Epigenetic mechanisms of asthma pathogenesis

EPIGENETINIAI ASTMOS PATOGENEZĖS MECHANIZMAI

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Summary. Asthma is a heterogeneous chronic lung disease linked with increased inflammation and reversible obstruction of the airways. The immunoregulatory pathways underlying asthma include many different cell types, and eosinophils are major effector cells in disease pathogenesis. Their development is regulated and dependent on epigenetic factors that are still incompletely understood. Because epigenetic mechanisms are related to the effects of the environment, such as air pollution and tobacco smoke, their contribution to asthma pathogenesis is under active investigation. Asthma does not follow Mendelian patterns of inheritance; consequently, mechanisms are more complex, and the genes responsible for disease inheriting remain unidentified. Asthma epigenetics covers the transient and heritable phenotypic changes in gene expression without directly changing the DNA sequence. Three epigenetic mechanisms are distinguished, including DNA methylation, histone modifications, and the expression of non-coding RNAs. DNA methylation sites are notably altered in the airway epithelium, in turn contributing to immune responses and eosinophil activation. Histone modifications suggest themselves as regulators for the relief of asthma symptoms. Non-coding RNA-based therapies show a promising diagnostic potential to indicate severity, heterogeneity, and phenotype of asthma. Identification of altered methylation sites, significant changes in histone status, and non-coding RNA signatures in asthma patients can provide potential biomarkers of new therapeutic options for the treatment. For this reason, asthma epigenetics has recently attracted substantial interest. In this scientific review, the main focus will be on the current understanding of connections between epigenetic mechanisms and asthma pathogenesis.
Keywords: DNA methylation, histone modification, non-coding RNA, asthma, epigenetics.
Santrauka. Astma yra heterogeninė lėtinė plaučių liga, susijusi su padidėjusiu uždegimu ir kvėpavimo takų obstrukcija. Į astmos imunoreguliacijos kelius yra įsitraukę daug skirtingų ląstelių tipų, tačiau eozinofilai yra laikomi pagrindinėmis uždegiminėmis ląstelėmis, dalyvaujančiomis ligos patogenezėje. Jų raida yra reguliuojama ir priklauso nuo epigenetinių veiksnių, kurie vis dar nėra visiškai suprantami. Kadangi epigenetiniai mechanizmai yra susiję su aplinkos poveikiu, pavyzdžiui, oro tarša ir tabako dūmais, jų indėlis į astmos vystymąsi yra aktyviai tiriamas. Astma neatitinka Mendelio nustatytų paveldimumo dėsnių, todėl mechanizmai yra sudėtingesni, o genai, atsakingi už ligos paveldėjimą, lieka nenustatyti. Astmos epigenetika apima trumpalaikius ir paveldimus fenotipinius genų raiškos pokyčius, tiesiogiai nepakeičiant DNR sekos. Išskiriami trys epigenetiniai mechanizmai, įskaitant DNR metilinimą, histonų modifikacijas ir nekoduojančiųjų RNR raišką. DNR metilinimo pokyčiai yra ypač dažnai aptinkami kvėpavimo takų epitelyje, todėl yra svarbūs imuniniam atsakui ir eozinofilų aktyvacijai. Histonų modifikacijos atlieka reguliacinį vaidmenį ir gali būti žymuo, prisidedantis prie astmos simptomų palengvinimo. Nekoduojančiomis RNR pagrįsta diagnostika leidžia nustatyti astmos sunkumą, heterogeniškumą ir fenotipą. Pakitusio DNR metilinimo nustatymas, reikšmingos histonų modifikacijos ir nekoduojančiųjų RNR genų raiškos pokyčiai astma sergančiuose pacientuose gali suteikti naujų terapinių žymenų. Dėl šios priežasties astmos epigenetika pastaruoju metu sulaukia didelio susidomėjimo. Pagrindinis šios mokslinės apžvalgos dėmesys bus skiriamas supratimui apie epigenetinių mechanizmų sąsajas su astmos patogeneze.
Reikšminiai žodžiai: DNR metilinimas, histonų modifikacijos, nekoduojančiosios RNR, astma, epigenetika.

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INTRODUCTION

Epigenetic regulation includes heritable changes in gene expression that occur without changes in DNA sequences and may partially mediate the complex gene-by-environment interactions leading to asthma development. Asthma is a heterogeneous chronic respiratory disease affecting over 340 million individuals worldwide, with different clinical phenotypes cause hypersensitivity of the airways. It is characterized by dyspnoea, wheezing, and cough symptoms. The disease

severity is determined by exposure to irritants, smoking, medication, exercise, or viral respiratory infections [1].

