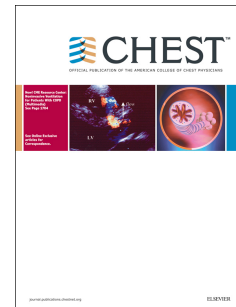


Accepted Manuscript

Epigenetic changes in airway smooth muscle as a driver of airway inflammation and remodelling in asthma

Klaudia A. Kaczmarek, MRes, Rachel L. Clifford, PhD, Alan J. Knox, MD



PII: S0012-3692(18)32719-3

DOI: <https://doi.org/10.1016/j.chest.2018.10.038>

Reference: CHEST 2036

To appear in: *CHEST*

Received Date: 29 August 2018

Revised Date: 10 October 2018

Accepted Date: 29 October 2018

Please cite this article as: Kaczmarek KA, Clifford RL, Knox AJ, Epigenetic changes in airway smooth muscle as a driver of airway inflammation and remodelling in asthma, *CHEST* (2018), doi: <https://doi.org/10.1016/j.chest.2018.10.038>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Word count (text): 2552

Word count (abstract): 142

For Translating Basic Research into Clinical Practice section.

Title: Epigenetic changes in airway smooth muscle as a driver of airway inflammation and remodelling in asthma

Authors: Klaudia A. Kaczmarek, MRes; Rachel L. Clifford, PhD; Alan J Knox, MD

Affiliation: Division of Respiratory Medicine, Clinical Sciences Building, Nottingham University Hospitals NHS Trust (City Hospital Campus), NG5 1PB. Nottingham NIHR Biomedical Research Centre. Nottingham MRC Molecular Pathology Node.

Corresponding Author: Alan J. Knox

Corresponding Author address:

Clinical Sciences Building
Nottingham City Hospital
Hucknall Road
Nottingham
NG5 1PB
UK

Email: alan.knox@nottingham.ac.uk

Funding information: Asthma UK, Nottingham NIHR Biomedical Research Centre

Key words: Asthma, airway smooth muscle, epigenetics

Abbreviations:

3'-UTR (3'-untranslated region), AHR (airway hyperresponsiveness), ASM (Airway smooth muscle), Aza-CdR (5-Aza-2'-deoxycytidine), BET (Bromodomain and extraterminal), BSM (bronchial smooth muscle), DNMT1 (DNA methyltransferase 1), DNMTi (DNA methyltransferase inhibitors), ECM (extracellular matrix), HAT (histone acetyltransferase), HDAC (histone deacetyltransferase), HKMT (histone lysine methyltransferase), miRNA (microRNA), mRNA (messenger RNA), MMP (Matrix metalloproteinase), ncRNA (Non-coding RNA), p/CAF (p300/CBP-associated factor), PDE (phosphodiesterase), *PDE4D* (Phosphodiesterase 4D), PDGF (platelet-derived growth factor), PKC (protein kinase C), PMA (phorbol myristate acetate), PRMT (protein arginine methyltransferase), SAHA (suberoylanilide hydroxamic acid), TSA (Trichostatin A), VCAM-1 (Vascular cell adhesion molecule-1),

Abstract

Epigenetic changes are heritable changes in gene expression, without changing the DNA sequence. Epigenetic processes provide a critical link between environmental insults to the airway and functional changes that determine how airway cells respond to future stimuli. There are three primary epigenetic processes: histone modifications, DNA modification, and non-coding RNAs. Airway smooth muscle (ASM) has several important roles in the development and maintenance of the pathological processes occurring in asthma, including inflammation, remodelling and contraction/hyperresponsiveness. In this review, we describe the evidence for the role of epigenetic changes in driving these processes in ASM cells in asthma, with a particular focus on histone modifications. We also discuss how existing therapies may target some of these changes and how epigenetic processes provide targets for the development of novel asthma therapeutics. Epigenetic marks may also provide a biomarker to assess phenotype and treatment responses.

Introduction

Asthma is characterised by chronic airway inflammation and tissue remodelling which contribute to the different clinical phenotypes of the disease. Airway smooth muscle (ASM), is present in the central and peripheral airways. The traditional view of ASM function was that it was merely a contractile organ, contracting in response to mediators and neurotransmitters and relaxing in response to endogenous relaxants or bronchodilator drugs. Over the last 30 years it has become clear that its function is more complex. Increased ASM thickness is a prominent feature of asthma and the increased ASM mass has several important synthetic functions and is an abundant source of chemokines, growth factors, lipid mediators and matrix modifiers. Collectively these functions contribute to airway inflammation, airway remodelling and airway hyperresponsiveness.

Epigenetics

When human cells are subjected to environmental stimuli an epigenetic signature is left which modifies the response to subsequent stimulation. Epigenetic changes are heritable changes that regulate gene expression, which do not involve changes in DNA sequence¹. Three primary epigenetic processes exist: DNA modification (methylation/hydroxymethylation); histone modifications and non-coding RNAs. A schematic diagram with the summary of key epigenetic modifications is shown below (Figure 1). Epigenetics is key in integrating environmental exposure and genetics and has become an important area of focus for asthma research. Epigenetic changes in ASM could prime the airways to respond abnormally to different asthmagens thus perpetuating inflammation and worsening remodelling.

Most epigenetic research on ASM cells in asthma has concentrated on histone modifications, particularly histone acetylation and histone methylation. The role of other histone modifications (phosphorylation, ubiquitylation, sumoylation, and citrullination) is less well explored. Histone acetylation occurs at lysine residues within the N-terminal tail of histone proteins, balanced by the competing activity of histone acetyltransferases (HATs) and histone deacetyltransferases (HDACs). Increased histone acetylation is associated with increased gene expression. Histone methylation is more complex, occurring at lysine and arginine residues with the histone N-terminal tail. Lysine residues can be mono, di or trimethylated, while arginine residues can be mono or di-methylated. Arginine dimethylation occurs symmetrically or asymmetrically. The effect of histone methylation on gene expression is dependent on the residue methylated and the extent/orientation of the methylation. The enzymes responsible are histone lysine methyltransferases (HKMTs) and protein arginine methyltransferases (PRMTs).

