



The Role of Epigenetics in the Chronic Sinusitis with Nasal Polyp

Tiancong Liu¹ · Yang Sun¹ · Weiliang Bai¹

Accepted: 24 October 2020

© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Purpose of Review Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common and heterogeneous inflammatory disease. The underlying epigenetic mechanisms and treatment of CRSwNP are partially understood. Of the different epigenetic changes in CRSwNP, histone deacetylases (HDACs), methylation of DNA, and the levels of miRNA are widely studied. Here, we review the human studies of epigenetic mechanisms in CRSwNP.

Recent Findings The promoters of COL18A1, PTGES, PLAT, and TSLP genes are hypermethylated in CRSwNP compared with those of controls, while the promoters of PGDS, ALOX5AP, LTB4R, IL-8, and FZD5 genes are hypomethylated in CRSwNP. Promoter hypermethylation suppresses the gene expression, while promoter hypomethylation increases the gene expression. Studies have shown the elevation in the levels of HDAC2, HDAC4, and H3K4me3 in CRSwNP. In CRSwNP patients, there is also an upregulation of certain miRNAs including miR-125b, miR-155, miR-19a, miR-142-3p, and miR-21 and downregulation of miR-4492. Epigenetics takes part in the immunology of CRSwNP and may give rise to endotypes of CRSwNP. Both HDAC2 and the miRNA including miR-18a, miR-124a, and miR-142-3p may take function in the regulation of glucocorticoid resistance. HDAC inhibitors and KDM2B have shown effectiveness in decreasing nasal polyp, and DNA methyltransferase (DNMT) or HDAC inhibitors may have a potential efficacy for the treatment of CRSwNP.

Summary Recent advances in the epigenetics of CRSwNP have led to the identification of several potential therapeutic targets for this disease. The use of epigenetics may provide novel and effective biomarkers and therapies for the treatment of nasal polyp.

Keywords Chronic rhinosinusitis with nasal polyps (CRSwNP) · Nasal polyp · Epigenetics · Methylation · Histone deacetylase (HDAC) · miRNA

Introduction

Chronic rhinosinusitis (CRS) is an increasingly important medical problem with significant adverse impact on patient quality of life and the current therapies often prove inadequate. For the purposes of evaluating and treating these patients, current recommendations divide CRS into two subsets defined by the presence or absence of nasal polyps (NPs): CRS with NP (CRSwNP) and CRS without NP (CRSsNP) [1•]. The prevalence of nasal polyps was 2.7% (95% confidence interval, 1.9–3.5), and polyps were more frequent in men (2.2 to 1), the elderly (5% at ≥ 60 years of age), and

asthmatics [2]. While nasal polyps are observed in a variety of clinical conditions including cystic fibrosis and malignancy, they are benign and typically develop bilaterally in the sinonasal cavity. Clinically, patients with CRSwNP have shown poor response to medical management and high post-operative recurrence because of NP regrowth [3, 4], and it is frequently associated with bronchial asthma [5–7]. Both genetic and environmental factors are believed to contribute to the pathogenesis of CRS. Epigenetics has recently become one of the most promising and expanding fields to unravel the gene expression pattern in several diseases. The most well-studied example is cancer, but other diseases like asthma, allergic rhinitis, inflammation-associated diseases such as lung injury, autoimmune disease, and type 2 diabetes display epigenetic regulation during their occurrence. CRSwNP formation is also identified as closely related to epigenetic processes. Epigenetic modification is essential for regulating tissue- and stage-specific gene expression during growth and development, and can serve as a mediator of gene environment interactions in the pathogenesis and progression of

This article is part of the Topical Collection on *Rhinosinusitis*

✉ Weiliang Bai
bweiliangcmu@163.com

¹ Department of Otolaryngology Head and Neck Surgery, Shengjing Hospital of China Medical University, Shenyang 110004, China

disease [8]. Gene expression patterns are strongly influenced by epigenetic changes, including DNA methylation, histone acetylation, and chromatin remodeling [9].

Basics of Epigenetics

DNA Methylation

Mammalian DNA methylation (the presence of 5-methylcytosine, 5mC) has important functions in a lot of biological processes, including genomic imprinting, cell fate determination, chromatin architecture organization, and regulation of gene expression [10–12]. More than half of the genes in vertebrate genomes contain short (approximately 1 kb) CpG-rich regions known as CpG islands (CGIs), and the rest of the genome lacks for CpGs. Gene regulation by 5mC was initially studied by analyzing CGIs. The presence of 5mC at CGI is usually related with inhibiting gene expression; however, the underlying mechanisms are not necessarily identical at gene promoters, gene bodies, or repeated sequences [13•]. Aberrant DNA methylation changes are linked with many human diseases, particularly with cancer. Recent studies showed that DNA methylation gave rise to the pathogenesis of airway allergic inflammation such as bronchial asthma [14, 15], aspirin-tolerant asthma [16], and allergic rhinitis (AR) [17]. Cardenas et al. reported that the nasal DNA methylation was a sensitive biomarker of asthma, allergic rhinitis, and airway inflammation [18].

Histone Modifications

Histones are the main proteins forming the DNA-protein complex chromatin. This close association with DNA is essential for providing genomic stability and regulation of gene expression. Regulation of histone function through post-translational modifications is another key epigenetic mechanism. Multiple enzymes are involved in histone modifications; however, they can be divided into two main categories, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Both HATs and HDACs are subdivided based on their location and specificity. A simple model for understanding the role of HATs and HDACs in histone modifications is their opposing effect on lysine acetylation. Due to their stabilizing effect on the chromatin architecture following reversal of lysine acetylation, HDACs act as transcriptional repressors [19]. In addition to acetylation, histone phosphorylation and methylation are complementary mechanisms involved in the regulation of gene expression. Histone core phosphorylation regulates DNA accessibility through nucleosome unwrapping [20], and histone methylation, controlled by multiple methyltransferases and demethylases, results in the addition and removal of methyl groups leading to dynamic changes in these

proteins affecting their role in the regulation of gene function. Importantly, DNA methylation affects histone methylation and vice-versa [21, 22]. There are several known mechanisms of epigenetic regulation, of which DNA methylation and histone modifications are the best understood to date (Fig. 1).

Non-coding RNAs

Non-coding RNAs (ncRNAs) are classified into four well-characterized forms: microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), and long non-coding RNAs (lncRNAs) [23]. miRNAs are short non-coding RNA molecules containing approximately 22 nucleotides that negatively regulate gene expression through interacting with the 3'-UTR (untranslated regions) of mRNA (messenger RNA) which results in the degradation of mRNA or repression of protein translation [24, 25]. The main function of piRNA was to protect the integrity of germ cell genome by silencing mRNA of transposable elements, which can interrupt the genome by insertion or transposition [26]. Additional roles of piRNA are their involvement in genome rearrangement and epigenetic programming [27]. siRNAs, which downregulate gene expression guided by sequence complementarity, can be used therapeutically to block the synthesis of disease-causing proteins [28]. Despite their classification under the ncRNA label, small and large ncRNAs are diverse and exhibit specific behaviors that characterize their function.

Evidences Revealing the Changes of DNA Methylation in CRSwNP

CRSwNP is a complex, multifactorial disease that involves multiple genetic, immunological, environmental, and mucosal factors, but its etiology remains unclear. Many potential contributing factors have been identified, including allergic responses, impaired mucociliary clearance, immune dysfunction, impaired epithelial defense, microbes, and environmental exposure [29]. Nevertheless, further research is needed to determine the role of genetic factors and their interaction with these contributing factors in the pathophysiology of CRSwNP.

The majority of human studies of epigenetic mechanisms in nasal polyp have concentrated in DNA methylation differences between nasal polyps and normal tissues with gene chip methods. These studies have revealed the methylation changes in CRSwNP. Zheng et al. screened the COL18A1 gene with methylation microarray. The methylation frequency of COL18A1 was significantly higher in NPs than in inferior turbinate mucosa samples [30]. COL18A1 encodes the type XVIII collagen α chain which constitutes endostatin. Endostatin can inhibit endothelial cell proliferation and angiogenesis. Methylation changes in COL18A1 may influence the production of endostatin. Suzuki et al. found that recombinant endostatin could prevent the development of asthma features in a mouse

