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An update on the role of epigenetics in systemic vasculitis

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Abstract

Purpose of review—The purpose of this review is to discuss recent observations of epigenetic changes related to the complex pathogenesis of systemic vasculitides and their contribution to the field.

Recent findings—There have been new observations of epigenetic changes in vasculitis and their potential role in disease pathogenesis in antineutrophil cytoplasmic antibody-associated vasculitis, giant-cell arteritis, Kawasaki disease, Behçet's disease, and IgA vasculitis. Some of this recent work has focused on the efficacy of using DNA methylation and miRNA expression as clinical biomarkers for disease activity and how DNA methylation and histone modifications interact to regulate disease-related gene expression.

Summary—DNA methylation, histone modification, and miRNA expression changes are all fruitful ground for biomarker discovery and therapeutic targets in vasculitis. Current knowledge has provided targeted and suggested effects, but in many cases, has relied upon small cohorts, cosmopolitan cell populations, and limited knowledge of functional interactions. Expanding our knowledge of how these epigenetic mechanisms interact in a disease-specific and cell-specific manner will help to better understand the pathogenesis of systemic vasculitis.

Keywords

DNA methylation; epigenetics; histone modification; microRNA; systemic vasculitis

INTRODUCTION

Systemic vasculitides are a heterogeneous group of complex inflammatory diseases of unknown cause. They are characterized by histological evidence of leukocyte infiltration, inflammation, and necrosis of the vessel wall and vascular occlusion [1]. Numerous genetic loci have been associated with increased risk of vasculitis, with human leukocyte antigen

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Conflicts of interest

There are no conflicts of interest.

(HLA) genes encoding major histocompatibility complex (MHC) proteins being most robust and pointing toward the importance of the immune system in pathogenesis [2]. Environmental risk factors include exposure to silica dust, unknown viral or bacterial infections, drugs, farming occupations as well as complex factors like age [3–6]. The contribution of genetics alone to the pathogenesis of a systemic vasculitis varies with manifestations, but does not account for the entirety of the risk. Epigenetic mechanisms governing gene expression sit at the interface of genetic and environmental factors related to a variety of diseases [7]. Epigenetics is the study of hereditary, phenotypic traits that can alter the chromosome without changing the underlying genetic sequence [8]. DNA methylation, histone modifications, and noncoding RNA are epigenetic mechanisms that control gene expression and regulate cellular development, differentiation and activity (extensively reviewed in [9▪]).

DNA methylation is an epigenetic mechanism that consists of the addition of a methyl group to cytosines, primarily within CpG dinucleotides, catalyzed by DNA methyltransferases (DNMTs). De-novo DNA methylation is conducted primarily by DNMT3A and DNMT3B which are essential during the gestational development of mammals. Although DNMT1 is primarily responsible for maintaining established methylation patterns from cell to cell, the extent to which their functions overlap is still being explored [10]. DNMT function and targeting are regulated by complex systems including DNMT expression levels, RNA molecules, amino-acid residue modifications, genomic sequence and methylation status, histone tail signatures, transcription factor availability, and chromatin accessibility [10]. DNMT1 is also capable of recruiting histone deacetylase 1 (HDAC1) which promotes the formation of heterochromatin through the removal of acetyl groups from histone proteins, silencing gene expression and providing a link between DNA methylation and histone modification [11].

Histone modification is an epigenetic mechanism that regulates the dynamic chromatin structure and subsequently gene expression [12]. The functional opposition to HDACs, histone acetyl-transferases (HATs), promote the formation of euchromatin that is permissive to protein-DNA interactions [12]. Acetylation is only one of a multitude of histone modifications found in the genome which can include ubiquitylation, residue-specific methylation, and phosphorylation [13]. Identifying patterns of histone modifications and their relationship to gene expression has provided a way to understand how chromatin structure controls the regulation of cellular functions, and has led to the identification of ‘bivalent chromatin’ that contains both permissive (e.g., H3K4me2) and repressive (e.g., H3K27me3) histone modifications poised for expression depending on cellular requirements, which is vital during cell development [9▪].

Noncoding RNAs are transcribed but not translated into proteins, and act as an epigenetic mechanism to regulate gene expression. The most studied are microRNAs (miRNAs), around 22 nucleotides in length that regulate posttranscriptional gene silencing through translational control of mRNA molecules. miRNAs target the 3′ untranslated region of their target mRNA molecule and control their stability and protein interactions [14]. miRNA expression is controlled by other epigenetic mechanisms and itself controls these

mechanisms as an ‘epigenetics-miRNA regulatory circuit’ that, when perturbed, can contribute to disease pathogenesis [15].