Histone Modification and Asthmatic airway inflammation

Airway inflammation is a prominent characteristic of asthma. Airway biopsies show increased numbers and activation of mast cells, macrophages, eosinophils, T-lymphocytes, basophils, dendritic cells and neutrophils². ASM cells contribute via the production of inflammatory mediators, including histamine, prostanoids, leukotrienes, cytokines, chemokines, and endothelins³. Several studies have explored how histone modifications regulate ASM's synthetic functions.

Histone acetylation

HAT activity is increased in bronchial biopsies from individuals with asthma compared to healthy controls subjects, while HDAC activity is reduced⁴. Abnormalities in the HAT/HDAC ratio may contribute to airway inflammation in asthma. Several chemokine genes responsible for inflammatory cell recruitment are regulated by histone modifications in ASM. These include eotaxin, CXCL-8 and CXCL-10. Eotaxin is a potent eosinophil chemoattractant⁵. Our group has demonstrated that the TNF- α induced expression of eotaxin in ASM cells occurs via protein kinase C (PKC) β II, which recruits histone acetyltransferase p300/CBP-associated factor (p/CAF) to increase histone H4 acetylation at the promoter⁶.

CXCL8 is a neutrophil chemoattractant ⁵. ASM from asthmatic subjects hypersecretes CXCL8 due to H3 lysine 18 acetylation at the CXCL8 promoter, mediated by increased recruitment of the HAT p300 ⁷. Bromodomain and extraterminal (BET) proteins are a family of proteins which recognise acetylated histone lysine residues, and BET inhibition reduces CXCL8 secretion from ASM ⁷, via disruption of BRD4 and RNA polymerase II association with the promoter (Figure 2b). TNF- α increases acetylated histone H4 at the CXCL8 promoter in human ASM cells, but it is not known if this is abnormal in asthma ⁸.

CXCL10, a mast cell and T lymphocyte chemoattractant, is induced by TNF- α and IFN- γ in ASM cells via histone H4 acetylation at its promoter ⁹. TNF- α and IFN- γ have a synergistic effect which is mediated by the transcriptional co-activator CREB binding protein.

Histone acetylation at cell adhesion molecule promoters may also contribute to airway inflammation. Vascular cell adhesion molecule-1 (VCAM-1) is responsible for recruitment and infiltration of polymorphonuclear leukocytes ¹⁰. VCAM-1 upregulation in human ASM by TNF- α , endothelin-1 and IL-1 β , is mediated by HAT p300 and histone H4 acetylation ¹⁰⁻¹². Moreover, HDAC4 regulates TNF- α - and IL-1 β - induced VCAM-1 expression via a p300-independent pathway ^{11,12}.

COX is responsible for prostaglandin synthesis from arachidonic acid. ⁵ Unlike constitutive COX-1, COX-2 is inducible by pro-inflammatory stimuli ⁵. The transcriptional regulation of COX-2 by bradykinin and IL-1 β in human ASM cells is associated with histone H4 lysine acetylation in a stimulus-specific manner ¹³. Bradykinin and IL-1 β both enrich acetylated histone H4 lysine residue AcK8-associated COX-2 promoter DNA in ASM cells whereas only Bradykinin enriches AcK16-, AcK5-associated COX-2 promoter DNA ¹³.

Collectively these studies show a close relationship between HAT activation, histone H3 and H4 acetylation and production of several chemokines and inflammatory proteins in ASM cells either in cells from patients with asthma or from cells from normal subjects stimulated with asthma-relevant cytokines.

Histone methylation

There is less information on how histone methylation regulates chemokine production in ASM. Expression of the histone arginine methyltransferase PRMT1 is increased in severe asthma, in lung sections and isolated ASM cells ¹⁴, due to miR-19a deficiency ¹⁴. Furthermore, PRMT1 expression is upregulated in the lungs of rats with antigen-induced pulmonary inflammation ¹⁵.

Histone Modifications and Asthmatic airway remodelling

Histone modifications also regulate ASM remodelling in asthma. BET bromodomain inhibitors prevent fetal calf serum and TGF- β -induced cell proliferation in ASM cells from asthma and healthy subjects ¹⁶. Interestingly asthmatic cells are partially resistant to the effect of BET inhibitors. HDAC8 regulates ASM cytoskeleton dynamics ¹⁷. Histone arginine modifications may also be important. PRMT1 inhibition (AMI-1) decreases ASM cell activity, proliferation, extracellular matrix (ECM) deposition, and cell migration and reduces α -SMA, collagen type I, and fibronectin expression ¹⁴. Furthermore, PRMT1 inhibition (AMI-1) decreases airway and alveoli lesions, mucus secretion, and collagen deposition in rats with antigen-induced pulmonary inflammation, suggesting a role for PRMT1 in asthmatic airway remodelling ¹⁸.

Several proteins involved in remodelling are also modified by histone changes and in some instances these processes are dysregulated in asthma. VEGF, an angiogenic molecule, contributes to bronchial vascular remodelling and chronic inflammation, and is overexpressed in asthmatic sputum and tissue ¹⁹. VEGF is hypersecreted by ASM cells in asthma due to abnormalities in histone lysine methylation at its promoter ²⁰, namely increased H3K4me3 and decreased H3K9me3. This is due to failed G9A

recruitment, which prevents trimethylation of lysine 9 on histone H3 and subsequently the repression of VEGF transcription via regulation of Sp1 and RNA polymerase II binding²⁰ (Figure 2a).

Matrix metalloproteinases (MMPs) degrade ECM and basement membranes²¹. MMP-9 is a gelatinase which is increased in the lungs in asthma¹⁹ and TNF- α - induced MMP-9 upregulation in ASM requires histone H3 acetylation, mediated by p300²¹.