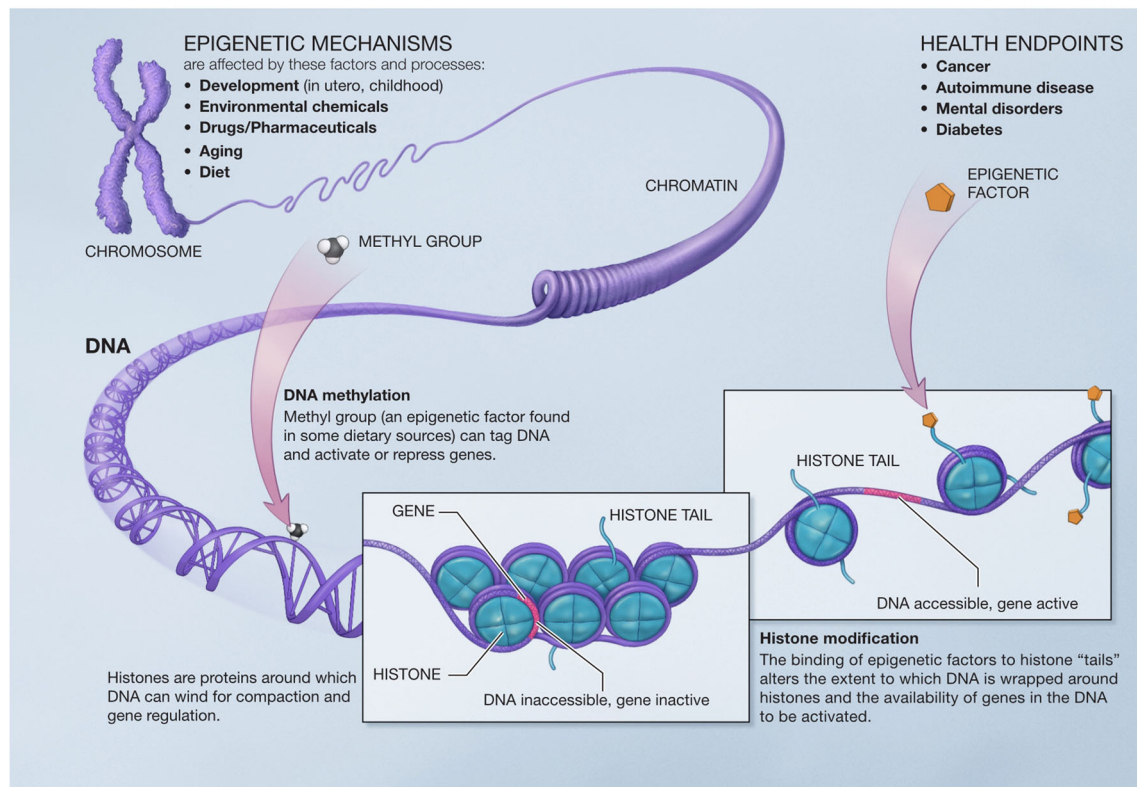


Fig. 1 Overview of mechanisms involved in epigenetic regulation of gene expression. There are several epigenetic mechanisms that regulate gene expression during development and in response to environmental and other factors. DNA methylation suppresses (downregulates) gene expression. Histone modification can occur via different pathways that

lead to different effects (e.g., histone acetylation facilitates gene expression). A third group of mechanisms, post-translational RNA modification, is not pictured here for clarity. Modified from the US National Institutes of Health (<https://commonfund.nih.gov/epigenomics/figure>)

model [31]. So COL18A1 might play an important role in the formation and development of CRSwNP. Kim et al. found that the promoter regions of 10 and 30 genes were hypermethylated and hypomethylated, respectively, in NP samples compared with controls [32]. The genes of KRT19, NR2F2, and ADAMTS1 were hypomethylated in CRSwNP, and their mRNA expressions were verified higher than normal tissues. This represents the direct involvement of a translational regulation mechanism in polyp formation. Moreover, differential methylation patterns were detected in nasal polyp tissue from patients with aspirin-intolerant asthma. Hypermethylation was detected at 332 loci in 296 genes, while hypomethylation was detected at 158 loci in 141 genes. In the arachidonate pathway, LTB4R, ALOX5AP, and PGDS were hypomethylated, whereas PTGES was hypermethylated [16]. The genes and pathways identified in this study may be attractive targets for further study.

NPs are associated with eosinophilic-dominant inflammation and the presence of Th2 cytokines, which is also observed in CRS with asthma and AR [33, 34]. Studies showed that Th2 cytokine negatively regulated the tissue-type plasminogen activator (t-PA; gene name PLAT) gene expression in cultured airway epithelial cells [35]. Kidoguchi et al. found that hypermethylation of the proximal PLAT promoter may lead to decreased gene expression levels in NP, leading to excessive fibrin

deposition by insufficient plasmin activation [36]. Infiltration of the sinus mucosa with inflammatory cells is a hallmark of CRS, with IL-8 released by airway epithelial cells in response to inflammatory stimuli possibly playing an important role in attracting neutrophils to the site of inflammation [37]. Indeed, increased IL-8 levels have been detected in significantly greater amounts in nasal mucosa of patients with CRS versus healthy control subjects [38]. Patients with CRSwNP have a more significant decrease of DNA methylation of 3 CpG sites in the IL-8 proximal promoter than both patients with CRSsNP and control subjects. The level of hypomethylation of IL-8 proximal promoter CpG sites is associated with tissue IL-8 protein expression and granulocyte activation [39]. Thymic stromal lymphopoietin (TSLP) is an IL-7-like and epithelial cell-derived cytokine that is commonly recognized as a driver of T_{H2} responses. TSLP stimulates dendritic cells (DCs) to induce naive CD_{4+} T cell differentiation into T_{H2} cells and cytokine production [40], indicating that TSLP plays an important role in allergic and immune disorders. While one study has demonstrated significant overexpression of TSLP mRNA in NP tissues from CRSwNP patients compared to nasal mucosa from CRSsNP and control subjects [41], another study has shown that the numbers of immunohistochemically stained TSLP⁺ nasal epithelial cells were significantly increased in NP tissues from atopic subjects

compared to non-atopic subjects and also strongly correlated with the number of tissue eosinophils and IgE levels in NP tissue [42•]. Li et al. found that increased DNA methylation at the TSLP locus is likely to be associated with CRSwNP pathogenesis; however, these findings need to be confirmed in multicenter studies with larger age-matched cohorts [43]. Nasal polyps and eosinophil/non-eosinophil counts are reported to be directly correlated. FZD5 is a receptor for Wnt5A, and its complex with Wnt5A contributes to activating inflammation and tissue modification. Kim et al. found that FZD5 expression was significantly higher in nasal polyp samples from patients with eosinophilic CRSwNP (E-CRSwNP) than in those from patients with non-eosinophilic CRSwNP, as indicated by immunohistochemistry. The FZD5 gene was hypomethylated in the E-CRSwNP tissues as compared with the controls [44].

Bacteria can affect the transcriptional program of host cells by influencing DNA methylation [45]. *Staphylococcus aureus* (*S. aureus*) is associated with CRSwNP, atopic dermatitis, and allergic rhinitis [46]. *S. aureus* could disrupt immunologic and mechanical barrier by promoting defective mucociliary clearance. *S. aureus* could promote nasal polyp formation, prolonged inflammation, and bacterial imbalance. The nasal polyp stimulated by *S. aureus* enterotoxin B (SEB) may have resulted in hypermethylation rather than in hypomethylation, and the methylation changes mainly took place at intragenic regions. The genes of CTSLL2, Y_RNA and AC022026.3 showed hypermethylation at promoter regions [47•]. These studies suggest superantigens may lead to DNA methylation resulting in some gene expression changes in the nasal mucosa. Gene promoter hypermethylation or hypomethylation plays an essential role in nasal polyp through maintaining of inflammation via epigenetic control of gene expression. Some genes are hypermethylated, and some other genes are hypomethylated in the nasal polyps compared with that of normal tissues (Table 1). The analysis of methylation changes has the potential to assist in the detection, management, and possible prevention of nasal polyp as diagnostic tools.

HDAC2, HDAC4, and H3K4me3 Maybe the Target in the Treatment of CRSwNP

Similar to DNA methylation, histone modifications do not affect DNA nucleotide sequence but can modify its availability to the transcriptional machinery. Histone acetylation, which is a type of chromatin modification, is regulated by HAT and HDAC. Histone acetylation by HAT is generally associated with increased transcription. In contrast, deacetylation of histones by HDAC is associated with transcriptional repression [48, 49]. HDACs have been divided into four classes based on their homology with yeast proteins [50]. Class I HDACs, which are homologous to the yeast protein RPD3 and expressed in all human cell lines and tissues, include HDAC 1, 2, 3, and 8 that have a nuclear localization [51, 52]. Class II can be subdivided into two subclasses: IIa (HDAC 4, 7, and 9) and IIb (HDAC 6 and 10). Class II exhibits tissue-specific expression and can shuttle between the nucleus and cytoplasm, which suggests that this class of HDACs is involved in acetylation of non-histone proteins [51]. Class I and class II HDACs are inhibited by trichostatin A (TSA). Class III HDACs, or sirtuins (SIRT1-7), are homologous with the yeast SIRT2 family of proteins [53]. The location and pattern of tissue-specific expression of this class are unknown [54–56]. HDAC11, the only member of class IV, is homologous to both class I and class II HDACs [57]. The varieties of HDACs reflect diversification of functions in different tissues and biological processes [58].

