling is a cardinal feature of asthma and the control of it is mediated in part by VEGF which in asthmatics is hypersecreted by human airway smooth muscle cells (HASM). In asthmatic HASM there is a decrease in the H3K9me3 repressive complex at the promoter of the VEGF gene. The methyltransferase G9a is vital for repression of VEGF in healthy patients HASM [106].

JMJD2D is an H3K9me3 demethylase which removes H3K9me3 repression complexes, activating transcription [107]. In dendritic cells and macrophages, JMJD2D is induced by external stimulus and is required for *Mdc* and *Il12b* transcription. This is an example of how H3K9me3 is able to broadly control functional enhancers linked to cell-type-specific gene expression [105].

Potential asthma therapies targeting H3K9

Inhibitors of both H3K9 methyltransferases and demethylases have been recently developed. These

tools that target the enzymes G9a and JMJD2D prevent activation of the inflammation in macrophages and dendritic cells of asthmatics undergoing allergen exposure [106]. Similar treatments may be of use to limit airway remodeling in HASM cells [106]. UNC0642, a recently discovered inhibitor of G9a, may be a useful tool in future studies [108]. However, G9a inhibition may result in detrimental side effects. For example, while knockout of G9a reduces inflammation in cell culture it is essential for embryogenesis [106,109]. Therefore further research will be required to allow more targeted inhibition of histone methylation at specific gene loci, for example understanding how H3K9 methylation is targeted to specific genes.

The role of H3K27 methylation

H3K27me3 can have different functional effects on gene transcription depending on the location of the histone relative to the gene [110]. First, when the modified residues are located within the body of the gene, H3K27me3 inhibits gene expression; however, when H3K27me3 is found at the transcriptional start site it is associated with the expression of bivalent genes and transcriptional pausing. Finally H3K27me3 in the promoter region of a gene is associated with an increase in transcription [110].

H3K27 methylation in asthma

Trimethylation of H3K27 can be catalyzed by EZH2, which is a subunit of the polycomb repressive complex 2 [97] and has been associated with gene repression. EZH2 is a histone lysine methyltransferase which is highly specific to K3K27, and is vital for $CD4^+$ differentiation and activation [111]. H3K27me3 blocks the production of IL-4 in Th1 cells while in Th2 cells the gene body of the repressed *IFNG* was marked by H3K27me3 [111].

The ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX) and JMJD3 are H3K27-specific demethylases [112] which remove the H3K27me3 repressive marks [113]. JMJD3 and UTX regulate chromatin complexes [114], macrophage plasticity in mice, pro-inflammatory gene regulation [115] and T helper cell development [112]. JMJD3 knockdown in THP-1 monocyte cell line and macrophages decreased the effect of inflammatory signaling pathways as measured by gene array, by increasing H3K27me3 at the promoters of NF-κB induced genes and many members of the CD40 and chemokine signaling pathways [113,114].

In a mouse model of asthma, Th2 cells from and *Ezh2*-/- mice were adoptively transferred to wild-type mice which underwent a subsequent acute ovalbumin (OVA) challenge. The transfer of *Ezh2*-KO Th2 cells resulted in in eosinophilia, IL-4, IL-5 and IL-13 and mucus hyperproduction and enhanced asthma-like pathology [111]. There was also overproduction of IFN-γ indicating activation of both the Th1 and Th2 pathways which is often seen in human asthma. However, other studies suggest that blockade of *Ezh2* is not sufficient to prevent the production of inflammatory cytokines [111].

Epithelial cells respond to IL-4, by demethylating H3K27me3 at the *ALOX15* gene promoter increasing its transcription [116]. *ALOX15* oxygenates polyunsaturated fatty acids to synthesize potent signaling mediators and has been shown to increase in expression with increasing severity of asthma [117]. The *ALOX15* promoter is associated with H3K27me3 and its expression is regulated by UTX [117]. Whether this is causal in asthma, however, is unknown. Tool compounds for this demethylase include the nonselective inhibitor GSK-J4 [68].

Potential asthma therapies targeting H3K27 methylation

As the loss of EZH2 is linked to the asthma phenotype inhibiting the demethylases JMJD3 or UTX may be beneficial in asthma (Table 1). There is currently a selection of new tools and potential drugs available which will target the H3K27me3 modifying enzymes (JMJD3, UTX, EZH2) such as 1-substituted cyclopropylamine [118] an irreversibly binding, nonspecific H3K27 demethylase inhibitor. Blocking JMJD3 in macrophages with the inhibitors GSK-J1 and GSK-J4 prevents LPS-induced inflammation [68].

The phenelzine analog compound 12d is a lysine-specific demethylase inhibitor, it has been shown to have additive effects when used with HDAC inhibitors in inhibiting cell proliferation and may be useful to inhibit inflammatory cytokines as well [69]. Compounds targeting H3K27 methylation have not been therapeutically assessed, nor are all their effects understood. However they do provide potential tools to investigate role of histone methylation in inflammatory gene expression.

Generally histone demethylation is essential for dramatic changes in epigenetic states such as in cell differentiation of T cells; however, demethylase proteins that antagonize repression such as JMJD3 are expressed in terminally differentiated cells. JMJD3 has a separate role in chromatin remodeling which is independent from its H3K27-demethylase function. This demonstrates a role for JMJD3 outside of epigenetic modifications and must be taken into account when using drugs to modify the effects of them [112].

DNA methylation

The role of DNA methylation in asthma

DNA methylation is generally associated with gene repression and occurs at complementary pairs of cys-

teine residues which are directly followed by guanine (CpG) in mammals [119]. DNA methylation regulates many processes including cell fate, inactive X chromosomes and gene-specific activation and silencing. During cellular replication, the methylation marks are maintained by the DNA methyltransferase DNMT1 which converts hemimethylated DNA into fully methylated DNA [120]. During germ cell development, cell-specific methylation is totally removed and is then re-established by DNMT3A and DNMT3B, which can give rise to specific maternal and paternal gene expression profiles, termed genetic imprinting [119].