The focus of this review is to discuss our current knowledge of the role epigenetics in systemic vasculitis, and more specifically to highlight new developments in the field of interest to clinicians and researchers.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic necrotizing vasculitis of small vessels characterized by the presence of autoantigens against neutrophil cytoplasmic proteins, specifically myeloperoxidase (MPO) and proteinase 3 (PR3) [1,16]. The presence of antineutrophil cytoplasmic antibodies against MPO and PR3 is used in the classification of AAV, though not all patients have ANCA. ANCAs have been implicated in vascular damage in AAV patients. Neutrophils in AAV patients are more sensitive to activation by ANCA, as demonstrated by the production of reactive oxygen species and neutrophil extracellular traps (NETs) [17,18]. In normal neutrophils, MPO and PR3 expression primarily occurs early in cell development, contributing to the formation of intracellular granules, but AAV cells continue to express MPO and PR3 into maturity which indicates a deviation from normal gene silencing [19,20].

Ciavatta *et al.*, Yang *et al.*, and Jones *et al.* [21,22▪▪,23▪▪] have provided excellent studies of how epigenetic mechanisms control *PRTN3* and *MPO* gene expression and their dysregulation in AAV (Fig. 1). The initial study of AAV granulocytes revealed a depletion of repressive H3K27me3 marks and an increase in mRNA expression of *PRTN3* and *MPO* [21]. In addition, a marked demethylation of a CpG island and the promoter region of *MPO* in AAV were observed, although *PRTN3* promoter region was constitutively demethylated in patients and controls. The authors then explored the regulatory mechanisms governing H3K27me3 and found enhancer of zeste homolog 2 (EZH2) interacted with Runt-related transcription factor 3 (RUNX3) to recruit H3K27 methyltransferase to *PRTN3* and *MPO*. The promoter region of the *RUNX3* gene was also hypermethylated in AAV granulocytes. This suggests a regulatory model whereby hypermethylation of *RUNX3* and the loss of EZH2 and H3K27 methyltransferase recruitment is coupled with overexpression of H3K27me3 demethylase jumonji C domain-containing protein 3 (JMJD3) in AAV neutrophils. JMJD3 removes the H3K27me3 marks from regulatory regions of *MPO* and *PRTN3* and increases chromatin accessibility aided by DNA demethylation allowing access to transcriptional machinery. Genomic regions containing genetic risk variants in AAV were found to be enriched for H3K27me3 marks that indicate a closed or poised state for the chromatin in Th17 cells, supporting the role of Th17 cells in AAV pathogenesis [24▪,25].

Yang *et al.* [23▪▪] investigated expression changes in genes encoding histone modification proteins and found a suite of four genes: euchromatic histone-lysine *N*-methyltransferase 1 and 2 (*EHMT1*, *EHMT2*) and male sex lethal 1 homolog and insulin growth factor (*MSL1* and *ING4*) with consistent expression changes in leukocytes and granulocytes from AAV patients compared with healthy controls. *EHMT1* and *EHMT2* are associated with H3K9me2, a mark of gene silencing, and were found to be underexpressed in AAV

leukocytes and granulocytes. *MSL1* and *ING4* are associated with H4K16ac, a mark of gene activation, *MSL1* was found to be overexpressed in AAV leukocytes and granulocytes, although *ING4* was underexpressed in leukocytes, but not significantly underexpressed in granulocytes. These expression changes were noted to be significantly different between leukocytes from active AAV patients with elevated *MPO/PRNT3* expression and inactive patients with low-expression *MPO/PRNT3*, making them potential disease activity biomarkers. H4K16ac and H3K9me2 were, respectively, enriched and depleted at *MPO* and *PRTN3* promoter regions in AAV granulocytes, especially in more active disease. *MLL2*, *MLL3*, and *MLL4* (mixed-lineage leukemia) genes that regulate H3K4me2 were overexpressed in AAV patients compared with healthy controls. H3K4me2 is a well-recognized mark of transcriptional activation and, along with H3K27me3, is part of a bivalent chromatin signature [9▪,26]. H3K4me2 was equally enriched in both patients and controls at the *MPO* and *PRTN3* promoter region, suggesting that they remain in a poised state at these loci even in healthy cells. Taken together, *MPO* and *PRTN3* seem to maintain areas of bivalent chromatin that contain both permissive and repressive marks that are poised for expression with the loss of repressive marks. This occurs in AAV patients and is enhanced during active disease leading to abnormal over-production of granule proteins and neutrophil-mediated vascular damage.