TSA is an inhibitor of HDAC which could suppress TGF- β 1-induced extracellular matrix (ECM) expression in kidney [59], cornea [60], and lung [61] fibrosis. In nasal polyp tissues, expression levels of α -SMA, fibronectin, and collagen type I RNA transcripts and proteins were increased by TGF- β 1 treatment. Cho et al. found that TSA could reverse the effect of TGF- β 1 on RNA and protein expression levels by reducing HDAC2 and HDAC4 [62•]. TSA could not only reduce airway inflammation but also decrease the

Table 1 The genes that were hypermethylated or hypomethylated in the nasal polyps compared with control tissues

Phenotype	Hypermethylated genes	Hypomethylated genes	Reference
Nasal polyp	COL18A1		[30]
Nasal polyp	RP1, ESPN, MAP6, MPND, SKA1, STAMBPL1, ARHGAP31, ARHGAP31-AS1, PTGER4, ZNF222	RP11-120J1.1, RUFY(4-16), DMRT3, NR2E1, PTPN3, OSTM1, AMN, SEPT10, ZNF609, FAM196A, KRT19, PDE3A, TMEM132E, ANKRD18A, NR2F2, ROBO2, ADAMTS1, SLC18A3	[32]
Nasal polyp (AERD)	PTGES	PGDS, ALOX5AP, LTB4R	[16]
Nasal polyp	PLAT		[36]
Nasal polyp		IL-8	[39]
Nasal polyp	TSLP		[43]
Eosinophilic CRSwNP		FZD5	[44]

expression of IL-4, IL-5, and TGF- β 1 [63], while IL-4, IL-5, and TGF- β 1 can give rise to the formation of CRSwNP. These results suggest that TSA plays a role in the inhibition of nasal polyp development and has clinical advantages for the treatment of CRSwNP. HDAC2 is expressed in airway epithelium and plays a pivotal role in inflammatory cells. However, the role of HDAC2 in allergic airway inflammation remains poorly understood. Lai et al. found that HDAC2 gene silencing further enhanced inflammatory cytokines in human bronchial epithelial cells [64]. HDAC2 overexpression could restore the enhanced inflammatory cytokines. It suggests that activation of HDAC2 could prevent the development of allergic airway inflammation. The effect of HDAC2 in nasal polyp is different from that of other diseases like asthma. The research results are controversial. The use of HDAC inhibitors has yielded conflicting results, with some studies showing anti-inflammatory effects and others pointing to enhanced inflammation, thus requiring further investigation of the use of this treatment [65, 66]. So the study of HDAC2 effect in nasal polyp needs to be further explored. HDAC4 may take a particular role in the allergy diseases. Activation of AMPK modulates miR-206/HDAC4/cyclin D1 signaling pathway to suppress smooth muscle cell (ASMC) proliferation [67]. Targeting on HDAC4 has a potential value in the prevention and treatment of asthma by alleviating airway remodeling. Studies showed that hsa-miR-20a-5p could attenuate allergic inflammation in HMC-1 cells by targeting HDAC4 [68]. Histone methylation may either activate or inhibit the expression of downstream genes, depending on the site and degree of methylation [69]. Histone H3 lysine 4 (H3K4) methylation refers to the modification occurring at the fourth lysine residue from the N-terminus of histone H3. H3K4 methylation is associated with transcriptional activation and elongation. The fourth lysine residues of histone H3 can be monomethylated (H3K4me1), di-methylated (H3K4me2), and trimethylated (H3K4me3), which greatly enhances the diversity of the regulatory function of histone methylation [70]. Studies showed that H3K4me3 expression was increased in nasal polyp samples compared with inferior turbinate samples, suggesting that histone methylation may play an important role in control of nasal epithelia. H3K4me3 expression markedly declined with MLL1 reduction [71]. MLL1 may be a potential therapeutic target for nasal inflammatory diseases. KDM2B could bind to the promoters of p15INK4B, p16INK4A, and p57KIP2 and thereby reduced the H3K4me3 and H3K36me2 levels [72]. KDM2B and transcriptional regulator Brg1 were significantly decreased in nasal mucosa of CRSwNP patients, and they were positively correlated [73]. KDM2B may have an inhibitory effect on the development of CRSwNP nasal mucosal epithelial inflammation through the regulation of H3K4me3, which needs to be further verified.

CRSwNP Is Associated with the Changes in the Levels of microRNA

microRNAs (miRNAs), a group of endogenous non-coding RNAs with highly conserved sequences [74], play a role in regulating gene expression by specifically binding to the 3'-untranslated region base sequence of target messenger RNAs (mRNAs) [75]. miRNAs are deeply involved in the regulation of normal and pathologic cellular responses [76]. Specific miRNA profiles were demonstrated in CRSwNP. In a miRNA microarray chip analysis-based study, Xuan et al. found that five upregulated miRNAs, including miR-210-5p, miR-3178, miR-585-3p, miR-3146, and miR-320e, and 19 downregulated miRNAs, including miR-32-3p, miR-1299, miR-3196, miR-3924, and miR-548e-3p, were differentially expressed in tissues of CRSwNP versus controls [77]. Another study showed that miR-125b and miR-155 were significantly upregulated while miR-92a, miR-26b, and miR-181b were downregulated in CRSwNP patients compared with that of CRSsNP [78].

Apart from the studies showing the widespread changes in miRNA expression, scientists have also focused on individual miRNAs to delineate their specific role in CRSwNP. The immune regulatory cells mainly include regulatory T cells and regulatory B cells [79, 80]. Some subfractions of dendritic cells (DCs) also have an immune regulatory function. IL-10 and transforming growth factor- β are the main immune regulatory mediators [81]. Studies showed that the IL-10 expression in peripheral DC was significantly lower in polyp patients, and the levels of miR-19a were markedly higher in DCs in the polyp group [82]. Exposure to recombinant IL-4 suppressed the IL-10 expression in DCs, which was abolished by knocking down the miR-19a gene in DCs. It indicated that miR-19a might play a critical role in the suppression of IL-10 in peripheral DCs, which may be a target in the immune therapy for nasal polyp. Eosinophilic chronic rhinosinusitis (CRS) with nasal polyps (CRSwNP) represents a hard-to-treat subtype of CRS. An important role of miR-125b has also been delineated as its increased levels have been detected in eosinophilic CRSwNP. The expression of miR-125b has been found in a diversity of organs including the trachea [83]. Overexpression of miR-125b in BEAS-2B cells may result in a downregulation of 4E-BP1 protein expression, but no change in 4E-BP1 mRNA expression. Consistently, miR125b inhibitor could increase 4E-BP1 protein expression without changing mRNA levels in HBECs. Studies showed that 4E-BP1 protein expression negatively correlated with miR-125b expression, indicating a link between miR-125b and 4E-BP1 in eosinophilic CRSwNP. Knockdown of 4E-BP1 can enhance virus evoked type I IFN production through increasing IRF-7 expression in mouse embryonic fibroblasts [84]. The miR-125b-mediated suppression of 4E-BP1 may play a critical role in the airway antiviral innate immunity.

Through promoting type I IFN expression, miR-125b-4E-BP1 pathway may contribute to mucosal eosinophilia in eosinophilic CRSwNP [85•]. Scientists have also shown an increase in the expression of miR-142-3p in nasal mucosa of chronic rhinosinusitis with polyps patients compared to normal human. TNF- α is mainly produced by mononuclear macrophages and eosinophils and could induce interleukin (IL)-6, IL-10, and interferon- γ (IFN- γ) release and play a role in cytotoxicity and growth inhibition [86]. In addition, TNF- α could induce nuclear factor kB (NF-kB), C-fos, and C-Jun expression and regulate the immunoreaction, which is a broad spectrum of pro-inflammatory cytokines [87]. miR-142-3p and TNF- α expression levels were increased after LPS stimulation in a dose- and time-dependent manner. Knockdown of miR-142-3p in HNEpCs downregulated tumor necrosis factor alpha (TNF- α) expression at both messenger RNA and protein levels [88]. It indicated that miR-142-3p might participate in the regulation of the body's inflammatory response through the LPS-TLR-TNF- α signaling pathway in chronic rhinosinusitis with nasal polyposis. In another study, the expression levels of miR-21 in CRSwNP were significantly higher than those in CRSsNP and controls. Downregulation of miR-21 resulted in increased PTEN and decreased Akt phosphorylation. Furthermore, overexpression of miR-21 had the opposite effects. So TGF- β 1-miR-21-PTEN-Akt axis may contribute to the pathogenesis of CRSwNP. miR-21 might be a reliable target for treating nasal polyp genesis [89•].

In contrast to the above described studies showing an increase in the levels of different types of miRNAs in CRSwNP, scientists have found an opposite role of miR-4492 in this disease. Studies showed that miR-4492 was downregulated and IL-10 was upregulated in CRSwNP versus CRSsNP tissues, and an inverse correlation between miR-4492 and IL-10 was determined in CRS tissues [90]. Deregulation of a number of cytokines, including IL-1, IL-2, IL-10, and TNF- α , has been detected in a large number of diseases, including cancer, autoimmune diseases, and inflammatory diseases. Increasing evidence suggests that cytokines are key mediators of the inflammation and immune response associated with NP, among which TNF- α and IL-10 are two predicted targets of miR-4492. Dysregulation of IL-10 is involved in the immunopathologic response to infection and the development of numerous autoimmune diseases [91]. Little is known about miR-4492 function. One study indicated that miR-4492 suppresses colorectal cancer progression [92]. Another study revealed that miR-4492 mimics significantly suppressed bladder cancer cell proliferation, migration, and invasion, suggesting that miR-4492 is a tumor suppressor in bladder cancer [93]. In KEGG pathway analysis, IL-10 was enriched in the Jak/STAT signaling pathway. It is indicated that miR-4492 may serve roles in nasal polyposis via the hsa-miR-4492/IL-10 interaction in the Jak/STAT signaling pathway [94]. The different expressions of miRNA in CRSwNP are seen in

Table 2. DNA methylation can inhibit the transcription of miRNAs, just like coding genes, by methylating the CpG islands in the promoter regions of miRNAs. Conversely, certain miRNAs can directly target DNA methyltransferases and bring about their inhibition, thereby affecting the whole genome methylation pattern [95•].