The importance of DNA methylation in allowing transcription is vital in the activation of the asthmatic immune response and restricting the DNA methylome in some cells may be of therapeutic benefit to patients. Naïve $CD4^+$ T cells show methylated CpG regions in the *IL-4* promoter which limits transcription. After house dust mite stimulation, DNA demethylation increases at the *IL-4* promoter in cells from patients with bronchial asthma, but not in controls [31]. To enforce differentiation to Th2 type $CD4^+$ T cells, Th1 type gene promoters, such as *IFNG*, are inhibited by enhancer DNA methylation. This is linked to decreased expression of IFN-γ in $CD4^+$ T cells [70]. Th2 cells can, however, reactivate the production of IFN-γ by demethylating the IFN-γ promoter, demonstrating the cell's epigenetic plasticity. The changes are mediated by GATA3 and T-bet, both of which are vital to T-cell development and differentiation [121].

DNMT1, the DNA methyltransferase that maintains DNA methylation, appears to be self-regulating in mouse asthma models. Following allergic stimulation, DNMT1 expression in the lung, trachea and bronchoalveolar lavage fluid cells was decreased in tandem with DNMT1 promoter DNA hypermethylation [122]. Studies examining zebrafish development have highlighted the cross-talk that occurs between epigenetic processes. The gene *lef1* is controlled by a network that includes Dnmt3 and G9a, the H3K9 methyltransferase. G9a and Dnmt3 seem to function simultaneously to silence critical regulators of cell fate [123].

The DNA methylation status at asthma-relevant SNPs within the IL-4R gene is associated with an increased risk of asthma at age 18 if the site was more highly methylated [22]. There are significant links between methylation status at 36 loci in peripheral blood eosinophils and the presence of serum IgE [23]. The implicated loci included eosinophil products and phospholipid inflammatory mediators and provide a list of potential new biomarkers and target genes for drug development against allergy and potentially allergic asthma.

Potential asthma therapies targeting DNA methylation

No DNA methylation targeting compounds have been investigated in relation to asthma *per se* (Table 1), however cancer drugs that target DNMT1-CFP1 and DNMT1-Stat3 complexes are able to demethylate specific regions of DNA associated with tumor suppressor genes without causing global DNA hypomethylation [71]. Drugs have been designed which are able to block DNMT1 complexes forming with HDAC1, STAT3, PCNA, CFP1 and USP7 which gives a broad scope for many other investigations into DNA methylation in T-cell differentiation. In addition to this targeting of DNMT at specific genes, knock-in mice that caused promoter hypermethylation at the INSL6 and the p16 genes and transcriptional suppression by adding a cis-acting regulatory element that attracts DNMT have been developed. This method could be applied to other genes as well [124].

Sections of RNA have been shown to modify the effects of DNMT. For example a region of RNA from the CEBPA locus (ecCEBPA) is able to interact with DNMT1 and prevent the methylation of its gene and increase the transcription of CEBPA. If this effect can be shown in other genes it may suggest a novel mechanism by which expressed RNA controls DNA methylation and will be a model to base new treatments off [125].

It is possibly that hyper and hypomethylation is modifiable so it is important that DNMT inhibitors may have beneficial effects in reducing allergen sensitization in asthmatics through the reversal of IFN-γ repression and the return of T cells to a Th1 phenotype [70]. At present DNMT1 inhibitors have been considered as an anticancer treatment, however making sure that they only inhibit hypermethylation of tumor suppressor genes without demethylating oncogenes is a technical challenge. The development of asthma therapeutics will also contain similar challenges. Excitingly as DNMTs act in complexes with other transcription factors, and their targeting is linked to the complexes they are associated with, such G9a [126], HDAC1 [127] and PCNA (proliferating cell nuclear antigen) [128], DNMT inhibitors have the potential to inhibit specific DNMTs that are associated with inflammation while leaving global DMNT activity unaffected.

miRNA in asthma

Noncoding RNAs (ncRNA) are functional RNAs which are not transcribed and may be important in respiratory disease [129]. They can be broadly subclassified into three groups; housekeeping RNAs (ribosomal, transfer, splicesomal), long noncoding (pseudogenes, intronic, intergenic) and the small noncoding

RNAs (PIWI-associated RNA, endogenous siRNA, microRNAs). microRNAs (miRNAs) are the most studied in respiratory disease, including asthma, cystic fibrosis [130] and lung carcinoma [131].

miRNAs are ~20 nt in length, are highly conserved across species and act as regulators of both genes and gene networks [132]. They induce mRNA degradation and/or inhibit mRNA translation, and it is predicted that as many as 60% of mRNAs are targets for miRNAs [133]. Conversely, long noncoding RNAs (lncRNAs) are greater than 200 nt in length and their mechanism of action can include regulation of both mRNA transcription and/or translation [129], and acting as 'sponges' for miRNAs [134]. Currently, the majority of studies of lncRNAs in the lung have been in relation to lung cancer [129].

The study of ncRNAs in disease typically involves examining the differential expression of the ncRNAs between different patient subsets (e.g., nondisease vs disease), concentrating upon a single ncRNA that is significantly changed in expression, and targeting said ncRNA to inhibit or overexpress its action (extensively reviewed by Booton and Lindsay 2014 [129]). A large number of miRNAs are differentially expressed in asthma in a predominantly cell-specific manner. These include miR-19 in T cells [24], miR-18 in epithelial cells [135] and miR-221 in HASM cells [136]. Work examining miRNA profiles show these are dramatically different in the bronchial epithelium of asthmatics compared with healthy subjects with 217 differentially expressed miRNA genes [137]. The use of corticosteroids (a standard asthma treatment) only had a limited effect in restoring normal miRNA expression in the asthmatic population, which still retained 200 differentially expressed genes compared with healthy subjects [137].