Jones *et al.* [22▪▪] investigated DNA methylation changes in *MPO* and *PRTN3* as potential disease biomarkers in AAV total leukocytes. They noted a decrease in *DNMT1* expression but no significant reduction in global DNA methylation in AAV. However, they detected many differentially methylated genes in AAV patients, among these were *MPO* and *PRTN3* which were both hypomethylated in patients with active disease compared with healthy controls. Methylation levels in both genes were also significantly lower in AAV patients with active compared with inactive disease. In addition, DNA methylation of both *MPO* and *PRTN3* was negatively correlated with gene expression. By comparing AAV subsets (PR3-AAV versus MPO-AAV) using longitudinally collected samples from the same patients, they observed that MPO- and PR3-AAV patients experience a significant and near identical increase in the methylation level of *PRTN3* promoter after disease remission. However, only patients with MPO-AAV, and not PR3-AAV, show evidence for a significant increase in the methylation level at *MPO*, suggesting that epigenetic changes at these two loci may provide a distinction between the two disease serotypes. At both *PRTN3* and *MOP*, AAV patients who entered remission and displayed increased site-specific methylation had a significant reduction in mRNA expression of both genes, whereas those patients who experienced decreased DNA methylation upon remission displayed no change in gene expression. Perhaps the most valuable observation in this study was that demethylation of the *PRTN3* promoter region was highly predictive of disease relapse in AAV patients regardless of ANCA-serotype; patients with demethylation in *PRTN3* were 4.55 times more likely to relapse. This effect was narrowed down to a single CpG site in the promoter region of *PRTN3*. These results are very promising, but a larger study will be needed to confirm the prognostic use of this CpG site as a biomarker for relapse in AAV patients.

One drawback to this study is the use of total leukocytes in which differences in cell population proportions can mask cell-specific methylation changes from being detected. One the other hand, total leukocytes represent an easily accessible source for clinical testing and

any effect that can be detected consistently in patients might have a potentially significant role in disease pathogenesis and will be valuable to develop into a disease biomarker.

GIANT-CELL ARTERITIS

Giant-cell arteritis (GCA) is an inflammatory disease of the large and medium arteries, occurring almost exclusively in people over 50 years of age, and characterized by granulomatous involvement which can lead to ischemia and necrosis or vision loss [27]. The arterial microenvironment at the site of inflammation in GCA is considerably complex with vessel-residing dendritic cells (DCs) acting as pathogen detectors and guiding T cell stimulation and the local inflammatory response, and Th1 and Th17 cells providing proinflammatory signals [28,29]. An exploration of DNA methylation changes occurring within the temporal artery of GCA patients revealed a strong T cell-specific signature consisting of hypomethylated loci in genes involved in TCR/CD28 signaling and calcineurin (Ca)/NFAT-regulatory pathways [30▪]. NFAT is a transcription factor regulating cytokine expression in T cells, including *IFNG* and *TNF*, and *CD40LG* expression, which were also demethylated in affected arterial tissue from GCA patients. NFAT1 was also found to be localized to the nucleus of cells (suggesting dephosphorylation and activation) in the vessel wall of GCA biopsies by immunohistochemistry. Hypomethylation of cytokine genes for Th1 (*IFNG*) and Th17 (*IL6*, *IL21*) cells, macrophages (*TNF*), and DCs (*CCR7*, *CCL18*) supported their presence in immune infiltration of the vessel wall. The Ca/NFAT pathway presents an intriguing therapeutic target. Dipyridamole, a highly specific calcineurin inhibitor, is suitable for targeting NFAT-regulated expression and has been shown to inhibit the production of interferon-gamma (IFNγ), IL-17, and IL-6 in T cells from MRL/lpr lupus mouse model [31]. Many proinflammatory genes regulated by NFAT were hypomethylated in GCA-affected arteries, and there is evidence that NFAT can interact with HDAC proteins to control histone modifications in specific contexts [32].

Croci *et al.* [33▪] identified miRNAs overex-pressed in GCA tissue by comparing active, non-active, and normal artery tissue. Of these miRNAs, miR-146a, miR-155, and miR-21 were overexpressed in inflamed temporal artery tissue compared with noninflamed and normal tissue. These miRNAs play a role in the regulation of the inflammatory response in T cells, macrophages, and DCs. They are also overexpressed in abdominal aortic aneurysms and atherosclerotic plaques and might play a role in vascular remodeling [34,35]. Although none of the known protein targets of these miRNAs were differentially expressed, miR-21 expression was found to be localized to cells in the medial-intimal layer of the artery in this study.

Age itself is a considerable risk factor for GCA and is likely due in part to changes in the immune function throughout the lifespan, which is known as immunosenescence [36,37]. Age-related DNA methylation changes in CD4+ T cells suggest a pro-inflammatory epigenetic architecture with age [38]. The miRNAs highlighted by Croci *et al.* [33▪,39] have also been implicated in immunesenescence and their increased expression in GCA tissue perhaps reflects an accelerated biological age that will need to be explored further.

KAWASAKI DISEASE

Kawasaki disease (KD) is a medium-vessel vasculitis that primarily occurs in children between ages 8 months and 5 years. It is characterized by inflammation of the coronary arteries, and is the leading cause of acquired heart disease in children from developed regions [40].