The Functions of Epigenetic Changes in the Endotypes of CRSwNP

There is currently increasing evidence to suggest that there are numerous endotypes of CRSwNP, with different pathophysiologies and different forms. CRSwNP can be divided into three different endotypes: the T cell-/Th2 cytokine-based endotype, the B cell-/IgE-based endotype, and the eosinophil-based endotype [96•]. This gives rise to different treatment approaches in CRSwNP. This type 2 inflammatory pattern is characterized by a high presence of eosinophils, mast cells, basophils, and T-helper 2 (Th2) cells, and comorbid associations with asthma and atopy [97]. Within the type 2 inflammatory milieu, multiple cytokines, including IL-5, IL-4, and IL-13, have also been found to drive the immunologic pathways central to CRSwNP pathophysiology. IL-33 is a chemoattractant for Th2 cells and promotes the production

Table 2 The expression of miRNA in the nasal polyps compared with control tissues

Phenotype	Overexpression	Lower expression	Reference
Nasal polyp	miR-210-5p, miR-3178, miR-585-3p, miR-3146, and miR-320e	miR-32-3p, miR-1299, miR-3196, miR-3924, miR-548e-3p, miR-3184-5p, miR-375, miR-23a-5p, miR-377-5p, miR-574-5p, miR-3149, miR-500a-5p, miR-125b-2-3p, miR-1914-5p, miR-532-3p, miR-612, miR-1298-5p, miR-1226-3p, miR-668-3p	[77]
Nasal polyp	miR-125b, miR-155	miR-92a, miR-26b, miR-181b	[78]
Nasal polyp	miR-19a		[82]
Eosinophilic CRSwNP	miR-125b		[85•]
Nasal polyp	miR-142-3p		[88]
Nasal polyp	miR-21		[89•]
Nasal polyp		miR-4492	[94]

of Th2 cytokines such as IL-4, IL-5, and IL-13. The cytokines IL-25, IL-33, and TSLP have an effect on type 2 innate lymphoid cells (ILC2) [98]. Both B cells and the antibody fractions IgA and IgE were found to be elevated in patients with CRSwNP [99, 100]. It was shown that the chemokines CXCL12 (SDF-1 α) and CXCL13 (BCA-1) are present at elevated levels in nasal polyps. Both attract B cells. Furthermore, the receptors for SDF-1 α (CXCR4 and CXCR7) and BCA-1 (CXCR5) are also present at elevated levels [101]. RANTES is a member of the CC chemokine family and a strong chemoattractant for eosinophils and T lymphocytes, but not for neutrophils, and is primarily secreted by nasal epithelial cells [102, 103]. Increased RANTES expression results in an increased number of eosinophils in tissue. Therefore, RANTES likely also plays an important role in the mobilization of eosinophils in nasal polyps. Poposki and colleagues were able to show strong production of CCL23 in nasal polyps, which was largely colocalized with ECP, indicating predominantly eosinophilic CCL23 production in nasal polyps [104].

Methylation, histone modifications, and microRNAs all take part in the regulation of Th2 cytokine type of CRSwNP. Gata3 acts as a master regulator for T-helper 2 (Th2) cell differentiation by inducing chromatin remodeling of the Th2 cytokine loci, accelerating Th2 cell proliferation, and repressing Th1 cell differentiation. Studies showed that methylation on Gata3 negatively regulates elongation of the IL-5 transcript in T-helper 2 cells [105]. The IL-4 and IL-5 DNA methylation was significantly lower, and IL-10 and IFN- γ DNA methylation was higher in active IgE-mediated cow's milk allergy (CMA) patients [106]. Studies showed that ILC2-expressed GATA-3 were able to produce IL-5, IL-13, and IL-4 by demethylation [107]. Endogenous HDAC activity plays a crucial role in maintaining the balance of pre-established T(H)1-like and T(H)2-like responses, inhibiting excessive T(H)2 immunity [108]. The HDAC inhibitor TSA could enhance the human IL-5 and IL-4 gene expression by altering histone acetylation [109, 110]. miR-1248 could interact with the IL-5 transcript in the 3'-untranslated region and serves as a positive regulator to increase IL-5 expression [111]. Studies showed that miR-21 levels positively correlated with IL-13 levels and miR-21 might play a role in the regulation of IL-13 [112]. Liu et al. found that miR-487b could inhibit the expression of IL-33 [113].

The B cell/IgE-based endotype was also regulated mainly by methylation and microRNAs. We and colleagues found that IgE levels are associated with DNA methylation levels [114]. The expression of CXCL12 and CXCR4 could be increased by increasing DNA demethylation [115, 116]. Studies showed that miR-146a could enhance the secretion of IgE in B cells by upregulating 14-3-3 σ expression [117]. miR-221 could promote IgE-mediated activation of mast cell degranulation by the PI3K/Akt/PLC γ /Ca(2+) pathway [118]. Zhang

et al. found that miR-192 could block the activation pathway of T follicular helper cells by decreasing the expression of CXCR5 [119].

In E-CRSwNP, the expression of FZD5 and IL-5 could be increased by hypomethylation [44, 97]. Studies showed that the upregulated expression of miR-125b may enhance type I IFN expression through suppressing 4E-BP1 protein expression in airway epithelial cells, which potentially contributes to mucosal eosinophilia in eosinophilic CRSwNP [76]. The relationship between epigenetics and the three endotypes of CRSwNP is seen in Fig. 2.

Epigenetics and Glucocorticoid Resistance in CRSwNP

CRSwNP is an inflammatory condition with heterogeneous pathophysiology. A cornerstone of the management of this condition is the use of anti-inflammatory agents. Corticosteroids are very effective and the most commonly used [120]. Although systemic corticosteroids have shown benefit in managing this condition, the frequency of use often required in this condition is associated with significant adverse effects. Topical corticosteroids, particularly when utilized after endoscopic sinus surgery and delivered in a high-volume, high-pressure manner, provide the desired anti-inflammatory effects with nearly negligible systemic absorption [121]. Patients who often respond most favorably to glucocorticoid (GC) treatment are those with a Th2-mediated, highly eosinophilic CRSwNP.

However, there are some patients who fail to respond to GC, so-called GC resistance. The functions of GC are mediated by the GC receptors (GRs), which consist of three isoforms: GR- α , GR- β , and GR- γ [122]. GR- α could regulate GC signaling transmission, while GR- β and GR- γ prevent the GC activation despite its binding to GCs [123]. One reason of GC resistance is GC receptor modification, which includes phosphorylation [124], nitrosylation [125], and ubiquitination [126]. Some studies have reported increased expression of GR- β in glucocorticoid-resistant patients of several diseases, including asthma, rheumatoid arthritis, and inflammatory bowel disease [123, 127]. Downregulation of GR- α mRNA suggests the existence of glucocorticoid insensitivity, and the expression of GR- β may play an important role in the evaluation of the glucocorticoid therapeutic effect in patients with nasal polyps [128]. IL-1 β is one of the important cytokines involved in the etiology of nasal mucosal inflammation. IL-1 β could induce a reduction of the GR α /GR β ratio through p38 MAPK and JNK pathways. Studies showed that specific inhibitors of the p38 MAPK and JNK pathways resulted in an increased GR α /GR β ratio and a recovery of GC affinity in IL-1 β -induced polyps [129].

Epigenetics may also take part in the GC resistance. HDAC2 is important for corticosteroids to switch off inflammatory gene transcription [130]. HDAC2 activity and

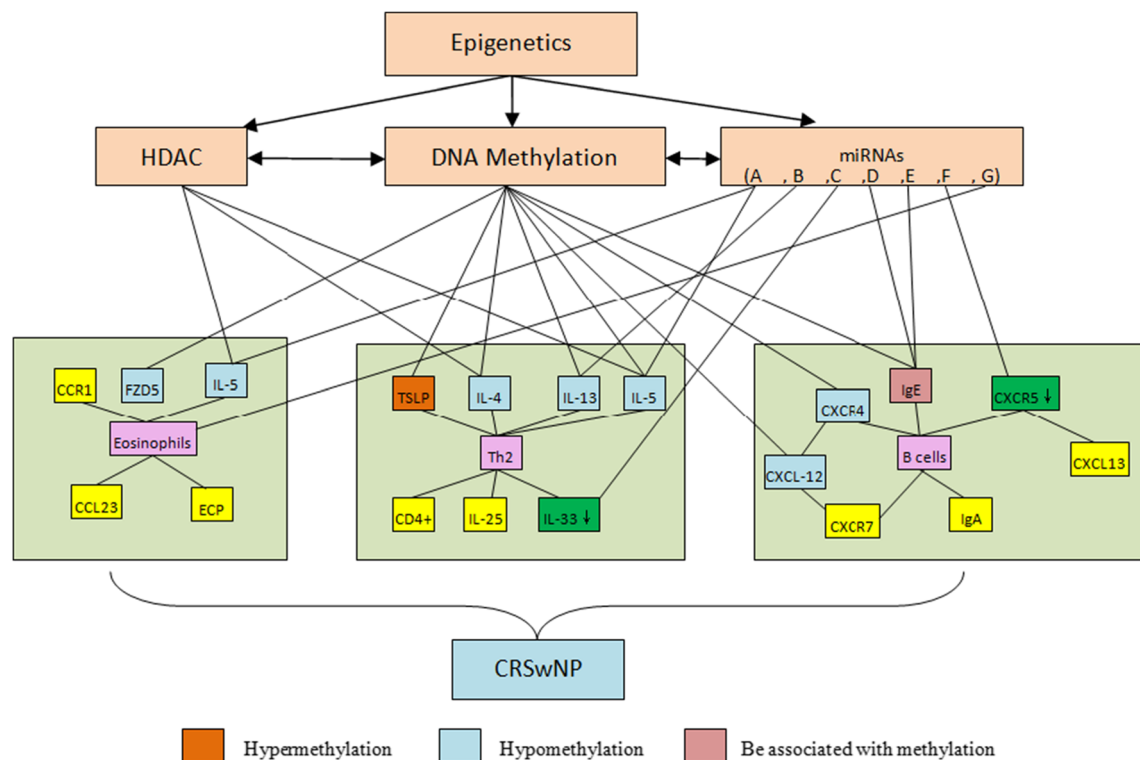


Fig. 2 CRSwNP can be divided into three different endotypes: the T cell-/Th2 cytokine-based endotype, the B cell-/IgE-based endotype, and the eosinophil-based endotype. All the three types are regulated by

epigenetics. (A: miR-1248, B: miR-21, C: miR-487b, D: miR-146a, E: miR-221, F: miR-192, G: miR-125b). The epigenetics takes regulation function in the three different endotypes

expression are reduced in patients with some diseases that respond poorly to glucocorticoid treatment. Overexpression of HDAC2 (by use of a plasmid vector) in bronchoalveolar macrophages from patients with GC-resistant COPD restored GC sensitivity to the level seen in controls [131]. Whether HDAC2 expression is also reduced in glucocorticoid-resistant patients with other inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, nasal polyp has not yet been investigated. Promoter methylation may silence the GR- α and take part in GC resistance. Mata-Greenwood et al. found that human endothelial GR mRNA expression is regulated by promoter methylation, and dexamethasone-sensitive and dexamethasone-resistant cells had different GR promoter methylation patterns [132].