The role of miRNA in asthma

In addition to studies on primary human cells, many current studies defining the potential role of miRNAs in asthma utilize mouse models (Table 1). Intranasal administration of miR-1 inhibits inflammatory responses to ovalbumin (OVA) and house dust mite (HDM) in mouse models of asthma by inhibiting the effect of VEGF [138]. VEGF is able to lead to Th2 type gene expression and recruitment [138]. In addition, miR-145 is as effective as dexamethasone in preventing airway hyper-responsiveness and inflammation in an HDM model of asthma [76]. The same group has reported that antagamirs to miR-126 are able to overcome inflammation and airway hyper-responsiveness (AHR) in mouse models of Th2-driven asthma [77] and that miR-9 antagamirs can reverse steroid insensitivity by targeting protein phosphatase (PP)2A and the glucocorticoid receptor in various mouse models of steroid-insensitive asthma [75].

MicroRNAs have also been used as tools to suppress target genes *in vivo*. Intranasal delivery of a siRNA against suppressors of cytokine signaling (SOCS3) reduces lung eosinophil and airway hyper-responsiveness to methacholine following OVA challenge [32]. Furthermore, a siRNA directed against CD86, involved in T-cell-dendritic cell interactions, inhibit OVA-induced hyper-responsiveness, lung eosinophilia and serum IgE in mice [139].

Potential asthma therapies targeting miRNA

Utilizing noncoding RNA molecules as novel therapies for treating disease *in vivo*, is problematic. At present there are only two ncRNAs in clinical trials. A microRNA mimic (MRX34) which inhibits tumor growth and increases overall survival in mouse models, and is currently in Phase I testing in patients with primary or metastatic liver cancer [72]. A miR-122 mimic is in human clinical trials for hepatitis C [73].

The problems when utilizing miRNAs as a therapy, are numerous. For example, modifying miRNAs with a locked nucleic acid (LNA) structure, although making the miRNA more biologically stable, also activates distinct mechanistic pathways, suggesting that toxicity issues may vary drastically with different miRNA sequences [140]. To address this issue, attaching ZEN (*N*,*N*-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine) to both ends of a miRNA considerably enhances its binding affinity, results in a greater degree of miRNA inhibition and has low toxicity *in vitro* [140].

Additionally, another limiting factor is determining how to reach a sufficient dose within the cell in order to achieve efficient miRNA targeting. An imaging-based analytical method using fluorescence and electron microscopy to track intracellular transport and release of ncRNAs demonstrated that lipid nanoparticles (LNPs) enter cells through clathrin-mediated endocytosis and macropinocytosis, and that less than 2% of the ncRNAs escaped from the endosomes, suggesting that the efficiency of internalization and release of ncRNAs is extremely low [141].

This problem may also be overcome, by utilizing nanovesicles as delivery vectors [142]. For example, nanovesicles containing miR-150 are capable of entering effector T cells and suppressing allergic contact dermatitis (ACD) and promoting antigen-specific tolerance in mice [74]. At the time of writing, the human application remains unknown.

Finally, the mechanism of action of ncRNAs (both miRNAs and lncRNAs) may not be as simple as first thought. Evidence suggests that miRNAs may have a range of functions, including regulation of transcrip-

tion through epigenetic mechanisms; of translation by acting as decoys and of acting as inhibitors of other long noncoding RNAs [134,143]. Also, lncRNA are thought to have a range of mechanisms including regulation of both mRNA transcription and/or translation [129], and acting as 'sponges' for miRNAs [134]. Therefore, using ncRNAs as a potential therapy may produce numerous 'off-target effects'. That said, improved techniques for targeting ncRNAs, and increasing knowledge of their biological function could lead to the production of specific gene expression modulators for respiratory disease in the future.

Conclusion

Asthma is a heterogeneous disease of the airways, which in cases of severe asthma is currently untreatable. Asthma has both environmental and heritable components, which has not been directly linked to specific genes, implying control through epigenetic mechanisms, which can regulate cell fate, secretion profiles, inflammation and proliferation.

At present study into the role of epigenetics in the development and maintenance of asthma is in its infancy. As new epigenetic tools and techniques become available, the role of different epigenetic mechanisms in asthma is becoming better understood, and may potentially allow for the future development of novel treatments for asthma. The dynamic nature of epigenetic modifications, which unlike DNA modifications can be reversed, offers hope that as some patients 'grow out' of asthma, a similar cure for asthma could be developed in the future.

The biggest challenge facing the development of epigenetic-based therapies for asthma, and other diseases, is the broad actions of the epigenetic modifying enzymes, which have multiple potential targets. Many of the existing early tool compounds are broadly active and therefore will have many undesired side effect proteins, however as our understanding of these enzymes increases so will specificity of the drugs which are available.

Recent developments in genome targeting tools, such as the development of CRISPR (clustered regularly interspaced short palindromic repeats) – Cas9, to provide highly selective RNA-guided endonucleases to modify the genome at specific sites [144], is overtaking standard genetic knockdown approaches in mice. This technology has been used successfully *in vivo* and allows specific targeting of DNA sequences and their associated epigenetic marks which can be directed at potentially clinically useful sites.

Many of the targets for epigenetic therapeutics need to be targeted to specific genes and areas of the genome. Drugs that inhibit DNA methylation are able to target specific regions of the genome as the methylases bind to transcription factors that bind specific regions of DNA. If a similar mechanism could be found to target genes such as histone modifiers then the degree of usefulness of many of these drugs would increase. While there is a long way to go, the future looks bright for new treatments for asthma based on epigenetics. New compounds will offer the possibility to investigate epigenetic pathways and may lead to a novel therapeutic direction of research.

Future perspective

Knowledge of the role of epigenetics in asthma will continue to grow and the links between asthma and specific combinations of epigenetic modifications will become clearer. As the cost of ChIP-sequencing and next-generation sequencing decreases, the affordability of investigating multiple epigenetic modifications and how they relate to gene and protein expression will increase. Investigating the role of epigenetic modifications will also become easier due to technological advances allowing much more complicated studies to be conducted with fewer primary cells from each patient.