DNA methylation studies of KD have revealed a relationship between *FCGR2A* methylation and response to intravenous immunoglobulin (IVIG) treatment. *FCGR2A* encodes the low-affinity immunoglobulin gamma Fc region receptor II-a protein that is expressed on the surface of macrophages, neutrophils, monocytes, and DCs, and acts to increase phagocytosis and inflammatory mediator production and contains a genetic risk variant for KD [41]. CpG sites within the promotor region of *FCGR2A* were hypomethylated in whole blood cells from KD patients compared with controls, and especially in patients resistant to IVIG treatment [42]. Another small-scale study found genome-wide, site-specific hypomethylation changes enriched in genes associated with the inflammatory immune response including *FCGR2A* [43]. This study demonstrated significant changes in DNA methylation patterns following IVIG treatment in KD, including reversal of the disease-associated hypomethylation in *FCGR2A* [43].

Toll-like receptors are a group of proteins that recognize molecular patterns, both exogenous and endogenous, and can interact with FCGR2A to induce a proinflammatory response [44,45]. A suite of TLR genes encoding TLR1, TLR2, TLR4, TLR6, TLR8, and TLR9 were found to be hypomethylated in KD patients compared with healthy and febrile controls [46]. The methylation levels of these genes were recovered within 3-week post-IVIG therapy, and mRNA expression levels maintained a negative correlation with DNA methylation.

Regulatory T cells (Tregs) play an important role in suppressing the proinflammatory activity and cytokine expression of Th17 cells through physical interactions or by releasing cytokines like IL-10 and TGF-beta. This regulatory balance seems to be skewed toward proinflammatory Th17 cells in acute KD patients where there is a reduction in FoxP3 expression, a critical transcription factor in Treg activity [47]. miR-31 expression was increased in Tregs from acute KD patients and suppresses FoxP3 expression, although miR-155, which promotes FoxP3 expression, was found to be decreased in Tregs from patients [48]. IVIG treatment partially recovered the abnormal expression of miR-31 and miR-155. Furthermore, miR-145, which might be involved in modulating TGF-beta signaling, was increased in whole blood and detected in plasma exosomes isolated from acute KD patients [49]. Exosomes are extracellular vesicles released from cells that can be taken in by other cells and are capable of transporting miRNAs as a theorized form of cell-to-cell communication [50,51]. Other proinflammatory microRNAs that also potentially target TGF-beta signaling and are involved in KD are miR-200c and miR-371-5p. miR-200c promotes endothelial cell apoptosis, inducing vascular smooth muscle cell inflammatory response and modulating TLR4 response [52]. Both miR-200c and -371-5p were shown to effectively distinguish between KD and healthy controls as well as IVIG-responsive and nonresponsive patients [53]. More recent research has identified new miRNAs with disease activity that potentially target vessel endothelial cell functions in KD patients [54–58]. Jia *et*

al. [55] performed a biomarker discovery screen on serum samples to detect exosome miRNAs in KD patients. After normalizing to internal control miRNA expression and recruiting an independent validation cohort, they identified two miRNA pairs (miR-1246/miR-4436b-5p and miR-197-3p/miR-671-5p) that when combined differentiated KD patients from healthy and febrile disease controls. These miRNA discovery studies are promising but generally relied upon small phenotypically varied cohorts of patient and controls. More work is required to validate these findings in KD, and to understand their cell-specific function and evaluate their efficacy as biomarkers.

BEHÇET'S DISEASE

Behçet's disease (BD) is a systemic, variable-vessel vasculitis of unknown cause characterized by recurrent acute inflammatory episodes with oral and genital ulcers, eye involvement, and skin involvement [59,60]. Although its pathogenesis is still currently under investigation, evidence points toward a combination of genetic and environmental triggers as contributing factors to the development of BD [59]. Genetic susceptibility for BD shows a very strong association with the *HLA-B/MICA* region, though non-MHC risk factors have been identified as well that support the involvement of Th1 and Th17 cells (*IL10*, *IL12A*, *STAT4*, and *IL23R-IL12RB2* locus) in pathogenesis [2,61–63]. This is supported by research showing that Th17, Th1, and Treg cell populations and cytokine production change with the disease state and can be found at inflammatory sites of BD patients [64–66].

DNA methylation of CD4+ T cells and monocytes extracted from the peripheral blood of BD patients are hypomethylated at genes associated with cytoskeletal remodeling processes such as actin and microtubule processing and cell adhesion [67]. Interestingly, some of the methylation deficiencies observed were returned to near those of healthy control levels at specific genes after treatment and disease remission. This recovery was more pronounced in monocytes than in T cells, but genes involved in microtubule formation and organization (*KIFA2* and *TPPP*) were affected in both cell types making them intriguing targets for clinical biomarkers and therapeutics.