miRNAs make an interesting candidate for the regulation of GC resistance because they are potent inhibitors of post-transcriptional gene expression via target cleavage or translational repression [133]. miR-29a and miR-18a could decrease GCs' responsiveness by diminishing GR expression levels [134]. miRs 18 and 124a could also reduce GR-mediated events by decreasing GR protein levels [135]. High levels of miR-142-3p resulted in low levels of GR- α protein expression, thus leading to T-leukemic cell resistance to GCs [136]. Expression of miR-221 and miR-142-3p was found to be elevated and combination of their levels could confirm diagnosis of allergic rhinitis with the sensitivity of 81% and

specificity of 65% [137]. Interestingly, miR-142-3p was significantly higher in nasal mucosa of chronic rhinosinusitis with polyps patients compared to normal human [138]. So the miR-142-3p may be responsible for the GC resistance in CRSwNP and need to be further studied. With increasing miRNA patterns emerging into researchers' vision, they worth more investigation as promising predictive biomarkers for drug effectiveness and disease prognosis.

Current Epigenetic Therapy in CRSwNP

With the advancement of new diagnostic tools, it reveals that there are many diseases that are caused by epigenetic modifications. Development of "epigenetic therapies" now has the immense potential towards the treatment of many diseases including cancer, cardiovascular disorders, and also inflammation. Recently, many agents have been found to modify epigenetic patterns by changing DNA methylation patterns or cause modification of histones. Many of these agents are currently being tested in clinical trials [139].

DNA methyltransferase (DNMT) inhibitors, 5-azacytidine (clinical name Vidaza), and 5-aza-2'-deoxy-5-azacytidine (clinical name decitabine) are pan inhibitors and are now used to increase fetal hemoglobin production by causing hypomethylation of γ -globin genes in patients with sickle cell disease [140]. Azacitidine (AZA) and decitabine (DAC) are

hypomethylating agents (HMAs) commonly used to treat myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) when not eligible for intensive chemotherapy and allogeneic transplant [141–144]. AZA was the first drug to demonstrate a survival benefit in a randomized trial for patients with MDS. Oral steroids are recommended for the treatment of nasal polyps (NPs), but prolonged use is avoided because of side effects. Topical steroids can also control NPs without significant complications; however, the response to this is partially successful, and additional therapies are needed to treat glucocorticoid-resistant NPs. Yeo et al. revealed that topical AZA decreases inflammatory cytokines on human NP explants and this could have future therapeutic implications for NPs [145]. Methyl-CpG-binding protein 2 (MeCP2), known as a transcriptional regulator, has been suggested to play an important role in myofibroblast differentiation in the lung. 5-Azacytidine significantly could not only inhibit the expression of α -SMA and fibronectin mRNA in nasal polyp-derived fibroblasts (NPdFs) but also suppress collagen production and the expression of MeCP2 in a dose-dependent manner [146••].

HDAC inhibitors have been found to be a novel category of anticancer drugs. To date, the United States Food and Drug Administration have approved four HDAC inhibitors, vorinostat, romidepsin, panobinostat, and belinostat. More specifically, these HDAC inhibitors have the least severe side effects and are mostly used for hematologic cancers. HDAC inhibitors are also used in various types of neurodegenerative diseases, inflammation disorders, and cardiovascular diseases [147]. Similar to AZA, zebularine is another cytidine analog that inhibits DNMT1 activity. Although not yet FDA approved, this isoform has found good results in mouse models [148]. Epigenetic modulation by histone deacetylase (HDAC) inhibitors including trichostatin A (TSA) has been reported to have inhibitory effects on myofibroblast differentiation in lung and renal fibroblasts. Studies showed that TSA suppressed TGF- β 1-induced myofibroblast differentiation and ECM production by inhibition of HDAC2 and HDAC4 and hyperacetylation of histone in nasal polyp organ cultures [62•]. TSA could be a candidate of a therapeutic agent for reversing the TGF- β 1-induced ECM synthesis that leads to nasal polyp development. Histone deacetylase inhibitors may have a potential efficacy for the treatment of neurodegenerative diseases, cardiac hypertrophy, and asthma [149, 150]. Since the drugs modulating epigenetic changes have not been rarely evaluated clinically in the nasal polyp, therefore, it is not possible to precisely describe the side effects and efficacy of these drugs in nasal polyp. Nevertheless, it is possible to hypothesize that the clinical effects of epigenetic modulators may be long lasting and these drugs may normalize the hyperactive immune system to inhibit the pathogenesis of nasal polyp. Accordingly, future clinical studies shall be planned to explore the efficacy and safety of these drugs in nasal polyp.

Conclusions

CRSwNP is a common and heterogeneous inflammatory disease, which may involve many genes and many pathways. Epigenetic modification is essential for regulating tissue- and stage-specific gene expression during growth and development. Some genes are hypermethylated, and some other genes are hypomethylated in nasal polyp. In CRSwNP patients, there is also an upregulation of certain miRNAs including miR-125b, miR-155, miR-19a, miR-142-3p, and miR-21 and downregulation of miR-4492. HDAC2, HDAC4, and H3K4me3 may be the target in the treatment of CRSwNP. Both HDAC2 and the miRNA may take function in the regulation of glucocorticoid resistance. DNMT or HDAC inhibitors may have a potential efficacy for the treatment of CRSwNP. The epigenetics could give rise to the classification of nasal polyp and may provide new candidate genes for the treatments. The use of epigenetics has emerged as a viable means to provide biomarker and novel therapy for the treatment of nasal polyp.

Funding This work was supported by the National Natural Science Fund (NO.: 81241083), the science public welfare fund of Liaoning Province (NO.: 2013001010), and the science plan program of Shenyang City (NO.: F13-220-9-21).

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. • Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European position paper on rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020;29:1–464 **It shows the new treatment and development in nasal polyp.**
2. Johansson L, Akerlund A, Holmberg K, Melen I, Bende M. Prevalence of nasal polyps in adults: the Skovde population based study. *Ann Otol Rhinol Laryngol*. 2003;112:625–9.
3. Wynn R, Har-El G. Recurrence rates after endoscopic sinus surgery for massive sinus polyposis. *Laryngoscope*. 2004;114:811–3.
4. Veloso-Teles R, Cerejeira R. Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: clinical outcome and predictive factors of recurrence. *Am J Rhinol Allergy*. 2017;31:56–62.
5. Tokunaga T, Sakashita M, Haruna T, Takeno S, Ikeda H, Nakayama T, et al. Novel scoring system and algorithm for