Studies are beginning to allow the investigation of how the epigenome changes over time particularly in response to environmental exposures. Changes in DNA methylation linked to SNP analysis (EWAS, epigenome-wide association studies) and deconvolution of transcriptomic data in single cell types may allow for greater determination of important targets for subsets of asthma. Linking these studies to longitudinal methylation analysis (LEWAS) will allow even greater insight into how environmental challenges may affect disease onset. Similar studies in blood, airway epithelial cells or smooth muscle cells focused on histone modifications or chromatin structure using ChIP-seq or ACAT-seq approaches may be of even greater benefit and help define new animal models or targets that will define the potential therapeutic benefits of drugs that target epigenetic mechanisms.

In terms of therapeutics, the technology for delivering miRNAs and other epigenetic drugs to the lungs will improve and provide a greater therapeutic window. Currently, targeting of epigenetic drugs to specific sites is more advantageous with DNA methylation but it is hoped that selective targeting will enable higher specificity of next-generation epigenetic drugs.

Current epigenetic drugs are really tool compounds which have off-target effects which limit their options for clinical trials. Animal models will be useful for pharmacokinetic studies but may lack the correct epigenetic components of human cells and tissues to allow predictive studies. The option of topical delivery to the airways and the development of highly lung resident drugs with very low systemic exposure may improve the chances of successful clinical studies in asthma.

Disclaimer

The views expressed in this publication are those of the authors(s) and not necessarily those of the NHS, The National Institute for Health Research or the Department of Health.

Financial & competing interests disclosure

MM Perry is an Imperial College Research Fellow. MM Perry is a member of Interuniversity Attraction Poles Program-Belgian State-Belgian Science Policy – project P7/30. IM Adcock is supported by the Wellcome Trust (093080/Z/10/Z), the MRC (G1001367/1), EU-IMI, the Dunhill Medical Trust and by the British Heart Foundation. AL Durham is supported by the British Lung Foundation and Pfizer. PO Brook is supported by the MRC-Asthma UK Centre for Asthma and Allergic Mechanisms. The authors are supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Open access

Executive summary

- Asthma is a heritable heterogeneous disease of the airways and severe asthma currently has no treatment.
- Epigenetics may account of the heritability of asthma through histone modifications, DNA methylation and interfering RNA.
- There are now a variety of tools available to target many epigenetic mechanisms.
- Modifying acetylation and methylation of histones and DNA methylation can change expression of inflammatory genes.
- DNA methylation modifiers can be targeted at particular regions of DNA as they are bound to specific complexes which allow specificity of drugs.
- miRNA treatments such as suppressors of cytokine signaling (SOCS3) siRNA are able to be targeted specifically to the lung and can reduce eosinophilia.
- Further work is required before these drugs progress into human clinical trials.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. *Allergy* 59, 469–478 (2004).

2 WHO. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach. www.who.int/gard

3 Bradding P, Green RH. Subclinical phenotypes of asthma. *Curr. Opin. Allergy Clin. Immunol.* 10(1), 54–59 (2010).

4 Chung KF, Wenzel SE, Brozek JL *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* 43(2), 343–373 (2014).

5 British Thoracic Society. British guideline on the management of asthma. *Thorax* 69(Suppl. 1), 1 –192 (2014).

6 Owen JA, Punt J, Stranford SA, Jones PP. *Kuby Immunology.* W. H. Freeman and Company, New York, NY, USA (2007).

7 Bottcher MF, Bjurstrom J, Mai X-M, Nilsson L, Jenmalm MC. Allergen-induced cytokine secretion in atopic and non atopic asthmatic children. *Pediatr. Allergy Immunol.* (14), 345–350 (2003).

8 Nurse B, Puterman AS, Haus M, Berman D, Weinberg EG, Potter PC. PBMCs from both atopic asthmatic and nonatopic children show a T(H)2 cytokine response to house dust mite allergen. *J. Allergy Clin. Immunol.* 106, 84–91 (2000).

9 Woodruff PG, Modrek B, Choy DF *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am. J. Respir. Crit. Care Med.* 180(5), 388–395 (2009).

10 Langrish CL, Chen Y, Blumenschein WM *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201(2), 233–240 (2005).

11 Al-Ramli W, Préfontaine D, Chouiali F *et al.* TH17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J. Allergy Clin. Immunol.* 123(5), 1185–1187 (2009).

12 Oren R, Hod-Marco M, Haus-Cohen M *et al.* Thymus and autoimmunity: production of $CD25^{+}CD4^{+}$ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J. Immunol.* 193, 5317–5326 (1999).

13 Zhou L, Lopes JE, Chong MMW *et al.* TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature* 453, 236–240 (2008).

14 Shi YH, Shi GC, Wan HY *et al.* Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. *Chin. Med. J. (Engl)* 124, 1951–1956 (2011).

15 Thomsen SF, Van Der Sluis S, Kyvik KO, Skytthe A, Backer V. Estimates of asthma heritability in a large twin sample. *Clin. Exp. Allergy* 40, 1054–1061 (2010).

16 McGeachie MJ, Stahl EA, Himes BE *et al.* Polygenic heritability estimates in pharmacogenetics: focus on asthma and related phenotypes. *Pharmacogenet. Genomics* 23(6), 324–328 (2013).

17 Woodruff PG, Boushey HA, Dolganov GM *et al.* Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc. Natl Acad. Sci. USA* 104(40), 1558–1563 (2007).

18 Moffatt M, Gut I, Demenais F *et al.* A large-scale, consortium-based genomewide association study of asthma. *N. Engl. J. Med.* 363(13), 1211–1221 (2010).

19 Durham AL, Wiegman C, Adcock IM. Epigenetics of asthma. *Biochim. Biophys. Acta.* 1810(11), 1103–1109 (2011).

20 Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. *Epigenetics* 9(9), 1302–1316 (2014).

21 Rastogi D, Suzuki M, Greally JM. Differential epigenome-wide DNA methylation patterns in childhood obesity-associated asthma. *Sci. Rep.* 3 (Table 1), 2164 (2013).