Research into changes in miRNA expression in BD has revealed a variety of potential targets and biomarkers. Regulation of Th17 cell activity has shown up as a theme in miRNA research in BD. miR-23b was underexpressed in CD4+ T cells from active BD patients [68]. When transfected into CD4+ T cells *in vitro*, miR-23b reduced the expression of Notch pathway genes and production of IFNγ and IL-17 [68]. As an example of genetic–epigenetic interaction, genetic variants also play a role in miRNA functions including expression and protein targeting [69]. Two such variants have been identified in BD patients: rs2910164 (*MIR146A*; miR-146a) and rs11614913 (*MIR196A2*; miR-196a2) [70,71]. Carriers of the rs2910164 CC genotype displayed a reduction in mature miR-146a transcripts and IL-17, TNF-alpha, and IL-1 beta at the protein level in PBMCs as compared with the GG genotype which was more frequent in BD patients [70]. Carriers of the rs11614913 TT allele had reduced expression of miR-196a2 in PBMCs and the T allele was significantly more frequent among BD patients compared with healthy and disease (Vogt–Koyanagi–Harada syndrome and acute anterior uveitis associated with ankylosing spondylitis) controls and more frequent among BD patients with arthritis compared with other subgroups [71].

Reduced expression of miR-196a2 coincided with a reduction in the target protein Bach1 and an increase in proinflammatory IL-1 beta and MCP-1 cytokines [71].

IGA VASCULITIS (HENOCH–SCHÖNLEIN PURPURA)

IgA vasculitis (IgAV) primarily targets small vessels and is characterized by the deposition of IgA immune complexes in the vessel wall, and disease onset is often associated with an infection of the upper airway or gastrointestinal tract [60]. IgAV is considered a geographically and ethnically ubiquitous disease predominantly of infants and children between 3 and 12 years of age [72]. Ascertaining genetic risk is difficult because of case studies of insufficient size, but a meta-analysis confirmed the risk associated with *HLA-DRB1*01* and *HLA-DRB1*07* variants [2].

Luo *et al.* [73,74] observed a genome-wide increase in H3 acetylation and H3K4 methylation, which are both marks of open and transcriptionally accessible chromatin, in PBMCs isolated from IgAV patients. These marks were positively correlated with disease activity and were significantly enriched in IgAV patients with renal involvement compared with IgAV patients without renal involvement and healthy controls [73]. Coinciding with this was an increase in HATs and histone methyltransferases in IgAV patients and a decrease in the opposing histone deacetylases and histone demethylases, indicating a shift in the transcriptional profile to support an abnormal transcriptionally active state [73]. IgAV patients with renal involvement had a marked increase in IL-4, IL-6, and IL-13 at the mRNA and protein levels [73]. The authors found an enrichment of H3 acetylation and H3K4me3 at promoter and enhancer regions of *IL4*, a Th2 cytokine, in CD4+ T cells from IgAV patients compared with controls and an increased expression of TIM-1, a suggested regulator of the Th2 response [73]. By comparison, *IFNG*, encoding IFNγ cytokine of Th1 cells, displayed no enrichment for these histone marks or elevated expression [73]. An IgAV-specific global increase in open chromatin marks coupled with the open chromatin state and overexpression of Th2-related genes points toward Th2 cells as being potential effectors in the pathogenesis of IgAV. Future studies would benefit from next-generation sequencing to gain a more holistic view in which these chromatin changes are occurring in both circulating immune cells as well as those residing in the kidney, which may have different disease-specific epigenetic profiles.

CONCLUSION

Epigenetic mechanisms provide a means to understand the pathogenesis of vasculitis, improve diagnosis, monitor disease progression, and the potential identification of novel therapeutic targets (Table 1). The highly interconnected nature of these mechanisms places the emphasis of future epigenetics research in systemic vasculitis on integrating data from disease-specific and cell-specific DNA methylation states, histone modifications, and miRNA activity.

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KEY POINTS

- Epigenetic characterization in vasculitis can provide insights into disease pathogenesis and identify novel potential therapeutic targets.
- The autoantigen gene loci in ANCA-associated vasculitis *PRTN3* and *MPO* undergo epigenetic changes that correlate with disease activity and with PR3 and MPO expression.
- Epigenetic changes and microRNA expression profiles can be developed into disease biomarkers in vasculitis, but larger cohorts and validation studies are needed.
- Cell-type specific epigenetic signatures can be used to map causal variants in genetic risk loci and identify genetic-epigenetic interactions in vasculitis.