Heritability of asthma has been established in many genetic studies, and it is already known that many genes with minor effects contribute to pathogenesis [2]. However, it is also clear that only genetic elements are not responsible. Monozygotic and dizygotic twin studies showed that genetic and environmental factors are important to asthma pathogenesis [3]. Environmental factors determine the epigenetics of asthma, and at the same time, it indicates the development and phenotype of the disease through gene expression [4].

Three epigenetic mechanisms are distinguished, including DNA methylation, histone modifications, and the expression of non-coding RNAs. DNA methylation represses gene transcription by coordinately regulating the chromatin status and hindering the transcription factors to bind either a gene's promoter or enhancer. Histones are associated with transcriptional repression at the post-translational level. Their modifications change the structure of the histone protein complex and recruit the chromatin remodeling. Non-coding RNAs, interacting with mRNA, modify and regulate gene expression, but differently from histone modifications, at a post-transcriptional level. A wide variety of recent studies have suggested that long non-coding RNAs, microRNAs, small interfering RNAs, and Piwi-interacting RNAs are the most common regulatory RNAs in epigenetic control. This review will concentrate on recent advances of significant asthma heterogeneity. Epigenetic of DNA methylation, histone modifications, and gene expression regulation by non-coding RNAs will be used to frame this discussion.

METHODS

The scientific review provides information from freely available foreign scientific periodicals with a citation rate in the Clarivate Analytics Web of Science, Scopus, and Springerlink databases. The information was collected using National Center for Biotechnology Information (NCBI) PubMed and PMC, Google Scholar, and the Wiley Online Library search systems. The following keywords were used in order to collect the information: asthma, asthma epigenetic, epigenetic mechanisms, DNA methylation, histone modifications, non-coding RNA, long non-coding RNA, microRNA, small interfering RNA, Piwi-interacting RNA.

Asthma-related key environmental factors

It is now well known that epigenetic mechanisms are important to control the cell cycle and gene expression pattern during the development in response to biological or environmental changes. Altered histone modification, aberrant DNA methylation, specific micro-RNA expression, and other chromatin alterations result in a complex early-life reprogramming of immune T-cell response, macrophage activation, dendritic cell function, and disruption of airway epithelial barrier that delivers asthma risk and severity in later life. The rapid and higher prevalence of asthma in the past few decades, especially in developed countries where asthma is the most common work-related disease, suggests that the surrounding environment is important for developing the disease. It is considered that most asthma cases originate at an early age, so they belong to a list of complex diseases that are "programmed" by specific factors in early life [5].

In the prenatal period, when airways grow, and the immune system develops, maternal exposure to certain nutritional factors, dust mites, viral infection, and tobacco smoke increases the risk of asthma in offspring (Figure 1). Severe viral infections of the lower respira-