- classifying chronic rhinosinusitis: the JESREC study. *Allergy*. 2015;70:995–1003.
6. Rosati MG, Peters AT. Relationships among allergic rhinitis, asthma, and chronic rhinosinusitis. *Am J Rhinol Allergy*. 2016;30:44–7.
 7. Van Zele T, Holtappels G, Gevaert P, Bachert C. Differences in initial immunoprofiles between recurrent and nonrecurrent chronic rhinosinusitis with nasal polyps. *Am J Rhinol Allergy*. 2014;28:192–8.
 8. Yang IV, Lozupone CA, Schwartz DA. The environment, epigenome, and asthma. *J Allergy Clin Immunol*. 2017;140:14–23.
 9. Plass C, Pfister SM, Lindroth AM, Bogatyrova O, Claus R, Lichter P. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. *Nat Rev Genet*. 2013;14:765–80.
 10. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev*. 2002;16:6–21.
 11. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*. 2003;33(Suppl):245–54.
 12. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. *Nat Rev Genet*. 2013;14:204–20.
 13. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet*. 2012;13:484–92 **Evaluation of different functions of DNA methylation in different gene positions.**
 14. Harb H, Renz H. Update on epigenetics in allergic disease. *J Allergy Clin Immunol*. 2015;135:15–24.
 15. Nicodemus-Johnson J, Naughton KA, Sudi J, Hogarth K, Naurekas ET, Nicolae DL, et al. Genome-wide methylation study identifies an IL-13-induced epigenetic signature in asthmatic airways. *Am J Respir Crit Care Med*. 2016;193:376–85.
 16. Cheong HS, Park SM, Kim MO, Park JS, Lee JY, Byun JY, et al. Genome-wide methylation profile of nasal polyps: relation to aspirin hypersensitivity in asthmatics. *Allergy*. 2011;66:637–44.
 17. Samowski C, Laprise C, Malerba G, Moffatt MF, Dizier MH, Morin A, et al. DNA methylation within melatonin receptor 1A (MTNR1A) mediates paternally transmitted genetic variant effect on asthma plus rhinitis. *J Allergy Clin Immunol*. 2016;138:748–53.
 18. Cardenas A, Sordillo JE, Rifas-Shiman SL, Chung W, Liang L, Coull BA, et al. The nasal methylome as a biomarker of asthma and airway inflammation in children. *Nat Commun*. 2019;10:3095.
 19. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011;21:381–95.
 20. Brehove M, Wang T, North J, Luo Y, Dreher SJ, Shimko JC, et al. Histone core phosphorylation regulates DNA accessibility. *J Biol Chem*. 2015;290:22612–21.
 21. Bartke T, Vermeulen M, Xhemalce B, Robson SC, Mann M, Kouzarides T. Nucleosome-interacting proteins regulated by DNA and histone methylation. *Cell*. 2010;143:470–84.
 22. Fuks F. DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev*. 2005;15:490–5.
 23. Jodar M, Selvaraju S, Sandler E, Diamond MP, Krawetz SA. The presence, role and clinical use of spermatozoal RNAs. *Hum Reprod Update*. 2013;19:604–24.
 24. Bartel D, MicroRNAs P. Genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281–97.
 25. Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nat Rev Genet*. 2008;9:831–42.
 26. Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol*. 2011;12:246–58.
 27. Ross RJ, Weiner MM, Lin H. PIWI proteins and PIWI-interacting RNAs in the soma. *Nature*. 2014;505:353–9.
 28. Wittup A, Lieberman J. Knocking down disease: a progress report on siRNA therapeutics. *Nat Rev Genet*. 2015;16:543–52.
 29. Hsu J, Avila PC, Kern RC, Hayes MG, Schleimer RP, Pinto JM. Genetics of chronic rhinosinusitis: state of the field and directions forward. *J Allergy Clin Immunol*. 2013;131:977–93.
 30. Zheng YB, Zhao Y, Yue LY, Lin P, Liu YF, Xian JM, et al. Pilot study of DNA methylation in the pathogenesis of chronic rhinosinusitis with nasal polyps. *Rhinology*. 2015;53:345–52.
 31. Suzuki Y, Hamada K, Sho M, Ito T, Miyamoto K, Akashi S, et al. A potent antiangiogenic factor, endostatin prevents the development of asthma in a murine model. *J Allergy Clin Immunol*. 2005;116:1220–7.
 32. Kim JY, Kim DK, Yu MS, Cha MJ, Yu SL, Kang J. Role of epigenetics in the pathogenesis of chronic rhinosinusitis with nasal polyps. *Mol Med Rep*. 2018;17:1219–27.
 33. Bachert C, Wagenmann M, Hauser U, Rudack C. IL-5 synthesis is upregulated in human nasal polyp tissue. *J Allergy Clin Immunol*. 1997;99:837–42.
 34. Stevens WW, Schleimer RP, Kern RC. Chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol Pract*. 2016;4:565–72.
 35. Takabayashi T, Kato A, Peters AT, Hulse KE, Suh LA, Carter R, et al. Excessive fibrin deposition in nasal polyps caused by fibrinolytic impairment through reduction of tissue plasminogen activator expression. *Am J Respir Crit Care Med*. 2013;187:49–57.
 36. Kidoguchi M, Noguchi E, Nakamura T, Ninomiya T, Morii W, Yoshida K, et al. DNA methylation of proximal PLAT promoter in chronic rhinosinusitis with nasal polyps. *Am J Rhinol Allergy*. 2018;32:374–9.
 37. Jundi K, Greene CM. Transcription of interleukin-8: how altered regulation can affect cystic fibrosis lung disease. *Biomolecules*. 2015;5:1386–98.
 38. van Drunen CM, Reinartz S, Wigman J, Fokkens WJ. Inflammation in chronic rhinosinusitis and nasal polyposis. *Immunol Allergy Clin N Am*. 2009;29:621–9.
 39. Li J, Jiao J, Wang M, Gao Y, Li Y, Wang Y, et al. Hypomethylation of the IL8 promoter in nasal epithelial cells of patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2019;144:993–1003.
 40. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol*. 2002;3:673–80.
 41. Nagarkar DR, Poposki JA, Tan BK, Comeau MR, Peters AT, Hulse KE, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol*. 2013;132:593–600.
 42. Kimura S, Pawankar R, Mori S, Nonaka M, Masuno S, Yagi T, et al. Increased expression and role of thymic stromal lymphopoietin in nasal polyposis. *Allergy Asthma Immunol Res*. 2011;3:186–93 **TSLP is overexpressed in nasal polyp and maybe become a novel biomarker; the methylation of TSLP may be a new treatment target.**
 43. Li J, Jiao J, Gao Y, Zhang Y, Zhang L. Association between methylation in nasal epithelial TSLP gene and chronic rhinosinusitis with nasal polyps. *Allergy Asthma Clin Immunol*. 2019. <https://doi.org/10.1186/s13223-019-0389-3>.
 44. Kim JY, Cha MJ, Park YS, Kang J, Choi JJ, In SM, et al. Upregulation of FZD5 in eosinophilic chronic rhinosinusitis with nasal polyps by epigenetic modification. *Mol Cells*. 2019;42:345–55.
 45. Bieme H, Hamon M, Cossart P. Epigenetics and bacterial infections. *Cold Spring Harb Perspect Med*. 2012;2:a010272.

46. Vickery TW, Ramakrishnan VR, Suh JD. The role of *Staphylococcus aureus* in patients with chronic sinusitis and nasal polyposis. *Curr Allergy Asthma Rep*. 2019;19:21.
47. Pérez-Novo CA, Zhang Y, Denil S, Trooskens G, De Meyer T, Van Criekinge W, et al. Staphylococcal enterotoxin B influences the DNA methylation pattern in nasal polyp tissue: a preliminary study. *Allergy Asthma Clin Immunol*. 2013;9:48 **The gene methylation of nasal polyp may take place with the stimulation of *S. aureus* enterotoxin B, which will lead to the changes of some gene expression.**
48. Barnes PJ, Adcock IM, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur Respir J*. 2005;25:552–63.
49. Adcock IM, Tsaprouni L, Bhavsar P, Ito K. Epigenetic regulation of airway inflammation. *Curr Opin Immunol*. 2007;19:694–700.
50. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov*. 2006;5:769–84.
51. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J*. 2003;370:737–49.
52. Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol*. 2007;1:19–25.
53. Sengupta N, Seto E. Regulation of histone deacetylase activities. *J Cell Biochem*. 2004;93:57–67.
54. Reichert N, Choukallah MA, Matthias P. Multiple roles of class I HDACs in proliferation, differentiation, and development. *Cell Mol Life Sci*. 2012;69:2173–87.
55. Witt O, Deubzer HE, Milde T, Oehme I. HDAC family: what are the cancer relevant targets? *Cancer Lett*. 2009;277:8–21.
56. Moser MA, Hagelkruys A, Seiser C. Transcription and beyond: the role of mammalian class I lysine deacetylases. *Chromosoma*. 2014;123:67–78.
57. Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. *J Biol Chem*. 2002;277:25748–55.
58. Segré CV, Chiocca S. Regulating the regulators: the post-translational code of class I HDAC1 and HDAC2. *J Biomed Biotechnol*. 2011;2011:690848.
59. Komorowsky C, Ocker M, Goppelt-Strube M. Differential regulation of connective tissue growth factor in renal cells by histone deacetylase inhibitors. *J Cell Mol Med*. 2009;13:2353–64.
60. Sharma A, Mehan MM, Sinha S, Cowden JW, Mohan RR. Trichostatin A inhibits corneal haze in vitro and in vivo. *Invest Ophthalmol Vis Sci*. 2009;50:2695–701.
61. Choi JH, Oh SW, Kang MS, Kwon HJ, Oh GT, Kim DY. Trichostatin A attenuates airway inflammation in mouse asthma model. *Clin Exp Allergy*. 2005;35:89–96.
62. Cho JS, Moon YM, Park IH, Um JY, Kang JH, Kim TH, et al. Effects of histone deacetylase inhibitor on extracellular matrix production in human nasal polyp organ cultures. *Am J Rhinol Allergy*. 2013;27:18–23 **TSA could reverse the effect of TGF- β 1 on RNA and protein expression levels by reducing HDAC2 and HDAC4, which offer new clues for the treatment of nasal polyp.**
63. Ren Y, Xinming S, Kong L, Li M, Zhao X, Yu N, et al. Therapeutic effects of histone deacetylase inhibitors in a murine asthma model. *Inflamm Res*. 2016;65:995–1008.
64. Lai T, Wu M, Zhang C, Che L, Xu F, Wang Y, et al. HDAC2 attenuates airway inflammation by suppressing IL-17A production in HDM-challenged mice. *Am J Physiol Lung Cell Mol Physiol*. 2019;316:L269–79.
65. Brook PO, Perry MM, Adcock IM, Durham AL. Epigenome-modifying tools in asthma. *Epigenomics*. 2015;7:1017–32.
66. Royce SG, Karagiannis TC. Histone deacetylases and their inhibitors: new implications for asthma and chronic respiratory conditions. *Curr Opin Allergy Clin Immunol*. 2014;14:44–8.
67. Pan Y, Liu L, Li S, Wang K, Ke R, Shi W, et al. Activation of AMPK inhibits TGF- β 1-induced airway smooth muscle cells proliferation and its potential mechanisms. *Sci Rep*. 2018;8:3624.
68. Lu Y, Li Z, Xie B, Song Y, Ye X, Liu P. hsa-miR-20a-5p attenuates allergic inflammation in HMC-1 cells by targeting HDAC4. *Mol Immunol*. 2019;107:84–90.
69. Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, part I: covalent histone modifications. *Trends Mol Med*. 2007;13:363–72.
70. Yang W, Ernst P. SET/MLL family proteins in hematopoiesis and leukemia. *Int J Hematol*. 2017;105:7–16.
71. Yu L, Li N, Zhang J, Jiang Y. IL-13 regulates human nasal epithelial cell differentiation via H3K4me3 modification. *J Inflamm Res*. 2017;10:181–8.
72. Zheng Q, Fan H, Meng Z, Yuan L, Liu C, Peng Y, et al. Histone demethylase KDM2B promotes triple negative breast cancer proliferation by suppressing p15INK4B, p16INK4A, and p57KIP2 transcription. *Acta Biochim Biophys Sin Shanghai*. 2018;50:897–904.
73. Liu CC, Sun C, Zheng X, Zhao MQ, Kong F, Xu FL, et al. Regulation of KDM2B and Brg1 on inflammatory response of nasal mucosa in CRSwNP. *Inflammation*. 2019;42:1389–400 **It suggests that KDM2B may have an inhibitory effect on the development of CRSwNP nasal mucosal epithelial inflammation through the regulation of H3K4me3.**
74. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science*. 2001;294:853–8.
75. Janga SC, Vallabhaneni S. MicroRNAs as posttranscriptional machines and their interplay with cellular networks. *Adv Exp Med Biol*. 2011;722:59–74.
76. Erson AE, Petty EM. MicroRNAs in development and disease. *Clin Genet*. 2008;74:296–306.
77. Xuan L, Luan G, Wang Y, Lan F, Zhang X, Hao Y, et al. MicroRNAs regulating mucin type O-glycan biosynthesis and transforming growth factor β signaling pathways in nasal mucosa of patients with chronic rhinosinusitis with nasal polyps in Northern China. *Int Forum Allergy Rhinol*. 2019;9:106–13.
78. Xia G, Bao L, Gao W, Liu S, Ji K, Li J. Differentially expressed miRNA in inflammatory mucosa of chronic rhinosinusitis. *J Nanosci Nanotechnol*. 2015;15:2132–9.
79. Buckner JH. Mechanisms of impaired regulation by CD4(+) CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat Rev Immunol*. 2010;10:849–59.
80. Mauri C, Blair PA. Regulatory B cells in autoimmunity: developments and controversies. *Nat Rev Rheumatol*. 2010;6:636–43.
81. Thomson AW, Zahorchak AF, Ezzelarab MB, Butterfield LH, Lakkis FG, Metes DM. Prospective clinical testing of regulatory dendritic cells in organ transplantation. *Front Immunol*. 2016;7:15.
82. Luo XQ, Shao JB, Xie RD, Zeng L, Li XX, Qiu SQ, et al. Micro RNA-19a interferes with IL-10 expression in peripheral dendritic cells of patients with nasal polyposis. *Oncotarget*. 2017;8:48915–21.
83. Sonkoly E, Wei T, Janson PC, Sääf A, Lundberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? *PLoS One*. 2007;2:e610.
84. Colina R, Costa-Mattioli M, Dowling RJ, Jaramillo M, Tai LH, Breitbach CJ, et al. Translational control of the innate immune response through IRF-7. *Nature*. 2008;452:323–8.
85. Zhang XH, Zhang YN, Li HB, Hu CY, Wang N, Cao PP, et al. Overexpression of miR-125b, a novel regulator of innate immunity, in eosinophilic chronic rhinosinusitis with nasal polyps. *Am J*