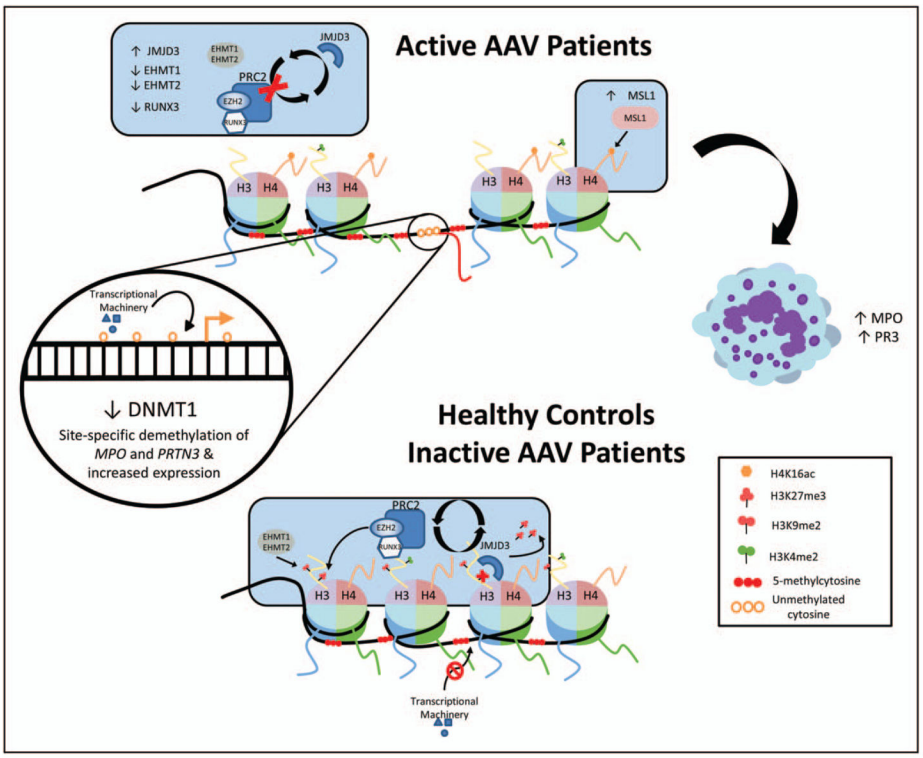

FIGURE 1.

A cartoon model of epigenetic control of *MPO* and *PRTN3* in ANCA-associated vasculitis. Ciavatta *et al.* and Yang *et al.* suggest that histone modifications surrounding the promoter and enhancer regions of *MPO* and *PRTN3* in AAV are in a bivalent state (presence of both repressive and permissive marks), maintaining gene silencing in mature neutrophils that is disrupted in AAV patients. In neutrophils from healthy controls and inactive patients with low MPO and PR3 expression, JMJD3 demethylates H3K27, although PRC2 remethylates it in kind to maintain a condensed silent state. *EHMT1* and *EHMT2* assist by maintaining H3K9me2 in the same region. Permissive H3K4me2 marks suggest an epigenetic poising and are present in both patients and controls, though the *MLL2*, *MLL3*, and *MLL4* genes that regulate this mark were overexpressed in patients compared with controls. DNA methylation of the gene promoter and enhancer regions provides a second method of epigenetic control, preventing the access of transcriptional machinery, and CpG islands can be targeted by PRC2 as well for H3K27me3. In patients with active disease, some disruptive process interrupts the gene silencing and a decrease in RUNX3 expression prevents the reestablishment of H3K27me3. Decreased expression of *EHMT1* and *EHMT2* correlates with depletion of H3K9me2 and an increase in *MSL1* expression correlates with enriched H4K16ac, a mark of gene activation. Jones *et al.* found that leukocytes from active AAV patients have decreased *DNMT1* expression and a site-specific decrease in DNA methylation, suggesting a process that targets specific loci including *MPO* and *PRTN3* and allows for gene expression. When AAV is inactive, methylation at these loci is returned to levels near that of healthy controls and expression is reduced. This suggests that *MPO* and *PRTN3* DNA methylation is a disease-specific process supported by the identification of a CpG site in the *PRTN3* promoter (CpG #13) that is demethylated in patients with a higher

risk of relapse. AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; proteinase 3; PR3, proteinase 3.

Table 1

Current knowledge of disease-specific epigenetic mechanisms in vasculitis

Epigenetic mechanism	Region (s) of interest	Disease-specific relationship	Function	Source	Reference
ANCA-associated vasculitis (AAV)					
Histone modification (H3K27me3)	*PRTN3, MPO*	Depleted H3K27me3 in AAV and corresponding increased mRNA expression; a mark of gene silencing	Neutrophil granule protein	Granulocytes	[21]
Histone modification (H3K9me2)	*PRTN3, MPO*	Depleted H3K9me2 at gene promoter in active AAV patients with high mRNA expression compared with healthy controls and inactive patients; regulated by EHMT1 and EHMT2 and a mark of gene silencing	Neutrophil granule protein	Neutrophils	[23▪▪]
Histone modification (H4K16ac)	*PRTN3, MPO*	Enriched H4K16ac at gene promoter in active AAV patients with high mRNA expression compared with healthy controls and inactive patients; regulated by ING4 and MSL1 and a mark of gene activation	Neutrophil granule protein	Neutrophils	[23▪▪]
Histone modification (H3K4me2)	*PRTN3, MPO*	H3K4me2 at promoter regions of both *PRTN3* and *MPO* in patients and controls; a mark of gene activation and suggests bivalent chromatin state	Neutrophil granule protein	Neutrophils	[23▪▪]
DNA methylation (promoter/CpG island)	*MPO*	Hypomethylation of promoter/CpG island of *MPO* in AAV patients	Neutrophil granule protein	Granulocytes	[21]
DNA methylation (promoter)	*RUNX3*	Hypermethylation of promoter region and reduced *RUNX3* mRNA expression in AAV patients	Recruits PRC2 that regulates H3K27 methylation of *MPO* and *PRTN3*	Granulocytes	[21]
DNA methylation (CGI/Exon 5–6)	*MPO*	Hypomethylation of CpG island/exon 5–6 of *MPO* gene of AAV patients; typically increased with disease remission and corresponded with decreased *MPO* mRNA	Neutrophil granule protein	Total leukocytes	[22▪▪]
DNA methylation (promoter: CpG #13)	*PRTN3*	Hypomethylation of promoter of *PRTN3* gene in AAV patients; typically increased with disease remission and	Neutrophil granule protein	Total leukocytes	[22▪▪]