Histone Modification and Asthmatic ASM contractility

Increased ASM contractility in asthma is associated with airway hyperresponsiveness (AHR)²². Histone modifications have a role in regulating ASM contractility. Trichostatin A (TSA), an HDAC inhibitor, abrogates methacholine-induced AHR in mice, and inhibits agonist-induced contraction in human precision-cut lung slices²³ possibly by disrupting Ca²⁺ release²³. TSA attenuates AHR, in a mouse asthma model⁴. HDAC8 also regulates ASM contractility¹⁷. HDAC inhibitors suberoylanilide hydroxamic acid (SAHA), diamide (1), and MGCD0103 inhibit antigen-induced contraction of sensitised guinea pig tracheal rings, as well as histamine, 5-hydroxytryptamine and carbachol induced contraction. MGCD0103 also reduces sodium fluoride-, KCl- and peroxy radical generator-induced contraction of guinea pig tracheal rings²⁴. However, it should be noted that These HDAC (and HAT) inhibitors are blunt pharmacological tools with potential off target effects and the results should probably be viewed with caution. HATs and HDACs can target proteins other than histones, which is an additional challenge to overcome when interpreting HAT/HDAC inhibitor effects.

Effect of current asthma treatments on histone modifications

Glucocorticoids and long and short-acting β_2 -agonists are the mainstay of the treatment of chronic asthma. Combination therapy with glucocorticoids and long-acting β_2 -agonists is effective in managing asthma symptoms and reducing asthma exacerbations²⁵. Both glucocorticoids and β_2 -agonists can modify histone acetylation in ASM. β_2 -agonists and glucocorticoids, repress TNF- α -induced eotaxin gene transcription by selective inhibition of histone H4 acetylation and NF- κ B p65 binding to the eotaxin promoter in human ASM cells²⁶. The combination of the two drug groups is additive, suggesting a mechanism whereby β_2 -agonists could potentiate the effect of glucocorticoids in reducing asthma exacerbations²⁷. However, there are unanswered questions: it is not clear whether glucocorticoids and β_2 -agonists act on HATs, whether β_2 -agonists effect is CREB- dependent or whether it is mediated via HDACs, as described in some cells^{28,29}.

Theophylline has been used in asthma treatment for over 70 years albeit mainly as an add on treatment³⁰. Theophylline relaxes ASM by phosphodiesterase (PDE) inhibition, but also induces HDACs which may contribute to its anti-inflammatory properties.³⁰ Montelukast, a cysteinyl leukotriene receptor antagonist, enhances the LPS-induced expression of the anti-inflammatory cytokine IL-10 in human myeloid dendritic cells, via histone H3 acetylation³¹. Montelukast inhibits TNF- α -stimulated CXCL8 expression via NF- κ B p65-associated HAT activity in phorbol myristate acetate (PMA)-differentiated U937 cells³². No studies have looked at histone modifying effects of theophylline and montelukast in ASM. PPAR γ agonists (which are used for diabetes) suppress TNF α -induced eotaxin gene transcription through inhibition of histone H4 acetylation in human ASM cells⁸ but a clinical study in asthma was cut short due to concerns regarding carcinogenic effects of this class of drugs.

Other epigenetic mechanisms and their role in mediating ASM changes in asthma

DNA methylation and non-coding RNAs have also been implicated in the regulation of ASM cell function in asthma with more published work on non-coding RNAs.

DNA methylation

The degree of DNA methylation differs between ASM from healthy and asthmatic individuals and varies with disease severity. Genes associated with differentially methylated positions in healthy versus non-severe asthma subjects at baseline are involved in the regulation of cell proliferation and apoptosis, while the differences between samples from severe asthma and healthy subjects are associated with ASM contraction, proliferation and endocytosis.³³ Human ASM Phosphodiesterase 4D (*PDE4D*) overexpression is increased in asthma, due to *PDE4D* promoter hypomethylation³⁴. *PDE4D* methylation decreases cell proliferation and migration and reduces the phosphorylation level of downstream effectors of Ca²⁺ signalling, including myosin light chain kinase and p38 MAP kinase. Furthermore in rat ASM, the DNA methyltransferase inhibitor (DNMTi) 5-Aza-2'-deoxycytidine (Aza-CdR, or Decitabine) inhibits PDGF-induced increase in contractility, migration and proliferation³⁵ via effects on DNA methyltransferase 1 (DNMT1).

Non-coding RNAs

Non-coding RNAs (ncRNAs) are transcribed from DNA but not translated but can regulate the transcription, stability or translation of protein-coding genes³⁶. The most widely studied ncRNAs are microRNAs (miRNAs). These are 19–24 nucleotide-long, single-stranded RNAs, which induce translational repression and/or messenger RNA (mRNA) degradation by sequence-specific binding to the 3'-untranslated region (3'-UTR) of target mRNA³⁶. Multiple ncRNAs can alter several ASM functions (summarised in Table 1³⁷⁻⁵¹). MiRNAs modify synthetic functions (miR-143-3p, miR-145, miR-708, miR-140-3p, miR-221, miR-146a, miR-155), contractility (miR-145, miR-133a, miR-708), and proliferative functions (miR-150, miR-139-5p, miR-23b, miR-143-3p, miR-145, miR-135a, miR-10a, miR-203, miR-221, miR-21, miR-138, miR-638, miR-217) in ASM cells, by acting on diverse cellular targets (*BCYRN1*, *SMAD3*, *NFATc1*, *TRPC1*, *PI3* kinase, *c-Abl*, *PDK1*, *RhoA*, *JNK* MAPK, *D1*, *NOR1*, *ZEB1*).

Therapeutic implications

Histone modifications and other epigenetic modifications are emerging targets for the development of novel asthma treatments. The reversibility of epigenetic changes makes them ideal targets. Interestingly, many drugs used in asthma treatment, including corticosteroids, β_2 -agonists, theophylline and montelukast, although not designed to target epigenetic changes, have some effects on histones and histone modifying enzymes. It would be beneficial if novel drugs for asthma were capable of targeting histone modifying enzymes at specific, pro-inflammatory promoters or possibly the promoters of several genes with similar cell functions.