- Respir Crit Care Med. 2012;185:140–51 **miR-125b is upregulated in nasal polyp, so the miR-125b–4E-BP1 pathway may be another new target for the treatment of nasal polyp.**
86. Mitoma H, Horiuchi T, Tsukamoto H, Ueda N. Molecular mechanisms of action of anti-TNF- α agents comparison among therapeutic TNF- α antagonists. *Cytokine*. 2018;101:56–63.
 87. Yang L, Wang Y, Shi S, Xie L, Liu T, Wang Y, et al. The TNF- α -induced expression of miR-130b protects cervical cancer cells from the cytotoxicity of TNF- α . *FEBS Open Bio*. 2018;8:614–27.
 88. Qing X, Zhang Y, Peng Y, He G, Liu A, Liu H. Mir-142-3p regulates inflammatory response by contributing to increased TNF- α in chronic rhinosinusitis with nasal polyposis. *Ear Nose Throat J*. 2019;9:145561319847972. <https://doi.org/10.1177/0145561319847972>.
 89. Li X, Li C, Zhu G, Yuan W, Xiao ZA. TGF- β 1 induces epithelial-mesenchymal transition of chronic sinusitis with nasal polyps through microRNA-21. *Int Arch Allergy Immunol*. 2019;179:304–19 **It showed that TGF- β 1-miR-21-PTEN-Akt axis might contribute to the pathogenesis of CRSwNP. miR-21 might be a reliable target for treating nasal polyp genesis.**
 90. Li L, Feng J, Zhang D, Yong J, Wang Y, Yao J, et al. Differential expression of miR-4492 and IL-10 is involved in chronic rhinosinusitis with nasal polyps. *Exp Ther Med*. 2019;18:3968–76.
 91. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Crit Rev Immunol*. 2012;32:23–63.
 92. Lu SR, Li Q, Lu JL, Liu C, Xu X, Li JZ. Long non-coding RNA LINC01503 promotes colorectal cancer cell proliferation and invasion by regulating miR-4492/FOXK1 signaling. *Exp Ther Med*. 2018;16:4879–85.
 93. Yang Y, Zhang F, Huang H, Xie Z, Huang W, Xie H, et al. Long noncoding RNA LINC00319 regulates ROMO1 expression and promotes bladder cancer progression via miR-4492/ROMO1 axis. *J Cell Physiol*. 2020;235:3768–75.
 94. Li LG, Ma T, YR C. MiR-200A-3P and miR4492 involved in regulation of chronic rhinosinusitis with nasal polyps. *Int J Genet*. 2018;41:108–14.
 95. Chhabra R. miRNA and methylation: a multifaceted liaison. *Chembiochem*. 2015;16:195–203 **The expression of miRNA may be controlled by methylation, and methylation may be influenced by miRNA. They have close relationship with each other.**
 96. Koennecke M, Klimek L, Mullol J, Gevaert P, Wollenberg B. Subtyping of polyposis nasi : phenotypes, endotypes and comorbidities. *Allergo J Int*. 2018;27:56–65 **CRSwNP is divided into three different endotypes: the T cell-/Th2 cytokine-based endotype, the B cell-/IgE-based endotype, and the eosinophil-based endotype.**
 97. Bachert C, Zhang L, Gevaert P. Current and future treatment options for adult chronic rhinosinusitis: focus on nasal polyposis. *J Allergy Clin Immunol*. 2015;136:1431–40.
 98. Mjösberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR2 and CD161. *Nat Immunol*. 2011;12:1055–62.
 99. Kato A, Peters A, Suh L, Carter R, Harris KE, Chandra R, et al. Evidence of a role for B cell-activating factor of the TNF family in the pathogenesis of chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2008;121:1385–92.e13922.
 100. Polzehl D, Moeller P, Riechelmann H, Perner S. Distinct features of chronic rhinosinusitis with and without nasal polyps. *Allergy*. 2006;61:1275–9.
 101. Patadia M, Dixon J, Conley D, Chandra R, Peters A, Suh LA, et al. Evaluation of the presence of B-cell attractant chemokines in chronic rhinosinusitis. *Am J Rhinol Allergy*. 2010;24:11–6.
 102. Allen JS, Eisma R, LaFreniere D, Leonard G, Kreutzer D. Characterization of the eosinophil chemokine RANTES in nasal polyps. *Ann Otol Rhinol Laryngol*. 1998;107:416–20.
 103. Meyer JE, Bartels J, Görög T, Sticherling M, Rudack C, Ross DA, et al. The role of RANTES in nasal polyposis. *Am J Rhinol*. 2005;19:15–20.
 104. Poposki JA, Uzzaman A, Nagarkar DR, Chustz RT, Peters AT, Suh LA, et al. Increased expression of the chemokine CCL23 in eosinophilic chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2011;128:73–81.e4.
 105. Hosokawa H, Kato M, Tohyama H, Tamaki Y, Endo Y, Kimura MY, et al. Methylation of Gata3 protein at Arg-261 regulates transactivation of the IL5 gene in T helper 2 cells. *J Biol Chem*. 2015;290:13095–103.
 106. Berni Canani R, Paparo L, Nocerino R, Cosenza L, Pezzella V, Di Costanzo M, et al. Differences in DNA methylation profile of Th1 and Th2 cytokine genes are associated with tolerance acquisition in children with IgE-mediated cow's milk allergy. *Clin Epigenetics*. 2015;7:38.
 107. Maggi L, Montaini G, Mazzoni A, Rossetti B, Capone M, Rossi MC, et al. Human circulating group 2 innate lymphoid cells can express CD154 and promote IgE production. *J Allergy Clin Immunol*. 2017;139:964–76 **Circulating innate lymphoid cells (ILC2s) from atopic patients expressed higher IL-4 levels than those from non-atopic subjects. ILC2s produce IL-5, IL-13, and IL-4 by demethylation.**
 108. Su RC, Becker AB, Kozyskyj AL, Hayglass KT. Epigenetic regulation of established human type 1 versus type 2 cytokine responses. *J Allergy Clin Immunol*. 2008;121:57–63.e3.
 109. Nakamaru Y, Takagi D, Homma A, Hatakeyama S, Fukuda S. Oxidative stress regulates IL-4 gene expression in mast cells through the reduction of histone deacetylase. *Otolaryngol Head Neck Surg*. 2015;152:48–52.
 110. Han S, Lu J, Zhang Y, Cheng C, Li L, Han L, et al. HDAC inhibitors TSA and sodium butyrate enhanced the human IL-5 expression by altering histone acetylation status at its promoter region. *Immunol Lett*. 2007;108(2):143–50.
 111. Panganiban RP, Pinkerton MH, Maru SY, Jefferson SJ, Roff AN, Ishmael FT. Differential microRNA expression in asthma and the role of miR-1248 in regulation of IL-5. *Am J Clin Exp Immunol*. 2012;1:154–65.
 112. Hammad Mahmoud Hammad R, Hamed DHED, Eldosoky MAER, Ahmad AAES, Osman HM, Abd Elgalil HM, et al. Plasma microRNA-21, microRNA-146a and IL-13 expression in asthmatic children. *Innate Immun*. 2018;24:171–9.
 113. Liu HC, Liao Y, Liu CQ. miR-487b mitigates allergic rhinitis through inhibition of the IL-33/ST2 signaling pathway. *Eur Rev Med Pharmacol Sci*. 2018;22:8076–83.
 114. Ek WE, Ahsan M, Rask-Andersen M, Liang L, Moffatt MF, Gyllenstein U, et al. Epigenome-wide DNA methylation study of IgE concentration in relation to self-reported allergies. *Epigenomics*. 2017;9:407–18.
 115. Tolić A, Grdović N, Dinić S, Rajić J, Đorđević M, Sinadinović M, et al. Absence of PARP-1 affects Cxcl12 expression by increasing DNA demethylation. *J Cell Mol Med*. 2019;23:2610–8.
 116. Jiang C, Guo J, Cheng H, Feng YH. Induced expression of endogenous CXCR4 in iPSCs by targeted CpG demethylation enhances cell migration toward the ligand CXCL12. *Inflammation*. 2019;42:20–34.
 117. Li F, Huang Y, Huang YY, Kuang YS, Wei YJ, Xiang L, et al. MicroRNA-146a promotes IgE class switch in B cells via upregulating 14-3-3 σ expression. *Mol Immunol*. 2017;92:180–9.
 118. Xu H, Gu LN, Yang QY, Zhao DY, Liu F. MiR-221 promotes IgE-mediated activation of mast cells degranulation by PI3K/Akt/PLC γ /Ca(2+) pathway. *J Bioenerg Biomembr*. 2016;48:293–9.