Epigenetic mechanism	Region (s) of interest	Disease-specific relationship	Function	Source	Reference
		corresponded with decreased *PRTN3* mRNA; hypomethylation of CpG #13 is associated with increased risk of disease relapse			
Giant-cell arteritis (GCA)					
DNA methylation (various loci)	*PPP3CC, NFATC1, NFATC2*	Hypomethylated in GCA temporal artery; nuclear localization of NFAT1 protein in GCA-positive biopsies	Calcineurin/NFAT-signaling pathway	Temporal artery biopsy	[30▪]
DNA methylation (cg09993145)	*RUNX3*	Hypomethylated in GCA temporal artery	Combines with T-bet for expression of IFNγ in Th1 cells	Temporal artery biopsy	[30▪,75]
DNA methylation (various loci)	*CD40LG, IL21, IL21R*	Hypomethylated in GCA temporal artery; protein present in GCA-positive biopsies	NFAT-regulated genes; involved in activation and maturation of Th cell lineages (*CD40LG*) and production of IL-17 in Th17 cells (*IL21, IL21R*)	Temporal artery biopsy	[30▪]
DNA methylation (various loci)	*CCR7, CCL18*	Hypomethylated in GCA temporal artery	Present on mature dendritic cells (*CCR7*) and attract naive T cells (*CCL18*)	Temporal artery biopsy	[30▪]
DNA methylation (various loci)	*CD3E, CD3G, CD3D, CD3Z, CD28, ZAP70*	Hypomethylated in GCA temporal artery	TCR/CD28-regulated T cell activation	Temporal artery biopsy	[30▪]
DNA methylation (various loci)	*TNF, IL2, IL1B, IL18, IFNG, LTA, LTB*	Hypomethylated in GCA temporal artery	Proinflammatory proteins and key Th1 cell cytokine (*IFNG*)	Temporal artery biopsy	[30▪]
Noncoding RNA (microRNA)	*MIR146A, MIR155, MIR21*	MicroRNAs overexpressed in inflamed GCA temporal artery	Regulation of inflammatory and vascular remodeling networks; miR-21 is localized to medial/intimal layers	Temporal artery biopsy	[33▪]
Kawasaki disease (KD)					
DNA methylation (promoter: cg24422489)	*FCGR2A*	Hypomethylated in KD patients, more so in IVIG-resistant cases; methylation increased after IVIG treatment and mRNA expression was reduced	Ig Fc receptor that regulates an array of immune responses and is a potent proinflammatory gene; genetic risk locus for KD	Whole blood	[42,43]
DNA methylation (various loci)	*TLR1, TLR2, TLR4, TLR6, TLR8, TLR9*	Hypomethylated in KD patients; methylation is restored with IVIG treatment and mRNA expression is reduced	Recognition of bacterial products and inflammatory signals	Total leukocytes	[46]
Noncoding RNA (microRNA)	*MIR145*	Increased miR-145 in whole blood of acute KD patients; found in extracellular vesicles from patient plasma	Differentiation of neutrophils and vascular smooth muscle cells and targets regulatory genes in TGF beta signaling pathway	Whole blood & plasma	[49]