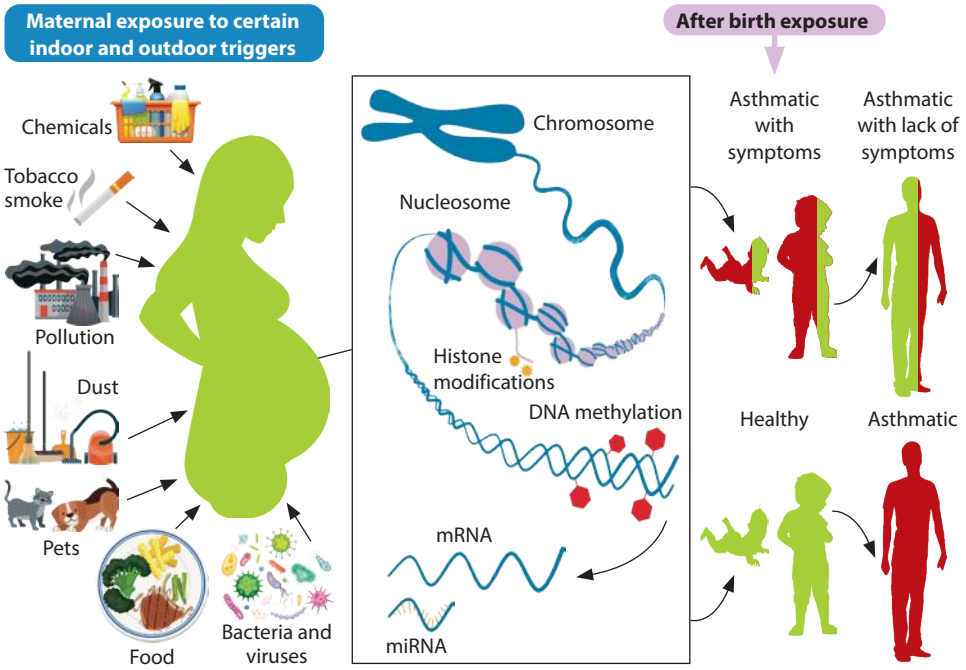

Figure 1. Epigenetic regulation after early environmental exposures

In the prenatal period, triggers such as tobacco smoking, dust, air pollution, nutritional factors, pets, and viral infection can increase the risk of asthma in offspring. Environment exposure can also affect a person after birth. For those who felt symptoms in adults, they may have less symptoms in adulthood. While those who were healthy children may become adults with asthmatic symptoms.

tory tract or exposure to air irritants increase the risk of asthma in early childhood, especially in the first years of life. It has been shown that outdoor microbial and viral pathogens, pollens, molds, airborne particulates, ozone, diesel exhaust particles, cold air, and humidity can cause or exacerbate asthma [2, 6]. Moreover, indoor allergens, such as dust mites, pets, mice, cockroaches, wood, particles generated from indoor combustion of tobacco, biological agents (indoor endotoxin), 1,3-β-glucans from molds, and gram-positive bacteria are also signs that can also contribute to the development of asthma [6]. Other asthma affecting factors are a variety of nutrients, such as omega-6 polyunsaturated fatty acids, vitamins C and D, saturated fat, β-carotene, selenium, sodium, and magnesium [7]; and pharmaceuticals, such as paracetamol [8]. Beyond question, variable asthma history (i.e., symptoms incidence and remission) may be the result of epigenetic regulation after early or subsequent environmental exposure. In early life, all these asthma triggers have an obvious reprogramming effect on immature airways and can lead to a change in asthma risk at a later age.

Overview of Epigenetic Mechanisms

Epigenetics covers the transient and heritable changes to gene expression in the setting of non-coding changes in the genome without affecting the DNA sequence. Epigenetic mechanisms have recently attracted substantial attention. They could significantly impact asthma-related immune and regulatory pathways, act as a potential biomarker and provide new therapeutic targets for the treatment of asthma. Asthma's epigenetic includes three major mechanisms: DNA methylation, histone modifications, and non-coding RNA expression. A visual scheme of the primary mechanisms is shown in Figure 2. Without these three main mechanisms, several chemical processes also may have epigenetic effects on DNA and protein modifications in asthma – phosphorylation, acetylation, and ubiquitin group transfer [9].

Epigenetics regulate immune response related to asthma

Asthma is a multifactorial disease, and epigenetics plays an important role in the regulation of immune response. Naive CD4+ T lymphocytes differentiation into the Th2 cells and expression of cytokines interleukin 4 (IL-4), IL-5, and IL-13 are essential for asthma pathogenesis. These cytokines recruit and activate allergic response primary effector cells, such as eosinophils and mast cells [10, 11]. Naive CD4+ T lymphocytes can differentiate into a Th2 lineage, depending on antigen-presenting cells, cytokine environmental, and antigen amounts. In naive CD4+ cells, the cytosine-phosphate-guanine (CpGs) in the promoter regions are methylated in the IL-4 genes. IL-4 is the major cytokine that affected the differentiation of naive CD4+ T lymphocytes into the Th2 lineage. The CD4+ T lymphocytes stimulation by allergen challenge enhances demethylation at the IL-4 locus, and the magnitude of methylation loss at the IL-4 promoter gene positively correlates with IL-4 expression [12].

Emerging evidence suggests that histone deacetylases (HDACs) maintain the balance of pre-established TH1-like and TH2-like responses in human T-cells. Interestingly, the HDAC inhibitor trichostatin A (TSA) triggered total cell hyperacetylation, resulting in increased Th2-related IL-5 and IL-13 cytokine expression. It was revealed that TSA treatment shifted the Th1:Th2 ratios 3 to 8-fold, skewing responses more a Th2-like phenotype [13]. Simultaneous activation