22 Zhang H, Tong X, Holloway JW *et al.* The interplay of DNA methylation over time with Th2 pathway genetic variants on asthma risk and temporal asthma transition. *Clin. Epigenetics* 6, 8 (2014).

23 Liang L, Willis-Owen SAG, Laprise C *et al.* An epigenome-wide association study of total serum immunoglobulin E concentration. 520(7549), 670 –674 *Nature*(2015).

24 Seumois G, Chavez L, Gerasimova A *et al.* Epigenomic analysis of primary human T cells reveals enhancers associated with TH2 memory cell differentiation and asthma susceptibility. *Nat. Immunol.* 15(8) 777 –788 (2014).

25 Gerasimova A, Chavez L, Li B *et al.* Predicting cell types and genetic variations contributing to disease by combining GWAS and epigenetic data. *PLoS ONE* 8(1), e54359 (2013).

26 Wei G, Wei L, Zhu J *et al.* Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating $CD4^+$ T cells. *Immunity* 30(1), 155–167 (2009).

27 Himes BE, Jiang X, Wagner P *et al.* RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene that modulates cytokine function in airway smooth muscle cells. *PLoS ONE* 9(6), e99625 (2014).

28 Pagdin T, Lavender P. MicroRNAs in lung diseases. *Thorax* 67, 183–184 (2012).

29 Clifford RL, Patel JK, John AE *et al.* CXCL8 Histone H3 acetylation is dysfunctional in airway smooth muscle in asthma: regulation by BET. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* doi:10.1152/ajplung.00021.2015 (2015) (Epub ahead of print).

30 Thurman RE, Rynes E, Humbert R *et al.* The accessible chromatin landscape of the human genome. *Nature* 489(7414), 75–82 (2012).

31 Kwon N-H, Kim J-S, Lee J-Y, Oh M-J, Choi D-C. DNA methylation and the expression of IL-4 and IFN-gamma promoter genes in patients with bronchial asthma. *J. Clin. Immunol.* 28(2), 139–146 (2008).

32 Zafra MP, Mazzeo C, Gámez C *et al.* Gene silencing of SOCS3 by siRNA intranasal delivery inhibits asthma phenotype in mice. *PLoS ONE* 9(3), e91996 (2014).

33 Consortium RE, Kundaje A, Meuleman W *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* 518, 317–330 (2015).

34 ENCODE: Encyclopedia of DNA Elements. www.encodeproject.org

35 Roadmap Epigenomics Project. www.roadmapepigenomics.org

36 Romanoski CE, Glass CK, Stunnenberg HG, Wilson L, Almouzni G. Epigenomics: roadmap for regulation. *Nature* 518(7539), 314–316 (2015).

37 Dunham I, Kundaje A, Aldred SF *et al.* An integrated encyclopedia of DNA elements in the human genome. *Nature* 489(7414), 57–74 (2012).

38 Ransom M, Dennehey B, Tyler J. Chaperoning histones during DNA replication and repair. *Cell* 140(2), 183–195 (2010).

39 Luger K, Mäder A, Richmond R. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 7, 251–260 (1997).

40 Ito K, Yamamura S, Essilfie-Quaye S *et al.* Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. *J. Exp. Med.* 203(1), 7–13 (2006).

41 Vollmuth F, Geyer M. Interaction of propionylated and butyrylated histone H3 lysine marks with Brd4 bromodomains. *Angew. Chem. Int. Ed. Engl.* 49(38), 6768–6772 (2010).

42 Huisinga KL, Brower-Toland B, Elgin SCR. The contradictory definitions of heterochromatin: transcription and silencing. *Chromosoma* 115(2), 110–122 (2006).

43 Tessarz P, Kouzarides T. Histone core modifications regulating nucleosome structure and dynamics. *Nat. Rev. Mol. Cell Biol.* 15(11), 703–708 (2014).

44 Dumuis-Kervabon A, Encontre I, Etienne G *et al.* A chromatin core particle obtained by selective cleavage of histones by clostripain. *EMBO J.* 5(7), 1735–1742 (1986).

45 Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene.* 26, 5541–5552 (2007).

46 Portela A, Esteller M. Epigenetic modifications and human disease. *Nat. Biotechnol.* 28(10), 1057–1068 (2010).

47 Ito K, Caramori G, Lim S *et al.* Expression and activity of histone deacetylases in human asthmatic airways. *Am. J. Respir. Crit. Care Med.* 166, 392–396 (2002).

48 Su R-C, Becker AB, Kozyrskyj AL, Hayglass KT. Epigenetic regulation of established human type 1 versus type 2 cytokine responses. *J. Allergy Clin. Immunol.* 121, 57–63.e3 (2008).

49 Creyghton MP, Cheng AW, Welstead GG *et al.* Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl Acad. Sci. USA* 107(50), 21931–21936 (2010).

50 Vijayanand P, Seumois G, Simpson LJ *et al.* Interleukin-4 production by follicular helper T cells requires the conserved

IL-4 enhancer Hypersensitivity Site V. *Immunity* 36(2), 175–187 (2012).

51 Yu Q, Zhou B, Zhang Y *et al.* DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. *Proc. Natl Acad. Sci.* 109, 541–546 (2012).

52 Imhof A, Feller C, Forne I, Becker PB, Imhof A, Becker PB. Global and specific responses of the histone acetylome to systematic perturbation resource global and specific responses of the histone acetylome to systematic perturbation. *Mol. Cell* 57(3), 559–571 (2015).

53 Chen L, Fischle W, Verdin E, Greene W. Duration of nuclear NF-κB action regulated by reversible acetylation. *Science* 293 (5535), 1653–1657 (2001).

54 Marwick J a, Caramori G, Stevenson CS *et al.* Inhibition of PI3Kdelta restores glucocorticoid function in smoking-induced airway inflammation in mice. *Am. J. Respir. Crit. Care Med.* 179(7), 542–548 (2009).