There are no selective HAT inhibitors currently available for human studies but there is some intriguing data using Curcumin which is a HAT inhibitor⁵². Curcumin decreases IL-1 β -induced eotaxin and MCP-1 release from HASM cells⁵³, inhibits platelet-derived growth factor (PDGF)-induced proliferation of rat ASM and reduces ASM mass in mouse asthma models by upregulating caveolin-1 expression and blocking ERK activation⁵⁴. Interestingly, a pilot study showed that curcumin reduces airway obstruction in asthma patients⁵⁴. Curcumin has off target effects, however, and more selective HAT inhibitors are needed.

The HDAC inhibitors Vorinostat (suberanilohydroxamic acid, SAHA) and Romidepsin, have been approved for cutaneous T-cell lymphoma treatment⁵⁵, and have shown promising results in HIV infection⁴. Belinostat and Panobinostat are HDACi approved for treatment of peripheral T-cell lymphoma and multiple myeloma respectively⁵⁵. BET inhibitors have been investigated for their potential use in several cancers and atherosclerosis⁵⁶. It would be interesting to study them in asthma.

Although there are no approved drugs targeting histone methylation, several are in development. Pinometostat, a H3K79 methyltransferase DOT1L inhibitor, shows promising results in several leukaemias⁵⁷. Tazemetostat is an EZH2 inhibitor is currently in trials for several cancers⁵⁷ as are inhibitors of histone demethylase LSD1⁵⁷. Histone arginine methylation and PRMTs are relatively new targets. However, already some compounds look promising GSK3326595, a PRMT5 inhibitor, is in clinical trials for treatment of solid tumours and non-Hodgkin's lymphoma⁵⁸.

Targeting other epigenetic modifications could also be beneficial in asthma. MiRNAs are small, with known sequences and are often conserved among species, makes them very attractive in terms of drug development⁵⁹. Both miRNA mimics and inhibitors are currently in clinical trials for use in cancers and HCV infection⁵⁹. MRG-201 a miRNA-29 mimic, is in phase II clinical trials in pulmonary fibrosis,⁶⁰. Decitabine (Aza-CdR) and azacytidine are DNMTi used for myelodysplastic syndrome⁶¹. Hydralazine and procainamide, which have a long history of use in treatment of hypertension and cardiac arrhythmia, respectively, also have DNMTi activity⁶¹.

As well as providing therapeutic targets epigenetic marks could be useful in disease phenotyping and as a biomarker of disease progression or response to treatment: nasal DNA methylation marks are related to disease severity and corticosteroid treatment response⁶².

Conclusions

ASM has a crucial role in the development and perpetuation of inflammation, remodelling and hyperresponsiveness in asthma. Although epigenetic research in asthma is still in its infancy evidence is emerging to suggest that histone modifications and other epigenetic mechanisms, have a crucial role in regulating ASM functions. We suggest that ASM epigenetic changes are drivers of inflammation and remodelling rather than just being adaptive responses to these processes. Consistent with this hypothesis ASM ablation by thermoplasty has beneficial effects in asthma. ASM cells from patients with asthma have dysregulated histone acetylation and methylation at the CXCL8 and VEGF promoters respectively which are likely to contribute to inflammatory cell influx and remodelling in asthmatic airways. Targeting these and other dysregulated epigenetic mechanisms could provide novel approaches for the future asthma treatments. Selective inhibitors of many epigenetic processes are in development for several chronic inflammatory diseases and cancers. Although our review has focussed on ASM changes, the effect of these agents in asthma will depend on the role of different epigenetic targets in regulating the functions of multiple cell types in the airways. This is an exciting area, which is likely to reap future rewards.

References

- 1. Dunn BK, Verma M, Umar A. Epigenetics in cancer prevention: early detection and risk assessment: introduction. *Ann N Y Acad Sci.* 2003;983:1-4.
- 2. Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. *Frontiers in Microbiology.* 2013;4:263.
- 3. Johnson SR, Knox AJ. Synthetic functions of airway smooth muscle in asthma. *Trends in pharmacological sciences.* 1997;18(8):288-292.
- 4. Ververis K, Karagiannis TC. Potential non-oncological applications of histone deacetylase inhibitors. *American Journal of Translational Research.* 2011;3(5):454-467.
- 5. Howarth PH, Knox AJ, Amrani Y, Tliba O, Panettieri RA, Jr., Johnson M. Synthetic responses in airway smooth muscle. *Journal of Allergy and Clinical Immunology.* 2004;114(2):S32-S50.
- 6. Clarke DL, Sutcliffe A, Deacon K, Bradbury D, Corbett L, Knox AJ. PKC β augments NF-kappaB-dependent transcription at the CCL11 promoter via p300/CBP-associated factor recruitment and histone H4 acetylation. *J Immunol.* 2008;181(5):3503-3514.
- 7. 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 C.* 2015;308(9):L962-L972.
- 8. Nie M, Corbett L, Knox AJ, Pang L. Differential regulation of chemokine expression by peroxisome proliferator-activated receptor gamma agonists: interactions with glucocorticoids and beta2-agonists. *J Biol Chem.* 2005;280(4):2550-2561.
- 9. Clarke DL, Clifford RL, Jindarat S, et al. TNF alpha and IFN gamma Synergistically Enhance Transcriptional Activation of CXCL10 in Human Airway Smooth Muscle Cells via STAT-1, NF-kappa B, and the Transcriptional Coactivator CREB-binding Protein. *Journal of Biological Chemistry.* 2010;285(38):29101-29110.
- 10. Lin CC, Lin WN, Hou WC, Hsiao LD, Yang CM. Endothelin-1 induces VCAM-1 expression-mediated inflammation via receptor tyrosine kinases and Elk/p300 in human tracheal smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol.* 2015;309(3):L211-225.
- 11. Lee CW, Lin CC, Luo SF, et al. Tumor necrosis factor-alpha enhances neutrophil adhesiveness: induction of vascular cell adhesion molecule-1 via activation of Akt and CaM kinase II and modifications of histone acetyltransferase and histone deacetylase 4 in human tracheal smooth muscle cells. *Mol Pharmacol.* 2008;73(5):1454-1464.
- 12. Luo SF, Chang CC, Lee IT, et al. Activation of ROS/NF-kappaB and Ca²⁺/CaM kinase II are necessary for VCAM-1 induction in IL-1beta-treated human tracheal smooth muscle cells. *Toxicol Appl Pharmacol.* 2009;237(1):8-21.
- 13. Nie M, Pang L, Inoue H, Knox AJ. Transcriptional Regulation of Cyclooxygenase 2 by Bradykinin and Interleukin-1 β in Human Airway Smooth Muscle Cells: Involvement of Different Promoter Elements, Transcription Factors, and Histone H4 Acetylation. *Molecular and Cellular Biology.* 2003;23(24):9233-9244.
- 14. Sun QZ, Liu L, Wang H, et al. Constitutive high expression of protein arginine methyltransferase 1 in asthmatic airway smooth muscle cells is caused by reduced microRNA-19a expression and leads to enhanced remodeling. *J Allergy Clin Immun.* 2017;140(2):510-+.
- 15. Sun QZ, Yang XD, Zhong B, et al. Upregulated Protein Arginine Methyltransferase 1 by IL-4 Increases Eotaxin-1 Expression in Airway Epithelial Cells and Participates in Antigen-Induced Pulmonary Inflammation in Rats. *J Immunol.* 2012;188(7):3506-3512.