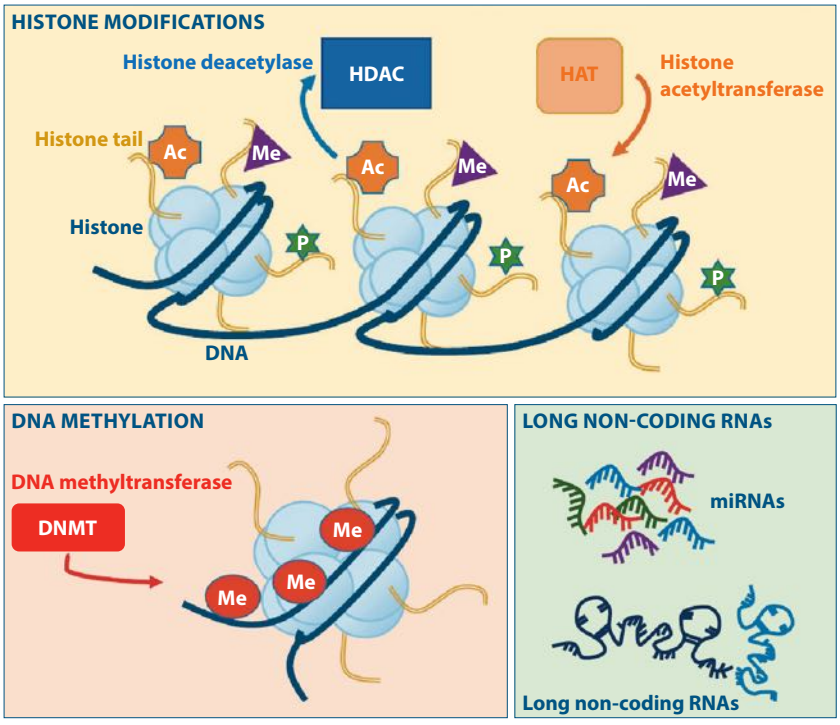

Figure 2. Three main epigenetic mechanisms: histone modifications, DNA methylation and non-coding RNAs.

DNA methylation occurs by adding a methyl group to the C-5 position of cytosine residues, primarily on the CpG site where a guanine nucleotide follows a cytosine nucleotide. Histones have N-terminal tails and globular domains, which may undergo post-translational modification, such as methylation, acetylation, sumoylation, phosphorylation, and ubiquitination. Non-coding RNAs are described as RNA molecules that are not coding proteins but are able to regulate gene expression by interacting with mRNA, histone-modifying complexes, DNA methyltransferases.

of T cells receptor (TCR) and IL-4 imbalanced Th2 homeostasis, which leads to the signal transducer and activator of transcription 6 (STAT6) phosphorylation and expression of GATA-binding protein 3 (GATA-3) and Th2 cytokines [12].

In general, inflammation is related to the activation of many major signaling pathways, particularly nuclear factor kappa B (NF-κB), which is activated in all asthma types, especially in severe asthma [14]. In the human airway smooth muscle cells, tumor necrosis factor α (TNF-α)-stimulated release of eotaxin is related to eotaxin gene promoter region histone H4 acetylation and NF-κB binding [15]. Nevertheless, IFN-γ is able to significantly inhibit TNF-α-stimulated expression of the NF-κB-sensitive genes IL-8, IL-6, and eotaxin. However, HDAC inhibitor TSA prevented the effects of IFN-γ on TNF-α-induced gene expression. Consequently, IFN-γ is closely related to HDAC activity or expression in airway smooth muscle cells [16]. This evidence suggests that inflammatory environmental stimuli can lead to Th2 skewing and increased inflammatory gene expression due to changes in histone acetylation status.

Altered DNA methylation

DNA methylation is the first epigenetic mechanism to be extensively studied in allergic diseases. It is the first identified and the most studied epigenetic alteration in human asthma due to the close relationship between DNA methylation and gene expression. It is a heritable epigenetic mechanism, catalyzed by DNA methyltransferases (DNMTs), that occurs by the transformation of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine (5mC) [17]. The presence of 5mC in different regions in the genome is associated with gene expression changes under the control of transcription factors [18]. CpG islands (CGI) are DNA-enriched segments in CG sequences; these CG-rich segments are often located near the gene transcription start site (TSS). Thus, changes in methylation of CGI are associated with gene expression activation or inhibition. In most cases, CGI is related to gene repression [19]. DNA methylation inhibits gene transcription by altering DNA conformation, stability, and activity, but it does not change DNA sequence [20].

The exploratory study revealed that prenatal exposure to the traffic-related polycyclic aromatic hydrocarbons is associated with umbilical cord white blood cell DNA methylation of acyl-CoA synthetase long-chain family member 3 (*ACSL3*) 5'-CGI and asthma symptoms in children up to age 5. It is suggested that methylated *ACSL3* 5'-CGI in umbilical cord white blood cell DNA may be a potential biomarker for environmentally-related asthma [21]. Another study showed that increased air pollution is associated with impaired Treg cell function. It occurs because of increased hypermethylation of CGI in the forkhead box transcription factor 3 (Foxp3) locus. Whereas Treg cells ensure protection from allergic diseases such as asthma by inhibiting IgE production in response to allergen exposure and proximal pathways of allergic sensitization [22].