Epigenetic mechanism	Region (s) of interest	Disease-specific relationship	Function	Source	Reference
Noncoding RNA (microRNA)	*MIR125A*	Increased miR-125a-5p circulating in acute and convalescent KD patient plasma	Inhibition of MKK7 expression which promotes caspase-3 expression and apoptosis in endothelial cells	Plasma	[54]
Noncoding RNA (microRNA)	*MIR1246/MIR4436B1* & *MIR197/MIR671*	MiRNA pairs that, when combined, can differentiate KD patients from controls and non-KD febrile cases	miR-197 is predictive of death in symptomatic coronary artery disease and miR-1246 is a biomarker for diastolic dysfunction	Serum exosomes	[55]
Noncoding RNA (microRNA)	*MIR155, MIR31*	Decreased miR-155 and increased miR-31 expression in CD4 + CD25 + Treg cells of acute KD patients; IVIG partially reverses expression difference	miRNAs expression correlated with FoxP3 mRNA in CD4 + CD25 + Treg cells suggesting common regulatory factors	CD4 + CD25 + Treg cells	[48]
Noncoding RNA (microRNA)	*MIR223*	Increased miR-223 in KD patient serum especially those with coronary artery lesions; identified as part of KD diagnostic miRNA panel in total leukocytes	Released by bone-marrow derived blood cells into serum; promotes apoptosis in endothelial cells by targeting IGF1R and suppresses cell proliferation	Serum/total leukocytes	[56▪,57]
Noncoding RNA (microRNA)	*MIR200C, MIR371*	Circulating miR-200c and miR-371-5p are increased in KD patients; reduced after IVIG therapy	Involved in proinflammatory response	Serum	[52,53]
Noncoding RNA (microRNA)	*MIR93*	Increased miR-93 in patients responding to IVIG; inverse correlation with *VEGFA* mRNA expression	Related to expression of VEGF-A	PBMCs	[58]
Behçet's disease (BD)					
DNA methylation	*RAC1, ARHGAP24, FSCN2, BAIAP2L1, FILIP1 SSH, ANK1, MYH15, MYO1C, MYO1D, MPRIP*	Methylation changes varies with cell type, but is distinct in BD patients; methylation at some sites restored after therapy	Actin processing and cytoskeletal remodeling	CD4 + T cells/ monocytes	[67]
DNA methylation	*TBCD, KIF1B, DNAH3, TUBB8, RGS14, TUBA3C, TUBA3D, TPPP, KIF2A*	Methylation changes varies with cell type, but is distinct in BD patients; methylation at some sites restored after therapy	Microtubule processing	CD4 + T cells/ monocytes	[67]
Noncoding RNA (microRNA)	*MIR155*	Conflicting reports of expression changes in BD patients	Overexpression inhibits production of IL-6 and IL-1 beta and promotes IL-10 in DCs which reduced CD4 + T cell expression of IL-17; targets Ets-1 which inhibits IL-17 production	PBMCs/DCs/CD4 + T cells	[76,77]

Epigenetic mechanism	Region (s) of interest	Disease-specific relationship	Function	Source	Reference
Noncoding RNA (microRNA)	*MIR146A*	Decreased miR-146a expression associated with rs2910164 (C >G) C allele which is less frequent in BD patients	Regulates proinflammatory cytokine production (IL-17, TNF-alpha, IL-1 beta)	PBMCs	[70]
Noncoding RNA (microRNA)	*MIR196A2*	Decreased miR-196a2 expression associated with rs11614913 (C >T) T allele which is more frequent in BD patients (specifically arthritis subgroup)	Presence of variant associated with decreased miR-196a expression and an increase in target gene *BACH1*; increased production of proinflammatory cytokines IL-1 beta and MCP-1	PBMCs	[71]
Noncoding RNA (microRNA)	*MIR638, MIR448, MIR3591*	Decreased miR-638 and miR-4488 expression in stable BD patients; Increased miR-3591-3p expression in active BD patients	Regulation of IL-6 production; differential expression associated with cancers, lupus and viral infection	CD11b + PBMCs	[78]
Noncoding RNA (microRNA)	*MIR23B*	Decreased miR-23b expression in CD4 + T cells of active BD patients	Regulation of Notch pathway and decreased expression of IL-17 and IFNγ in CD4 + T cells	CD4 + T cells	[68]
Noncoding RNA (microRNA)	*MIR139, MIR720*	Increased miR-139-3p and miR-720 expression in BD patients regardless of disease activity	Target Toll-like receptor and T cell receptor signaling pathways	PBMCs	[79]
IgA vasculitis (IgAV; Henoch–Schönlein purpura)					
Histone modification (H3 acetylation)	*IL4*	Increased H3 acetylation at *IL4* promoter and enhancer regions of CD4 + T cells from IgAV patients	Th2 cell cytokine	CD4 + T cells	[73]
Histone modification (H3K4me3)	*IL4*	Increased H3K4me3 at *IL4* promoter and enhancer regions of CD4 + T cells from IgAV patients	Th2 cell cytokine	CD4 + T cells	[73]
Histone modification (H3K4me)	Genome-wide	Globally increased H3K4me in PBMCs of IgAV patients with kidney disease; positive correlation with disease activity	Suggests that abnormal levels of H3K4me and maintenance proteins contributes to kidney disease in IgAV patients	PBMCs	[73]
Histone modification (H3 acetylation)	Genome-wide	Globally increased H3 acetylation in PBMCs of IgAV patients with kidney disease; positive correlation with disease activity	Suggests that abnormal levels of H3 acetylation and maintenance proteins contributes to kidney disease in IgAV patients	PBMCs	[73]

ANCA, antineutrophil cytoplasmic antibody.