- 16. Perry MM, Durham AL, Austin PJ, Adcock IM, Chung KF. BET Bromodomains Regulate Transforming Growth Factor-beta-induced Proliferation and Cytokine Release in Asthmatic Airway Smooth Muscle. *Journal of Biological Chemistry*. 2015;290(14):9111-9121.
- 17. Waltregny D, Glenisson W, Tran SL, et al. Histone deacetylase HDAC8 associates with smooth muscle alpha-actin and is essential for smooth muscle cell contractility. *Faseb Journal*. 2005;19(3):966-+.
- 18. Sun QZ, Liu L, Roth M, et al. PRMT1 Upregulated by Epithelial Proinflammatory Cytokines Participates in COX2 Expression in Fibroblasts and Chronic Antigen-Induced Pulmonary Inflammation. *J Immunol*. 2015;195(1):298-306.
- 19. Tagaya E, Tamaoki J. Mechanisms of Airway Remodeling in Asthma. *Allergology International*. 2007;56(4):331-340.
- 20. Clifford RL, John AE, Brightling CE, Knox AJ. Abnormal Histone Methylation Is Responsible for Increased Vascular Endothelial Growth Factor 165a Secretion from Airway Smooth Muscle Cells in Asthma. *J Immunol*. 2012;189(2):819-831.
- 21. Lee C-W, Lin C-C, Lin W-N, et al. TNF- α induces MMP-9 expression via activation of Src/EGFR, PDGFR/PI3K/Akt cascade and promotion of NF- κ B/p300 binding in human tracheal smooth muscle cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2007;292(3):L799-L812.
- 22. Berair R, Hollins F, Brightling C. Airway Smooth Muscle Hypercontractility in Asthma. *Journal of Allergy*. 2013;2013:185971.
- 23. 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*. 2012;46(2):132-138.
- 24. Assem E-SK, Peh KH, Wan BYC, Middleton BJ, Dines J, Marson CM. Effects of a selection of histone deacetylase inhibitors on mast cell activation and airway and colonic smooth muscle contraction. *International Immunopharmacology*. 2008;8(13):1793-1801.
- 25. Johnson M. Interactions between corticosteroids and beta2-agonists in asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2004;1(3):200-206.
- 26. Nie M, Knox AJ, Pang LH. B-2-Adrenoceptor agonists, like glucocorticoids, repress eotaxin gene transcription by selective inhibition of histone H4 acetylation. *J Immunol*. 2005;175(1):478-486.
- 27. Pauwels RA, Lofdahl CG, Postma DS, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. *New Engl J Med*. 1997;337(20):1405-1411.
- 28. Perng D-W, Su K-C, Chou K-T, et al. Long-acting β 2 agonists and corticosteroids restore the reduction of histone deacetylase activity and inhibit H2O2-induced mediator release from alveolar macrophages. *Pulmonary Pharmacology & Therapeutics*. 2012;25(4):312-318.
- 29. Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol*. 2000;20(18):6891-6903.
- 30. Barnes PJ. Theophylline. *Pharmaceuticals*. 2010;3(3):725-747.
- 31. Kuo C-H, Yang S-N, Kuo H-F, et al. Cysteinyl leukotriene receptor antagonist epigenetically modulates cytokine expression and maturation of human myeloid dendritic cells. *Pulmonary Pharmacology & Therapeutics*. 2016;39:28-37.
- 32. Tahan F, Jazrawi E, Moodley T, Rovati GE, Adcock IM. Montelukast inhibits tumour necrosis factor- α -mediated interleukin-8 expression through inhibition of nuclear factor- κ B p65-associated histone acetyltransferase activity. *Clinical & Experimental Allergy*. 2008;38(5):805-811.
- 33. Perry MM, Lavender P, Kuo C-hS, et al. DNA methylation modules in airway smooth muscle are associated with asthma severity. *European Respiratory Journal*. 2018;51(4).