Although DNA methylation in adults with asthma has been poorly studied, several studies suggest that smoking asthmatics have enhanced protocadherin-20 (*PCDH20*) methylation in sputum cells. Signatures of specific methylation were related to IL-13 response modules, eosinophil counts, fractional exhaled nitric oxide (FeNO), and an inhaled corticosteroid [23].

Since the airway epithelium has direct contact with antigens and bearing in mind that epigenetic changes are sensitive to environmental insults, DNA methylation may participate in the immune response. Bronchial epithelium cells were selected to investigate the methylation and expression levels of IL33 and two of its downstream targets – *IL1RL1* and *CCL26*. It was reported that the gene of IL33 and *CCL26* are less methylated in asthmatic bronchial epithelium cells than in bronchial epithelium cells in healthy individuals [24]. As the *CCL26* methylation level is reduced, the expression of this IL33 target is increased. Consequently, it contributes to asthma exacerbation and correlates with increased blood eosinophil count.

A large-scale epigenome-wide association study (EWAS) has uncovered several epigenome-wide methylation variants related to childhood-onset asthma with a reason to answer a question of how and which cell activity is affected by DNA methylated sites and whether these methylation models are already present at birth or develop during childhood. This meta-analysis identified 14 reduced DNA methylation CGI associated with childhood (4–16 years) asthma, but not at birth. Activation of purified eosinophils was increased, emphasizing that they are epigenetically altered in asthma [25]. This eosinophil methylation level could be used as a diagnostic tool for identifying children with asthma. This study also indicated that in the whole-blood CGI effector, memory CD8 T cells and natural killer cells activity was also increased, concurrent – the number of naive T cells was reduced [26].

Another valid research ascertained a decrease in expression of *ITGB4* by DNA methylation in asthmatic airway epithelial cells. Both in humans and mice, CGI is contained in the proximal promoters and exons of *ITGB4*, and for this reason, the asthma mice model was chosen for further indication. Results have demonstrated that the expression along with hypermethylation of *ITGB4* was strongly downregulated [27]. It can be related to the early identification of the

disease and a change of asthma phenotype. Besides, detection of DNA methylation in the specific CGI (chr17:73717720 and chr17:73717636) suggested it as a blood marker for asthma identification between asthmatics and healthy individuals.

Table 1. Summary of DNA methylation

DNA methylation	Relation to asthma	References
Methylated *ACSL3* 5'-CGI in umbilical cord white blood cell DNA	Related to asthma symptoms in children up to age 5; May be a potential biomarker for environmentally-related asthma	[21]
Increased hypermethylation of CGI in the Foxp3 locus	Is associated with impaired Treg cell function in the lungs, which can lead to worsening asthma pathology	[22]
Enhanced *PCDH20* methylation in sputum cells	Related to IL-13 response modules, eosinophil counts, fractional exhaled nitric oxide (FeNO), and an inhaled corticosteroid	[23]
The gene of IL33 and *CCL26* are less methylated in asthmatic bronchial epithelium cells than in healthy individuals	Contributes to asthma exacerbation and correlates with increased blood eosinophil count	[24]
DNA methylation level of purified eosinophils	Eosinophil methylation level could be used as a diagnostic tool for identifying children with asthma	[25]
Downregulated expression and hypermethylation of *ITGB4* in asthmatic airway epithelial cells than in healthy individuals	Blood marker for early asthma identification and phenotype determination	[27]

Altered histone modification

Histones are the main proteins, forming a DNA-protein complex, and modifications are essential epigenetic alterations. This association between histones and DNA is critical for DNA packaging, ensuring genome stability, and regulating gene expression. Most epigenetic research in asthma has been focused on histone acetylation and methylation; meanwhile, sumoylation, phosphorylation, citrullination, and ubiquitination are less investigated. Histone acetylation plays a key role in gene transcription and protein expression. It occurs when the lysine residues within the N-terminal tail are acetylated, and it is mediated by two main histone modifications enzymes – acetyltransferase (HAT) and histone deacetyltransferase (HDAC). Histone methylation is more complex. The modification comprises lysine and arginine residues, which occur within the histone N-terminal tail. Lysine residues can be mono-, di- or trimethylated, while arginine residues mono- or dimethylated [28]. This is important for gene transcription because depending on which amino acids are methylated and how many methyl groups are involved, the transcription of genes can either increase or decrease.