55 Wang Y, Tian Y, Morley M, Lu M. Development and regeneration of $Sox2^+$ endoderm progenitors are regulated by a Hdac1/2-Bmp4/Rb1 regulatory pathway. *Dev. Cell.* 24(4), 345–358 (2013).

56 Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest* 111, 852–857 (1997).

57 Butler CA, McQuaid S, Taggart CC *et al.* Glucocorticoid receptor β and histone deacetylase 1 and 2 expression in the airways of severe asthma. *Thorax* 67(5), 392–398 (2012).

58 Belkina AC, Nikolajczyk BS, Denis GV. BET protein function is required for inflammation: Brd2 genetic disruption and BET inhibitor JQ1 impair mouse macrophage inflammatory responses. *J. Immunol.* 190(7), 3670–3678 (2013).

59 Huang B, Yang X-D, Zhou M-M, Ozato K, Chen L-F. Brd4 coactivates transcriptional activation of NF-kappaB via specific binding to acetylated RelA. *Mol. Cell. Biol.* 29(5), 1375–1387 (2009).

60 Wu S-Y, Chiang C-M. The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation. *J. Biol. Chem.* 282(18), 13141–13145 (2007).

61 Royce S. Histone deacetylase inhibitors: can we consider potent anti-neoplastic agents for the treatment of asthma? *Ann. Clin. Lab. Sci.* 42(3), 338–345 (2012).

62 Hou X, Wan H, Ai X *et al.* Histone deacetylase inhibitor regulates the balance of Th17/Treg in allergic asthma. *Clin. Respir. J.* doi:10.1111/crj.12227 (2014) (Epub ahead of print).

63 Choi SW, Gatza E, Hou G *et al.* Histone deacetylase inhibition regulates inflammation and enhances Tregs after allogeneic hematopoietic cell transplantation in humans. *Blood* 125(5), 815 –819 (2014).

64 Cheng Z, Gong Y, Ma Y *et al.* Inhibition of BET bromodomain targets genetically diverse glioblastoma. *Clin. Cancer Res.* 19, 1748–1759 (2013).

65 Delmore JE, Issa GC, Lemieux ME *et al.* BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 146(6), 904–917 (2011).

66 Lewis MD, Miller SA, Miazgowicz MM, Beima KM, Weinmann AS. T-bet's ability to regulate individual target genes requires the conserved T-box domain to recruit histone methyltransferase activity and a separate family member-specific transactivation domain. *Mol. Cell. Biol.* 27(24), 8510–8521 (2007).

67 Rai G, Kawamura A, Anthony Tumber YL *et al.* ML324, a JMJD2 demethylase inhibitor with demonstrated antiviral activity. In: *Probe Reports from the NIH Molecular Libraries Program,* National Center for Biotechnology Information, MD, USA (2013).

68 Kruidenier L, Chung C, Cheng Z *et al.* A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature* 488(7411), 404–408 (2012).

69 Prusevich P, Kalin J, Ming S. A selective phenelzine analogue inhibitor of histone demethylase LSD1. *ACS Chem. Biol.* 9(6), 1284–1293 (2014).

70 Brand S, Kesper DA, Teich R *et al.* DNA methylation of TH1/TH2 cytokine genes affects sensitization and progress of experimental asthma. *J. Allergy Clin. Immunol.* 129(6), 1602–1610.e6 (2012).

71 Cheray M, Nadaradjane A, Bonnet P, Routier S, Vallette FM, Cartron P-F. Specific inhibition of DNMT1/CFP1 reduces cancer phenotypes and enhances chemotherapy effectiveness. *Epigenomics* 6(3), 267–275 (2014).

•• **One of the first targeted approach to limit the methylation of specific genomic loci.**

72 Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. *Nat. Rev. Drug Discov.* 12, 847–865 (2013).

73 Janssen HLA, Reesink HW, Lawitz EJ *et al.* Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* 368, 1685–1694 (2013).

74 Bryniarski K, Ptak W, Jayakumar A *et al.* Antigen-specific, antibody-coated, exosome-like nanovesicles deliver suppressor T-cell microRNA-150 to effector T cells to inhibit contact sensitivity. *J. Allergy Clin. Immunol.* 132(1), 170–181 (2013).

75 Li JJ, Tay HL, Maltby S *et al.* MicroRNA-9 regulates steroid-resistant airway hyperresponsiveness by reducing protein phosphatase 2A activity. *J. Allergy Clin. Immunol.* 136(2), 462 –473 (2015)

76 Collison A, Mattes J, Plank M, Foster PS. Inhibition of house dust mite-induced allergic airways disease by antagonism of microRNA-145 is comparable to glucocorticoid treatment. *J. Allergy Clin. Immunol.* 128(1), 160–167 (2011).

77 Mattes J, Collison A, Plank M, Phipps S, Foster PS. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. *Proc. Natl Acad. Sci. USA* 106(44), 18704–18709 (2009).

78 Banerjee A, Trivedi CM, Damera G *et al.* Trichostatin A abrogates airway constriction, but not inflammation, in murine and human asthma models. *Am. J. Respir. Cell Mol. Biol.* 46(2), 132–138 (2012).

79 Kim Y, Kim K, Park D *et al.* Histone deacetylase 3 mediates allergic skin inflammation by regulating expression of MCP1 protein. *J. Biol. Chem.* 287(31), 25844–25859 (2012).

80 Kienzler A-K, Rizzi M, Reith M, Nutt SL, Eibel H. Inhibition of human B-cell development into plasmablasts by histone deacetylase inhibitor valproic acid. *J. Allergy Clin. Immunol.* 131(6), 1695–1699 (2013).

81 Shi YL, Gu J, Park JY *et al.* Histone deacetylases inhibitor Trichostatin A ameliorates DNFB-induced allergic contact dermatitis and reduces epidermal Langerhans cells in mice. *J. Dermatol. Sci.* 68(2), 99–107 (2012).