- 34. Lin AHY, Shang Y, Mitzner W, Sham JSK, Tang W-y. Aberrant DNA Methylation of Phosphodiesterase 4D Alters Airway Smooth Muscle Cell Phenotypes. *American Journal of Respiratory Cell and Molecular Biology*. 2016;54(2):241-249.
- 35. Ning Y, Huang H, Dong Y, et al. 5-Aza-2'-deoxycytidine inhibited PDGF-induced rat airway smooth muscle cell phenotypic switching. *Arch Toxicol*. 2013;87(5):871-881.
- 36. Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. *Nature reviews Drug discovery*. 2013;12(11):847-865.
- 37. Sun M, Lu Q. MicroRNA regulation of airway smooth muscle function. *Biol Chem*. 2016;397(6):507-511.
- 38. Chiba Y, Misawa M. MicroRNAs and their therapeutic potential for human diseases: MiR-133a and bronchial smooth muscle hyperresponsiveness in asthma. *J Pharmacol Sci*. 2010;114(3):264-268.
- 39. Dileepan M, Jude JA, Rao SP, et al. MicroRNA-708 regulates CD38 expression through signaling pathways JNK MAP kinase and PTEN/AKT in human airway smooth muscle cells. *Respiratory Research*. 2014;15(1):107.
- 40. Liu Y, Yang K, Sun X, et al. MiR-138 suppresses airway smooth muscle cell proliferation through the PI3K/AKT signaling pathway by targeting PDK1. *Exp Lung Res*. 2015;41(7):363-369.
- 41. Liu Y, Sun X, Wu Y, et al. Effects of miRNA-145 on airway smooth muscle cells function. *Mol Cell Biochem*. 2015;409(1-2):135-143.
- 42. O'Leary L, Sevinc K, Papazoglou IM, et al. Airway smooth muscle inflammation is regulated by microRNA-145 in COPD. *FEBS Lett*. 2016;590(9):1324-1334.
- 43. Liu Y, Yang K, Shi H, et al. MiR-21 modulates human airway smooth muscle cell proliferation and migration in asthma through regulation of PTEN expression. *Exp Lung Res*. 2015;41(10):535-545.
- 44. Dileepan M, Sarver AE, Rao SP, Panettieri RA, Jr., Subramanian S, Kannan MS. MicroRNA Mediated Chemokine Responses in Human Airway Smooth Muscle Cells. *PLoS One*. 2016;11(3):e0150842.
- 45. Zhang XY, Zhang LX, Guo YL, et al. Schisandrin B inhibits the proliferation of airway smooth muscle cells via microRNA-135a suppressing the expression of transient receptor potential channel 1. *Cell Biol Int*. 2016;40(7):742-749.
- 46. Cheng W, Yan K, Xie LY, et al. MiR-143-3p controls TGF-beta1-induced cell proliferation and extracellular matrix production in airway smooth muscle via negative regulation of the nuclear factor of activated T cells 1. *Mol Immunol*. 2016;78:133-139.
- 47. Chen M, Shi J, Zhang W, et al. MiR-23b controls TGF-beta1 induced airway smooth muscle cell proliferation via direct targeting of Smad3. *Pulm Pharmacol Ther*. 2017;42:33-42.
- 48. Zhang H, Sun Z, Yu L, Sun J. MiR-139-5p inhibits proliferation and promoted apoptosis of human airway smooth muscle cells by downregulating the Brg1 gene. *Respir Physiol Neurobiol*. 2017;246:9-16.
- 49. Zhang XY, Tang XY, Ma LJ, et al. Schisandrin B down-regulated lncRNA BCYRN1 expression of airway smooth muscle cells by improving miR-150 expression to inhibit the proliferation and migration of ASMC in asthmatic rats. *Cell Prolif*. 2017;50(6).
- 50. Wang H, Yao H, Yi B, et al. MicroRNA-638 inhibits human airway smooth muscle cell proliferation and migration through targeting cyclin D1 and NOR1. *J Cell Physiol*. 2018.
- 51. Gao Y, Wang B, Luo H, Zhang Q, Xu M. miR-217 represses TGF-beta1-induced airway smooth muscle cell proliferation and migration through targeting ZEB1. *Biomed Pharmacother*. 2018;108:27-35.
- 52. Reuter S, Gupta SC, Park B, Goel A, Aggarwal BB. Epigenetic changes induced by curcumin and other natural compounds. *Genes & Nutrition*. 2011;6(2):93-108.

- 53. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Demedts MG, Verleden GM. Involvement of p38 MAPK, JNK, p42/p44 ERK and NF- κ B in IL-1 β -induced chemokine release in human airway smooth muscle cells. *Respiratory Medicine*. 2003;97(7):811-817.
- 54. Abidi A, Gupta S, Agarwal M, Bhalla HL, Saluja M. Evaluation of Efficacy of Curcumin as an Add-on therapy in Patients of Bronchial Asthma. *Journal of Clinical and Diagnostic Research : JCDR*. 2014;8(8):HC19-HC24.
- 55. Li Y, Seto E. HDACs and HDAC Inhibitors in Cancer Development and Therapy. *Cold Spring Harb Perspect Med*. 2016;6(10).
- 56. Perez-Salvia M, Esteller M. Bromodomain inhibitors and cancer therapy: From structures to applications. *Epigenetics*. 2017;12(5):323-339.
- 57. Morera L, Lübbert M, Jung M. Targeting histone methyltransferases and demethylases in clinical trials for cancer therapy. *Clinical Epigenetics*. 2016;8:57.
- 58. Rasco D, Tolcher A, Siu LL, et al. Abstract CT038: A phase I, open-label, dose-escalation study to investigate the safety, pharmacokinetics, pharmacodynamics, and clinical activity of GSK3326595 in subjects with solid tumors and non-Hodgkin's lymphoma. *Cancer Research*. 2017;77(13 Supplement):CT038.
- 59. Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V, Bansal P. MicroRNA therapeutics: Discovering novel targets and developing specific therapy. *Perspectives in Clinical Research*. 2016;7(2):68-74.
- 60. Montgomery R, Yu G, Ahangari F, et al. Feasibility, distribution, and efficacy of an inhaled oligonucleotide mimic of miR-29 for pulmonary fibrosis induced by bleomycin in rats. *European Respiratory Journal*. 2017;50(suppl 61).
- 61. Qi Y, Wang D, Wang D, et al. HEDD: the human epigenetic drug database. *Database: The Journal of Biological Databases and Curation*. 2016;2016:baw159.
- 62. Zhang X, Biagini Myers JM, Yadagiri VK, et al. Nasal DNA methylation differentiates corticosteroid treatment response in pediatric asthma: A pilot study. *PLoS One*. 2017;12(10):e0186150.