In present studies, enzyme histone acetyltransferase p300 (p300 HAT) transfection induced the acetylation of histone H3 in asthmatic lung tissues and regulated the expression of orosomucoid 1-like protein 3 (ORMDL3) gene [29]. *ORMDL3* correlates with an increased quantity of eosinophils, $CD4^+$, and T helper 2 (Th2) cytokines [30]. p300 HAT has also been reported as a promoter at the *VEGF* [31]. Increased secretion of *VEGF* from asthmatic airway smooth muscle cells contributes to the bronchial vascular remodeling. Aberrantly high levels of *VEGF* occur because of a transcription repression signal, which is dependent on histone methyltransferase G9A and histone H3K9 trimethylation. Meanwhile, in non-asthmatics, the *VEGF* promoter complex was repressed [32].

In patients with asthma, IL-8 or chemokine (C-X-C motif) ligand 8 (CXCL8) is expressed in the airway smooth muscle cells and contributes to airway remodeling. Children with severe asthma who constantly are in a smoking environment have a higher concentration of CXCL8. As a consequence, passive smoking impaired histone deacetylase-2 (HDAC2) activation via phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) (PI3K/AKT) pathway [33]. Although this study has been done in alveolar macrophages, it is relevant to airway smooth muscle epithelium as well. In asthmatic airway smooth muscle cells it was observed that acetylation of histone H3, histone H3K18, and binding of p300 HAT were increased compared with healthy donor cells. Methylation in CXCL8 showed no differences [34].

Airway inflammation, hyperresponsiveness, also higher eosinophil and IgE levels may be affected by histone H3 acetylation and histone H3K4 methylation. It occurs because of the deletion of the Th2 locus control region, which regulates the genes of Th2 cytokines [35]. Less than 2.5 micrometers in diameter particle matters and cold stress induce acetylation of H3K9 and H314, leading to an increased percentage of Th2 cells and H3K9/H3K14 hyperacetylation in IL-4 gene promoter. Likewise, a significant impact of p300 HAT and HDAC1 was detected in $CD4^+$ cells. Therefore it might contribute to histone modifications combined effects [36]. It is previously known that *GATA3* and *STAT6* are required to induce Th2 cell differentiation [37, 38], but *de novo* DNA methyltransferase-3A (DNMT3A) is also identified as a regulator of Th2 cell expression and epigenetic modifier. The deficiency of DNMT3A correlated with a decrease in DNA methylation and changes in the histone H3K27 at the IL-13 gene locus [39]. According to these observations, it can be noted that DNMT3A is also necessary for the

regular expression of IL-13. In general, several histone modifications regulate many asthma-related genes that initiate and maintain the symptoms of asthma. Table 2 summarizes the histone modifications and their association with this disease.

Table 2. Asthma-related histone modifications

Histone modification/ enzyme	Gene(s) or systems impacted	Relation to asthma	References
p300 HAT	ORMDL3	p300-mediated HAT modulates the expression of ORMDL3, thereby improving the airway remodeling in asthma	[29]
H3K9me3	VEGF	VEGF hypersecretion activates H3K9me3 in asthmatic bronchial smooth muscle cells	[32]
HDAC2	PI3K-AKT signaling pathway	HDAC2 function is impaired by passive smoking	[33]
H3ac, H3K18ac	CXCL8	Acetylation of H3ac, particularly H3K18, was increased in asthmatic airway smooth muscle cells CXCL8 promoter	[34]
H3ac, H3K4me	Th2 cytokine genes	Deletion of the Th2 locus control region causes a loss of general histone H3 acetylation, H3K4 methylation, and demethylation	[35]
H3K9ac, H3K14ac	CD4+ cells	Acetylation of H3K9 and H3K14 is increased in IL-4 gene promoter during particle matters exposure and cold stress on asthma	[36]
H3K27ac	IL- 13	Loss of DNMT3 leads to decreased DNA methylation and changes in the H3K27ac status at the IL-13 locus	[39]
Histone hyperacetylation	IL-13, IL-5	Increases Th2-related cytokines expression	[40]

Non-coding RNA

Non-coding RNAs (ncRNA) are functional RNA molecules transcribed from DNA but do not encode proteins. Mainly it includes long non-coding RNAs (lncRNAs) and small RNAs such as microRNAs (miRNAs), small interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs), which play a vital role in the pathogenesis and regulation of respiratory diseases, such as asthma [41].