82 Barnes PJ. Theophylline. *Am. J. Respir. Crit. Care Med.* 188(5), 901–906 (2013).

83 Ford PA, Durham AL, Russell REK, Gordon F, Adcock IM, Barnes PJ. Treatment effects of low-dose theophylline combined with an inhaled corticosteroid in COPD. *Chest.* 137, 1338–1344 (2010).

84 Barnes P, Adcock I, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur. Respir. J.* 25(3), 552–563 (2005).

85 Pinz S, Unser S, Buob D, Fischer P, Jobst B, Rascle A. Deacetylase inhibitors repress STAT5-mediated transcription by interfering with bromodomain and extra-terminal (BET) protein function. *Nucleic Acids Res.* 43 (7), 3524–3545 (2015).

86 Royce SG, Karagiannis TC. Histone deacetylases and their inhibitors: new implications for asthma and chronic respiratory conditions. *Curr. Opin. Allergy Clin. Immunol.* 14(1), 44–8 (2014).

87 Hakim A, Adcock I, Usmani O. Corticosteroid resistance and novel anti-inflammatory therapies in chronic obstructive pulmonary disease. 72(10), 1299 –1312 *Drugs* (2012).

88 Heller EA, Cates HM, Peña CJ *et al.* Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. *Nat. Neurosci.* 17(12), 1720–1727 (2014).

89 Konermann S, Brigham MD, Trevino AE *et al.* Optical control of mammalian endogenous transcription and epigenetic states. *Nature* 500(7463), 472–476 (2013).

• **The development of light-sensitive histone-distribution altering tools will enable in the short term new models of disease and in the long-term potentially novel spatially targeted therapeutics.**

90 Berkovits BD, Wolgemuth DJ. The role of the double bromodomain-containing BET genes during mammalian spermatogenesis. *Curr. Top. Dev. Biol.* 102, 293–326 (2013).

91 Moon KJ, Mochizuki K, Zhou M, Jeong HS, Brady JN, Ozato K. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Mol. Cell.* 19, 523–534 (2005).

92 Zeng L, Zhou MM. Bromodomain: an acetyl-lysine binding domain. *FEBS Lett.* 513(1), 124–128 (2002).

93 Wu SY, Lee AY, Hou SY *et al.* Brd4 links chromatin targeting to HPV transcriptional silencing. *Genes Dev.* 20, 2383–2396 (2006).

94 Filippakopoulos P, Qi J, Picaud S *et al.* Selective inhibition of BET bromodomains. *Nature* 468(7327), 1067–1073 (2010).

95 Khan YM, Kirkham P, Barnes PJ, Adcock IM. Brd4 is essential for IL-1B-induced inflammation in human airway epithelial cells. *PLoS ONE* 9(4), 1–17 (2014).

96 Perry MM, Durham AL, Austin PJ, Adcock IM, Chung KF. BET bromodomains regulate TGF-β-induced proliferation and cytokine release in asthmatic airway smooth muscle. *J. Biol. Chem.* 290(14), 9111 –9121 (2015).

97 Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat. Res.* 647(1–2), 21–29 (2008).

98 Koche RP, Smith ZD, Adli M *et al.* Reprogramming factor expression induces rapid and widespread targeted chromatin remodeling. *Cell Stem Cell.* 8(1), 96–105 (2011).

99 Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and IL-4 locus accessibility. *Annu. Rev. Immunol.* 24, 607–656 (2006).

100 Ernst J, Kheradpour P, Mikkelsen TS *et al.* Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature* 473(7345), 43–49 (2011).

101 Perros F, Hoogsteden HC, Coyle AJ, Lambrecht BN, Hammad H. Blockade of CCR4 in a humanized model of asthma reveals a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation. *Allergy* 64(7), 995–1002 (2009).

102 Chan O, Burke JD, Gao DF, Fish EN. The chemokine CCL5 regulates glucose uptake and AMP kinase signaling in activated T cells to facilitate chemotaxis. *J. Biol. Chem.* 287(35), 29406–29416 (2012).

103 Barsyte-Lovejoy D, Li F, Oudhoff MJ *et al.* (R)-PFI-2 is a potent and selective inhibitor of SETD7 methyltransferase activity in cells. *Proc. Natl Acad. Sci. USA* 111(35), 12853–12858 (2014).

104 Wang H, Cao R, Xia L *et al.* Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Mol. Cell.* 8, 1207–1217 (2001).

105 Zhu Y, van Essen D, Saccani S. Cell-type-specific control of enhancer activity by H3K9 trimethylation. *Mol. Cell.* 46(4), 408–423 (2012).

106 Clifford RL, John AE, Brightling CE, Knox AJ. Abnormal histone methylation is responsible for increased VEGF165a secretion from airway smooth muscle cells in asthma. *J. Immunol.* 189(2), 819–831 (2012).

107 Whetstine JR, Nottke A, Lan F *et al.* Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell* 125(3), 467–481 (2006).

108 Liu F, Barsyte-Lovejoy D, Li F *et al.* Discovery of an *in vivo* chemical probe of the lysine methyltransferases G9a and GLP. *J. Med. Chem.* 56(21), 8931–8942 (2013).

109 Tachibana M, Sugimoto K, Nozaki M *et al.* G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev.* 16, 1779–1791 (2002).

110 Young MD, Willson TA, Wakefield MJ *et al.* ChIP-seq analysis reveals distinct H3K27me3 profiles that correlate with transcriptional activity. *Nucleic Acids Res.* 39(17), 7415–7427 (2011).

111 Tumes DJ, Onodera A, Suzuki A *et al.* The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. *Immunity* 39(5), 819–832 (2013).

112 Miller S, Mohn S, Weinmann A. Jmjd3 and UTX play a demethylase-independent role in chromatin remodeling to regulate T-box family member- dependent gene expression. *Mol. Cell.* 40(4), 594–605 (2010).

113 Xiang Y, Zhu Z, Han G, Lin H, Xu L, Chen CD. JMJD3 is a histone H3K27 demethylase. *Cell Res.* 17(10), 850–857 (2007).