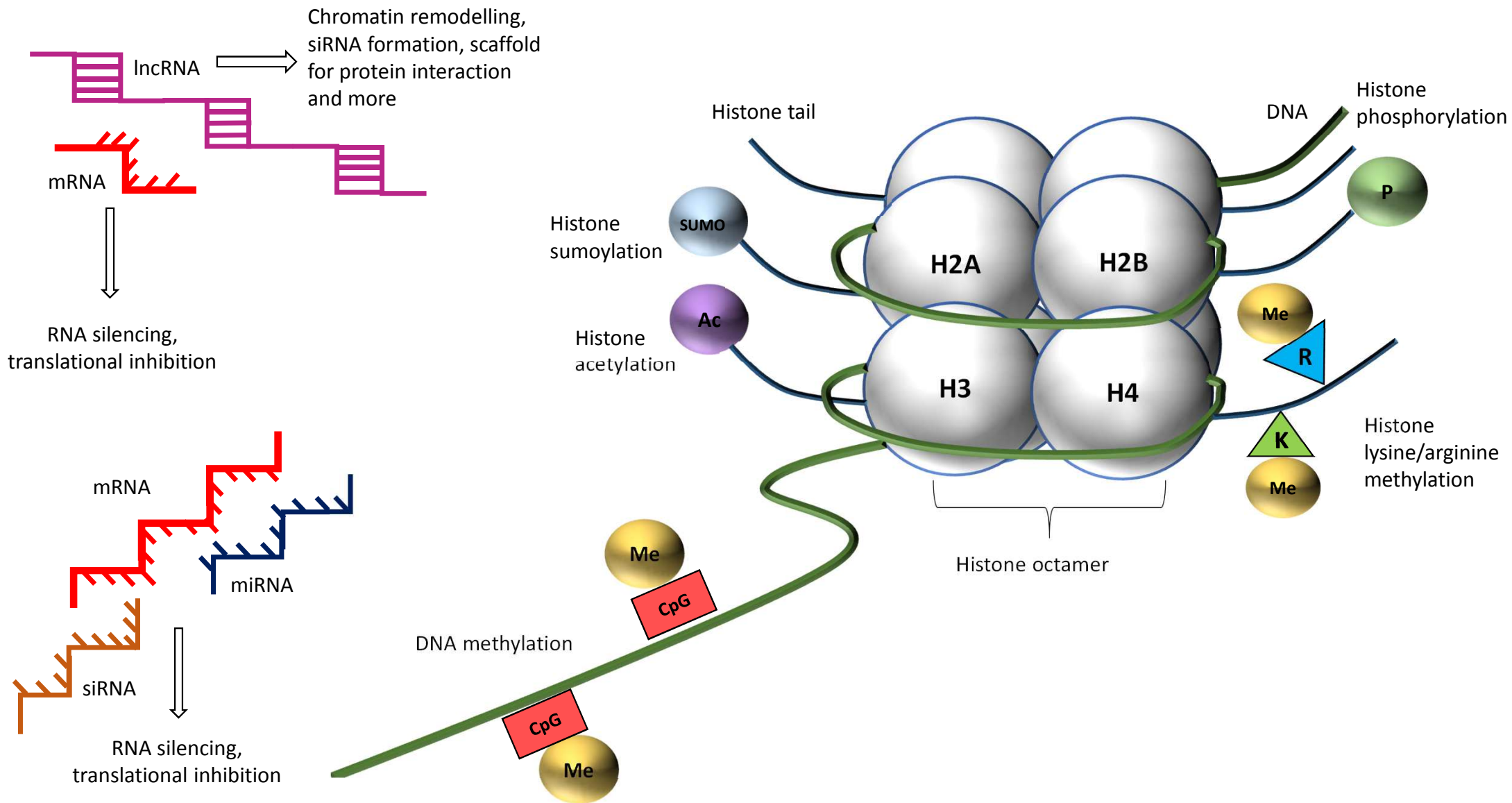
Table 1: Review of the role of miRNAs in ASM. Species is denoted as human (H), rat (R), mice (M)

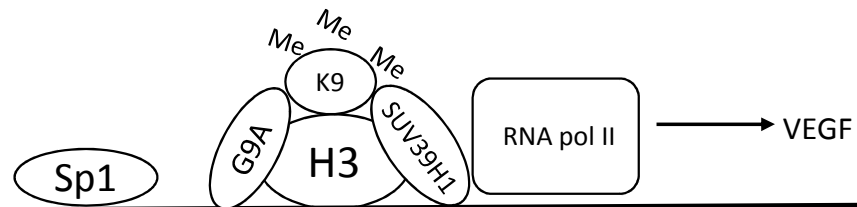
miRNA	Role in ASM	reference
miR-25	Upregulates KLF4 expression in cytokine-stimulated ASM (H)	37
miR-133a	Regulates bronchial smooth muscle (BSM) hyperresponsiveness, by negatively regulating the expression of RhoA, one of the key proteins of BSM contraction (M)	38
miR-221	Enhances proliferation and IL-6 release in ASM from patients with severe asthma (H)	37
miR-10a	Reduces mitogen-induced proliferation, via targeting PI3 kinase (H)	37
miR-708	Inhibits TNF- α -induced CD38 expression in ASM, through binding to 3'UTR of CD38 and regulating JNK MAPK and PI3K/AKT signalling (H)	39
miR-146a	Negatively regulates COX-2 and IL-1 β (H)	37
miR-155	Enhances COX-2 expression and PGE2 secretion (H)	37
miR-138	Reduces ASM proliferation via altering the expression of PDK1 and regulation of PI3K signalling (H)	40
miR-203	Inhibits ASM cell proliferation, controlling c-Abl expression, which in turn modulates ERK1/2 activation (H)	37
miR-145	Increases cell proliferation and migration and upregulates type I collagen and contractile protein MHC, negatively regulates pro-inflammatory cytokine release by targeting SMAD3 (H)	41,42
miR-21	Enhances proliferation and migration by reducing PTEN expression activation of PI3 kinase (H)	43
miR-708, miR-140-3p	Downregulates TNF- α – induced pro-inflammatory gene expression (H)	44
miR-135a	Negatively modulate the proliferation via TRPC1 (R)	45
miR-143-3p	Inhibits airway remodelling by suppressing proliferation and ECM protein deposition in TGF- β 1-activated cells through the negative regulation of NFATc1 signalling (H)	46
miR-23b	Inhibits airway remodelling, by suppressing proliferation via SMAD3 targeting (M)	47
miR-26a	Induces ASM cell hypertrophy (H)	37
miR-139-5p	Suppresses proliferation and promotes apoptosis (H)	48
miR-150	Reduces viability, proliferation and migration, via downregulation of <i>BCYRN1</i> (R)	49
miR-638	Inhibits ASM cell proliferation and migration through targeting cyclin D1 and NOR1 (H)	50
miR-217	Represses TGF- β 1-induced ASM cell proliferation and migration through targeting ZEB1 (H)	51