Long non-coding RNA

Long non-coding RNA (lncRNA) is described as a large class of transcribed RNA molecules more than 200 bp in length [42]. Dysregulation of differentially expressed lncRNA was studied and associated with asthma. 159 lncRNAs were differentially expressed and found in bronchial mucosa samples obtained from asthmatic patients compared with samples received from healthy controls. Results demonstrated that 8 of 159 lncRNAs interacted with 52 differentially expressed asthma-related genes. The expression level of one of lncRNA, ZNF667 antisense RNA 1 (ZNF667-AS1), was downregulated in asthma patients [43]. Therefore, it might play an important role in the pathogenesis of asthma, but considering that these studies are performed recently, further investigation of ZNF667-AS1 is needed.

Other studies have shown that the lncRNA nuclear-enriched abundant transcript 1 (NEAT1) relative expression was increased in patients with asthma both in disease exacerbation and remission. In patients with asthma, *NEAT1* relative expression was positively correlated with pro-inflammatory cytokines (TNF- α, IL-1β, and IL-17) during the exacerbation, while negatively correlated with anti-inflammatory cytokine [44]. It is already known that the upregulation of *NEAT1* increased serum level of reactive oxygen species (ROS) [45]; due to this, it can be concluded that *NEAT1* might stimulate asthma exacerbation, leading to a high degree of airway obstruction and worsen immune response [46].

Airway smooth muscle cell proliferation and migration in asthma depend on lncRNA Transcription Factor 7 (TCF7), which expression is controlled by Translocase Of Inner Mitochondrial Membrane Domain Containing 1 (TIMMDC1) via AKT signaling pathway. The first observation was that lncTCF7/TIMMDC1/AKT regulatory mechanism has an epigenetic impact on the development and progression of asthma [47]. Also, the first was indicated on $CD4^+$ T cells with next-generation sequencing in acute asthma patients. 36 differentially expressed lncRNAs were upregulated, and 98 lncRNAs were downregulated compared with controls. The measurements were done by fluorescence *in situ* hybridization and decreased expression of lncRNA fantom3_9230106C11 was observed [48]. It is previously known that $CD4^+$ T cells are responsible for IgE-mediated sensitization, airway hyperreactivity, and eosinophilia promotion [49]. These described studies assessed the significance of altered lncRNA expression and provided novel evidence. Some of this evidence is new, and more detailed analysis is necessary. Nevertheless, the use of lncRNA could reverse asthma progression and therefore be provided as biomarkers and therapeutic targets.

MicroRNA

miRNA is actively involved in the normal and pathological cellular responses. miRNA are small, single-

stranded, and highly conserved RNA molecules that typically consist of 18-25 nucleotides in length [50]. Comparison of miRNA profiles in normal lung tissue and tissue from asthma patients indicated substantial differences. These differences were evaluated by measuring expression levels of miRNA-21 and miRNA-155 and correlating them with serum IL-4. It was found that levels of both miRNA-21 and miRNA-155 were considerably increased in asthmatic patients than in healthy people. Moreover, serum IL-4 was found as the only essential predictor for the expression of miRNA-21, and meanwhile, asthma the only significant factor for the expression of miRNA-155 [51]. Besides, similar research was conducted by Rodrigo-Munoz with colleagues, which have recently published that expression levels of miRNA-21 in blood eosinophils were higher in asthmatics versus healthy controls [52].

miRNA-1165-3p is also considered as a potential non-invasive biomarker for the clinical management of asthma. First of all, the expression of miRNA-1165-3p was significantly elevated in asthmatics compared to healthy patients. In a comparison of asthma severity, expression of miRNA-1165-3p was higher in patients with severe and acute asthma [53]. Accordingly, miRNA-1165-3p is assessed as an indicator of asthma severity, phenotype determination, and heterogeneity of the disease.

Several miRNAs are associated with smooth muscle cell function and proliferation and are implicated with severe asthma. For instance, miRNAs let-7 family in human and animal is associated with TH2 inflammation modulation [54]; miR-221 controls increased airway smooth muscle cell proliferation in severe asthma patients [55]; miR-146a/b and miR-28-5p downregulation lead to the circulating CD8+ T cells activation in severe asthma [56]; and miR-629-3p, miR-223-3p, and miR-142-3p is associated with severe neutrophilic asthma [57]. All these observations about the role of many miRNAs in asthma emphasize their effects on inflammatory responses, cell function, or disease severity.

Small interfering RNA

Small interfering RNAs (siRNAs) are double-stranded RNA molecules of 21–25 base pairs involved in the RNA interference pathway. siRNA is one of the major targets in RNA-induced silencing complex (RISC), essential in gene regulation [58].