114 Ishii M, Wen H, Corsa CAS *et al.* Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood* 114(15), 3244–3254 (2009).

115 Satoh T, Takeuchi O, Vandenbon A *et al.* The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat. Immunol.* 11(10), 936–944 (2010).

116 Han H, Xu D, Liu C, Claesson H-E, Björkholm M, Sjöberg J. Interleukin-4-mediated 15-lipoxygenase-1 trans-activation requires UTX recruitment and H3K27me3 demethylation at the promoter in A549 cells. *PLoS ONE* 9(1), e85085 (2014).

117 Kuperman DA, Lewis CC, Woodruff PG *et al.* Dissecting asthma using focused transgenic modeling and functional genomics. *J. Allergy Clin. Immunol.* 116(2), 305–311 (2005).

118 Vianello P, Botrugno OA, Cappa A *et al.* Synthesis, biological activity and mechanistic insights of 1-substituted cyclopropylamine derivatives: a novel class of irreversible inhibitors of histone demethylase KDM1A. *Eur. J. Med. Chem.* 86, 352–363 (2014).

119 Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* 11(3), 204–220 (2010).

120 Hermann A, Goyal R, Jeltsch A. The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J. Biol. Chem.* 279(46), 48350–48359 (2004).

121 Williams CL, Schilling MM, Cho SH *et al.* STAT4 and T-bet are required for the plasticity of IFN-γ expression across Th2 ontogeny and influence changes in Ifng promoter DNA methylation1. *J. Immunol.* 191(2), 678–687 (2013).

122 Verma M, Chattopadhyay BD, Paul BN. Epigenetic regulation of DNMT1 gene in mouse model of asthma disease. *Mol. Biol. Rep.* 40(3), 2357–2368 (2013).

123 Rai K, Jafri IF, Chidester S *et al.* Dnmt3 and G9a cooperate for tissue-specific development in zebrafish. *J. Biol. Chem.* 285(6), 4110–4121 (2010).

124 Yu D, Waterland RA, Zhang P *et al.* Targeted p16Ink4a epimutation causes tumorigenesis and reduces survival in mice. *J. Clin. Invest.* 124(9), 3708–3712 (2014).

- **Demonstration of a model that is able to hypermethylate specific genomic loci and may be a targeted way to limit expression of inflammatory mediators among other things.**

125 Di Ruscio A, Ebralidze AK, Benoukraf T *et al.* DNMT1-interacting RNAs block gene-specific DNA methylation. *Nature* 503(7476), 371–6 (2013).

126 Estève P, Chin H. Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Genes Dev.* 3089–3103 (2006).

127 Robertson K, Ait-Si-Ali S, Yokochi T. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat. Genet.* 25(3), 338 –342 (2000).

128 Chuang L, Ian H, Koh T, Ng H, Xu G, Li B. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* 277(5334), 80 (1997).

129 Booton R, Lindsay MA. Emerging role of microRNAs and long noncoding RNAs in respiratory disease. *Chest* 146, 193–204 (2014).

130 Xu W, Hui C, Yu SSB, Jing C, Chan HC. MicroRNAs and cystic fibrosis--an epigenetic perspective. *Cell Biol. Int.* 35, 463–466 (2011).

131 Yu JCC, Martin S, Nasr J, Stafford K, Thompson D, Petrikovics I. MicroRNAs in lung cancer. *World J. Methodol.* 2(2), 33–41 (2012).

132 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297 (2004).

133 Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 19, 92–105 (2009).

134 Perry MM, Tsitsiou E, Austin PJ *et al.* Role of non-coding RNAs in maintaining primary airway smooth muscle cells. *Respir. Res.* 15, 58 (2014).

135 Martinez-Nunez RT, Bondanese VP, Louafi F *et al.* A MicroRNA network dysregulated in asthma controls IL-6 production in bronchial epithelial cells. *PLoS ONE* 9(10), e111659 (2014).

136 Perry MM, Baker JE, Gibeon DS, Adcock IM, Chung KF. Airway smooth muscle hyperproliferation is regulated by MicroRNA-221 in severe asthma. *Am. J. Respir. Cell Mol. Biol.* 50, 7–17 (2014).

137 Solberg OD, Ostrin EJ, Love MI *et al.* Airway epithelial miRNA expression is altered in asthma. *Am. J. Respir. Crit. Care Med.* 186(10), 965–974 (2012).

138 Takyar S, Vasavada H, Zhang J *et al.* VEGF controls lung Th2 inflammation via the miR-1-Mpl (myeloproliferative leukemia virus oncogene)-P-selectin axis. *J. Exp. Med.* 210, 1993–2010 (2013).

139 Asai-Tajiri Y, Matsumoto K, Fukuyama S *et al.* Small interfering RNA against CD86 during allergen challenge blocks experimental allergic asthma. *Respir. Res.* 15(1), 132 (2014).

140 Kakiuchi-Kiyota S, Koza-Taylor PH, Mantena SR *et al.* Comparison of hepatic transcription profiles of locked ribonucleic acid antisense oligonucleotides: evidence of distinct pathways contributing to non-target mediated toxicity in mice. *Toxicol. Sci.* 138, 234–248 (2014).

141 Gilleron J, Querbes W, Zeigerer A *et al.* Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. *Nat. Biotechnol.* 31, 638–646 (2013).

142 Jang SC, Kim OY, Yoon CM *et al.* Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. *ACS Nano.* 7, 7698–7710 (2013).

- **The potential for targeting nanovesicles containing ncRNA demonstrates an exciting opportunity for ground-breaking therapeutics.**

143 Taulli R, Loretelli C, Pandolfi P. From pseudo-ceRNAs to circ-ceRNAs: a tale of cross-talk and competition. *Nat. Struct. Mol. Biol.* 20(5), 541–543 (2013).

144 Cong L, Ran F, Cox D, Lin S, Barretto R. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339(6121), 819–823 (2013).