119. Zhang D, Wu Y, Sun G. miR-192 suppresses T follicular helper cell differentiation by targeting CXCR5 in childhood asthma. *Scand J Clin Lab Invest*. 2018;78:236–42.
120. Poetker DM, Jakubowski LA, Lal D, Hwang PH, Wright ED, Smith TL. Oral corticosteroids in the management of adult chronic rhinosinusitis with and without nasal polyps: an evidence-based review with recommendations. *Int Forum Allergy Rhinol*. 2013;3:104–20.
121. Kalish L, Snidvongs K, Sivasubramaniam R, Cope D, Harvey RJ. Topical steroids for nasal polyps. *Cochrane Database Syst Rev*. 2012;12:CD006549.
122. Zou YF, Xu JH, Wang F, Liu S, Tao JH, Cai J, et al. Association study of glucocorticoid receptor genetic polymorphisms with efficacy of glucocorticoids in systemic lupus erythematosus: a prospective cohort study. *Autoimmunity*. 2013;46:531–6.
123. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. *J Allergy Clin Immunol*. 2000;105:943–50 **The imbalance of different glucocorticoid receptors is the main reason of glucocorticoid resistance.**
124. Weigel NL, Moore NL. Steroid receptor phosphorylation: a key modulator of multiple receptor functions. *Mol Endocrinol*. 2007;21:2311–9.
125. Galigniana MD, Piwien-Pilipuk G, Assreuy J. Inhibition of glucocorticoid receptor binding by nitric oxide. *Mol Pharmacol*. 1999;55:317–23.
126. Wallace AD, Cidlowski JA. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. *J Biol Chem*. 2001;276:42714–21.
127. Kozaci DL, Chemajovsky Y, Chikanza IC. The differential expression of corticosteroid receptor isoforms in corticosteroid-resistant and -sensitive patients with rheumatoid arthritis. *Rheumatology (Oxford)*. 2007;46:579–85.
128. Li P, Li Y, Li YQ, Yang QT, Zhang GH. Glucocorticoid receptor expression and glucocorticoid therapeutic effect in nasal polyps. *Clin Invest Med*. 2010;33(3):E181–8.
129. Wang Z, Li P, Zhang Q, Lv H, Liu J, Si J. Interleukin-1 β regulates the expression of glucocorticoid receptor isoforms in nasal polyps in vitro via p38 MAPK and JNK signal transduction pathways. *J Inflamm (Lond)*. 2015;12:3.
130. Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary disease: inactivation of histone deacetylase. *Lancet*. 2004;363:731–3.
131. Ito K, Yamamura S, Essilfi e-Quaye S, Cosio B, Ito M, Barnes PJ, et al. Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF- κ B suppression. *J Exp Med*. 2006;203:7–13.
132. Mata-Greenwood E, Jackson PN PWJ, Zhang L. Endothelial glucocorticoid receptor promoter methylation according to dexamethasone sensitivity. *J Mol Endocrinol*. 2015;55:133–46.
133. Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. *Annu Rev Biophys*. 2013;42:217–39.
134. Glantschnig C, Koenen M, Gil-Lozano M, Karbiener M, Pickrahn I, William-Dautovich J, et al. A miR-29a-driven negative feedback loop regulates peripheral glucocorticoid receptor signaling. *FASEB J*. 2019;33:5924–41.
135. Vreugdenhil E, Verissimo CS, Mariman R, Kamphorst JT, Barbosa JS, Zweers T, et al. MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. *Endocrinology*. 2009;150:2220–8.
136. Lv M, Zhang X, Jia H, Li D, Zhang B, Zhang H, et al. An oncogenic role of miR-142-3p in human T-cell acute lymphoblastic leukemia (T-ALL) by targeting glucocorticoid receptor- α and cAMP/PKA pathways. *Leukemia*. 2012;26:769–77.
137. Specjalski K, Jassem E. MicroRNAs: potential biomarkers and targets of therapy in allergic diseases? *Arch Immunol Ther Exp*. 2019;67:213–23.
138. Qing X, Zhang Y, Peng Y, He G, Liu A, Liu H. Mir-142-3p regulates inflammatory response by contributing to increased TNF- α in chronic rhinosinusitis with nasal polyposis [published online ahead of print, 2019 Sep 9]. *Ear Nose Throat J*. 2019;145561319847972. <https://doi.org/10.1177/0145561319847972>.
139. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429:457–63.
140. Mabaera R, Greene MR, Richardson CA, Conine SJ, Kozul CD, Lowrey CH. Neither DNA hypomethylation nor changes in the kinetics of erythroid differentiation explain 5-azacytidine's ability to induce human fetal hemoglobin. *Blood*. 2008;111:411–20.
141. Diesch J, Zwick A, Garz AK, Palau A, Buschbeck M, Götze KS. A clinical-molecular update on azanucleoside-based therapy for the treatment of hematologic cancers. *Clin Epigenetics*. 2016;8:71.
142. Fenaux P, Mufti GJ, Hellström-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. International Vidaza High-Risk MDS Survival Study Group Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol*. 2009;10:223–32.
143. Fenaux P, Mufti GJ, Hellström-Lindberg E, Santini V, Gattermann N, Germing U, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol*. 2010;28:562–9.
144. Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, Beach CL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. *J Clin Oncol*. 2006;24:3895–903.
145. Yeo NK, Park WJ, Eom DW, Oh MY, Lee JH. Effects of azathioprine and its metabolites on inflammatory cytokines in human nasal polyp organ cultures. *Int Forum Allergy Rhinol*. 2019;9:648–55.
146. Shin JM, Um JY, Lee SA, Park IH, Lee SH, Lee HM. Effect of MeCP2 on TGF- β 1-induced extracellular matrix production in nasal polyp-derived fibroblasts. *Am J Rhinol Allergy*. 2018;32:228–35 **It suggests that 5-azacytidine could not only inhibit the expression of α -SMA and fibronectin mRNA in nasal polyp but also suppress the collagen production and the expression of MeCP2.**
147. Yoon S, Eom GH. HDAC and HDAC inhibitor: from cancer to cardiovascular diseases. *Chonnam Med J*. 2016;52:1–11.
148. Heerboth S, Lapinska K, Snyder N, Leary M, Rollinson S, Sarkar S. Use of epigenetic drugs in disease: an overview. *Genet Epigenetics*. 2014;6:9–19.
149. Ververis K, Karagiannis TC. Potential non-oncological applications of histone deacetylase inhibitors. *Am J Transl Res*. 2011;3:454–67.
150. Royce SG, Ververis K, Karagiannis TC. Histone deacetylase inhibitors: can we consider potent anti-neoplastic agents for the treatment of asthma? *Ann Clin Lab Sci*. 2012;42:338–45.