Figure legends

Figure 1: Epigenetic modifications- summary. Three main epigenetic mechanisms are DNA methylation, histone modifications, and noncoding RNAs. Histones are organized in the units of octamers, which are composed of two copies each of histones H2A, H2B, H3, and H4, wrapped by 147 base pairs of DNA. Histones have N- terminal tails, which protrude through the DNA and contain multiple residues, susceptible to undergo a series of posttranslational modifications, such as acetylation, phosphorylation, sumoylation, ubiquitination and methylation. These modifications regulate gene expression, and can lead to either gene transcription or repression, depending on the type of modification and its site. DNA methylation describes the addition of the methyl group to the C5 position of cytosine residues, primarily where cytosine is followed by a guanine (CpG sites). CpG islands are the regions of human genome with the high content of CpG sites - hypermethylation of CpG islands is associated with transcriptional repression, while hypomethylation in these regions leads to active transcription. Finally, non-coding RNAs (ncRNAs) are RNA molecules, which are not translated into proteins, but are able to regulate the transcription, stability or translation of protein-coding genes. They include long non-coding RNA (lncRNA), microRNA (miRNA), short interfering RNA (siRNA), and others, such as piwi-interacting RNA (piRNA). They exert their effect by interacting with messenger RNA (mRNA), histone modifying complexes, DNA methyltransferases, and various other proteins.

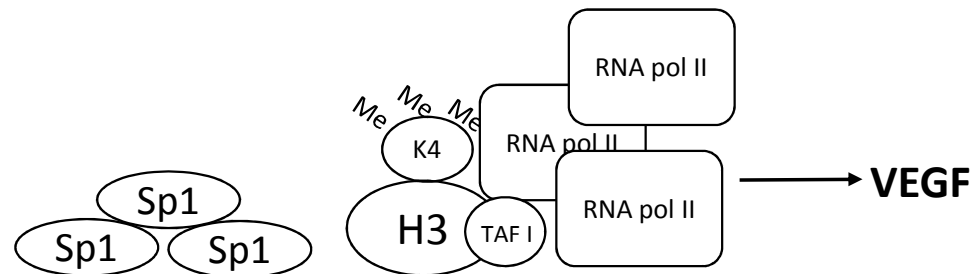
Figure 2: Abnormalities in histone modifications lead to hypersecretion of (A) VEGF and (B) CXCL8, from asthmatic airway smooth muscle (ASM) cells. (A) In non-asthmatic cells, a repressive complex is composed of H3K9me3, the histone methyltransferases G9A and SUV39H1, and low levels of Sp1. H3K9me3 and G9A recruitment repress VEGF transcription and maintain low levels of VEGF secretion. The asthmatic VEGF promoter lacks G9A recruitment and the repressive complex. Instead, transcriptionally active complex is formed at the promoter, with H3K4me3, increased RNA polymerase II, TAF1, and elevated levels of Sp1. (B) The hypersecretion of CXCL8 from asthmatic ASM cells is caused by increased RNA polymerase II, C/EBP β , and p65 binding and by aberrant presence of H3K18Ac and p300 at the asthmatic promoter. Then histone acetylation reader proteins Brd3 and Brd4 are necessary to induce CXCL8 transcription.



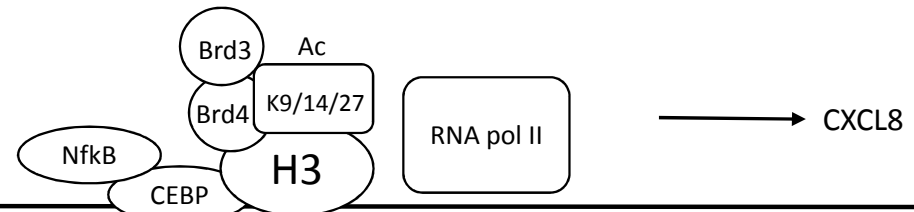
A Non-asthmatic

CpG methylation

Asthmatic

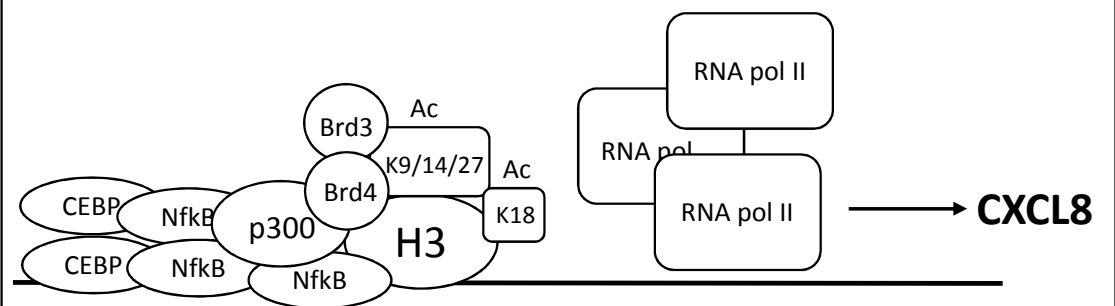


CpG methylation

B Non-asthmatic

CpG methylation

Asthmatic



CpG methylation