In the pathogenesis of asthma, Th2 cells play a crucial role by producing pro-inflammatory cytokines (IL-4, IL-5, IL-6, IL-9, IL-13, IL-25) [59]. The secretion of these interleukins is promoted by *GATA3*, which can be therapeutic downregulated by post-transcriptional gene silencing. In this instance, the downstream release of all Th2 cytokines would be prevented in the same way.

Consequently, siRNA-based therapy is a potential tool to target any single mRNA and intermediate its downregulation [60]. This strategy has been proposed recently, so no further research has been published yet. Targeting *GATA3* in activated Th2 cells by RNA interference could be a promising treatment of asthma. Promising results are expected in the future, as no significant research has been conducted up to date.

Piwi-interacting RNA

The largest group of small non-coding RNAs (26–31 base pairs) [61], recently found in human $CD4^+$ cells [62], are Piwi-interacting RNA (piRNA). piRNA interacts with piwi-subfamily Argonaute proteins and forms RNA-protein complexes [61].

Inhibition of piRNA-30840 was measured on IL-4 in the samples of asthma patients and normal controls. Obtained results demonstrated that piRNA30840

Table 3. Overview of non-coding RNA's

Non-coding RNA	Relation to asthma	References
Long non-coding RNA		
lncRNA ZNF667-AS1	A potential identification biomarker in bronchial mucosa samples	[43]
lncRNA NEAT1	Might stimulate asthma exacerbation	[46]
lncRNA TCF7	An indicator for airway smooth muscle cell proliferation and migration	[47]
MicroRNA		
miRNA-21 and miRNA-155	Expression level differences indicate in asthma patients	[51]
miRNA-1165-3p	A potential non-invasive biomarker for the clinical management of asthma	[53]
miRNAs let-7	Associated with Th2 inflammation modulation	[54]
miR-221	Controls increase airway smooth muscle cell proliferation in severe asthma	[55]
miR-146a/b and miR-28-5p	Downregulation leads to the circulating CD8+ T cells activation in severe asthma	[56]
miR-629-3p, miR-223-3p, and miR-142-3p	Associated with severe neutrophilic asthma	[57]
Small interfering RNA		
siRNAs	A potential tool to target any single mRNA and intermediate its downregulation	[60]
Piwi-interacting RNA		
piRNA-30840	Down-regulated IL-4 expression and CD4 T-lymphocytes development	[62]

down-regulated IL-4 expression and CD4 T-lymphocytes development through sequence-specific binding to pre-mRNA intron [62]. These findings revealed a new possible gene expression pathway for piRNA, and interestingly, this inhibition suggested a new mechanism during pathologic asthma conditions. Detailed piRNA studies influencing the pathogenesis of asthma have not been performed yet.

PERSPECTIVES AND CONCLUSIONS

Epigenetics is a new field for research of asthma and allergies that has developed strongly in recent decades. Epigenetics helps to provide novel perspectives on the disease treatment and understanding of the complexities mechanisms. Moreover, it provided an implication of gene-environment interaction in the pathogenesis of asthma.

At the cellular level, altered DNA methylated sites are related to eosinophil activation and increased quantity. To investigate the eosinophils' recruitment in asthma, it is necessary to analyze methylation levels in the asthmatic bronchial epithelial cells compared with healthy individuals. Numerous histone modifications and their interactions with asthma-related genes are important biological markers for the immune response and relief of asthma symptoms. Studies of non-coding RNA expression are novel but promising in the future; for instance, an indication of lncRNA's different expression on CD4+ T cells and siRNA-based therapy by downregulating *GATA3* on Th2 cells. A better understanding of these and other mechanisms would bring new insights into new therapeutics development for asthma. These observations need to be translated into better prediction models, developing biomarkers, and therapies targeting dysregulated pathways.

A significant limitation to our current understanding of asthma is the lack of studies in adults, the effect of histone modifications in asthma, environmental data, traditional gene-by-environment interactions, environment-by-environment interactions, effects of birth order, other factors such as obesity, and the extent of associations with specific asthma phenotypes. Despite these limitations, it is promised in the future that epigenetics will help us understand the theoretically avoidable environmental disease. A better understanding of asthma pathogenesis would help better assign an asthma patient to the predominant asthma phenotype, thereby improving the course of the disease and reducing adverse drug reactions. Data integration of omics (genome-wide variation, transcriptome, methylome, and microRNAs) in well-characterized asthma risk cohorts can improve our understanding of these associations.

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