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DNA Methylation: a New Player in Multiple Sclerosis

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Abstract Multiple sclerosis (MS) is a neurological and chronic inflammatory disease that is mediated by demyelination and axonal degeneration in the central nervous system (CNS). Studies have shown that immune system components such as CD4+, CD8+, CD44+ T cells, B lymphatic cells, and inflammatory cytokines play a critical role in inflammatory processes and myelin damage associated with MS. Nevertheless, the pathogenesis of MS remains poorly defined. DNA methylation, a significant epigenetic modification, is reported to be extensively involved in MS pathogenesis through the regulation of gene expression. This review focuses on DNA methylation involved in MS pathogenesis. Evidence showed the hypermethylation of human leukocyte antigen-DRB1 (HLA-DRB1) in CD4+ T cells, the genome-wide DNA methylation in CD8+ T cells, the hypermethylation of interleukin-4 (IL-4)/forkhead winged helix transcription factor 3 (Foxp3), and the demethylation of interferon-γ (IFN-γ)/IL-17a in CD44+ encephalitogenic T cells. Studies also showed the hypermethylation of SH2-containing protein tyrosine phosphatase-1 (SHP-1) in peripheral blood mononuclear cells (PBMCs) and methylated changes of genes regulating oligodendrocyte and neuronal function in normal-appearing white matter. Clarifying the mechanism of aberrant methylation on MS may explain part of the pathology and will lead to the development of a new therapeutic target for the treatment of MS in the future.

Keywords Multiple sclerosis · Epigenetics · DNA methylation

Abbreviations

5hmC	5-Hydroxymethylcytosine
5mC	5-Methylcytosine
APC	Antigen-presenting cell
BBB	Blood-brain barrier
BCL2L2	Bcl-2-like protein 2
CIS	Clinically isolated syndrome
CNS	Central nervous system
Co-rep	Co-repressors
CTSZ	Cathepsin z
DNHD1	Dynein heavy chain domain 1
Dnmts	DNA methyltransferases
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
Foxp3	Forkhead winged helix transcription factor-3
HAGHL	Hydroxyacylglutathione hydrolase-like
HDACs	Histone deacetylases
HERV-W	Human endogenous retrovirus W
HLA-DRB1	Human leukocyte antigen-DRB1
Hlx	H2.0-like homeobox
IFN-γ	Interferon-γ
IL	Interleukin
LGMN	Legumain
MBDs	Methyl-binding domain proteins
MBP	Myelin basic protein
MeCP2	Methyl-CpG-binding protein 2

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MHC	Major histocompatibility complex
MHC2TA	MHC class II transactivator
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NAWM	Normal-appearing white matter
NDRG1	N-myc downstream regulated gene 1
OPCs	Oligodendrocyte precursors
PAD2/4	Peptidyl arginine deiminase 2/4
PBMCs	Peripheral blood mononuclear cells
PPMS	Primary-progressive multiple sclerosis
RIS	Radiologically isolated syndrome
RPS2	Ribosomal protein S2
RRMS	Relapsing-remitting multiple sclerosis
SHP-1	SH2-containing protein tyrosine phosphatase-1
TCF-1	T-cell factor-1
TNFα	Tumor necrosis factor α

Introduction

Multiple sclerosis (MS) is a neurological and chronic inflammatory disease in which immune cell-mediated inflammation leads to demyelination and axonal degeneration in the central nervous system (CNS) [1]. There are many symptoms of MS, including balance and mobility impairment, weakness, reduced cardiovascular fitness, ataxia, fatigue, bladder dysfunction, spasticity, pain, cognitive deficits, depression, and pseudobulbar affect [2]. The phenotypic classifications of MS include relapsing-remitting multiple sclerosis (RRMS), clinically isolated syndrome (CIS), radiologically isolated syndrome (RIS), primary-progressive multiple sclerosis (PPMS), and secondary-progressive multiple sclerosis (SPMS) [3].

Studies have shown that inflammation mediated by immune cells results in myelin damage and axonal degeneration, especially in CD4+, CD8+ T cells; B lymphatic cells are also broadly involved in the pathogenesis of MS [4, 5]. Activated T cells enter the CNS and produce inflammatory cytokines, including tumor necrosis factor α (TNFα), interferon-γ (IFN-γ), interleukin (IL)-4, and IL-17. Those inflammatory cytokines contribute to the fenestration of the blood-brain barrier (BBB) and subsequent damage to myelin and axons [6–11]. Besides, the deregulation of transcription factors such as SH2-containing protein tyrosine phosphatase-1 (SHP-1) and forkhead winged helix transcription factor-3 (Foxp3) affect the severity of MS [12–14]. Moreover, studies also show that the aberrant modulation of neuronal and oligodendrocyte survival may hinder the response to the impairment in normal-appearing white matter (NAWM) [15, 16].

Although the pathogenesis of MS is not well known, accumulated evidence has shown that genes and environmental factors are likely to collectively confer susceptibility to MS [17]. Epigenetic factors, including DNA methylation, histone modification, chromatin remodeling, and noncoding RNA regulation, have been reported to cause MS by altering the interaction between genes and the environment [18–20]. Vitamin D deficiency, smoking, and Epstein-Barr virus have been shown to affect the risk of MS via epigenetic changes [21]. In recent years, DNA methylation has been reported to be linked with MS through the regulation of gene expression. Evidence showed the hypermethylation of the human leukocyte antigen-DRB1 (HLA-DRB1) gene in CD4+ T cells, the genome-wide DNA methylation in CD8+ T cells, the hypermethylation of IL-4/Foxp3 genes, and the demethylation of IFN-γ/IL-17a in CD44+ encephalitogenic T cells. Evidence also showed the hypermethylation of SHP-1 in peripheral blood mononuclear cells (PBMCs) and methylated changes of genes regulating oligodendrocyte and neuronal function in NAWM [18–20]. This review focuses on an overview of current evidence for methylation/demethylation changes in MS and how they contribute to this disease.

Pathogenesis of MS

Studies have shown that myelin-reactive T-cell blasts transgress the BBB and create a proinflammatory environment by promoting the expression of various genes in the CNS, thereby facilitating autoimmune attacks [22]. In particular, CD4+ and CD8+ T-cell surface molecules play a role in T-cell recognition [23]. CD8+ T cells, also called cytotoxic T lymphocytes (CTLA), specifically kill mutant cells and target cells infected by a virus through cytotoxicity. Meanwhile, CD4+ T cells, which are commonly referred to as T helper cells (Th) and consist of the Th1, Th2, and Th17 subtypes, aid the immune response by synthesizing and secreting inflammatory cytokines. T cells may enter the CNS by crossing the BBB in a process promoted by inflammatory cytokines [24]. Autoreactive CD4+ T helper cells orchestrate MS-induced myelin damage, and CD8+ T cells have been shown to undergo oligoclonal expansion at the site of pathology [25]. Both cell types play a pivotal role in orchestrating self-reactive immune responses in MS patients [26, 27]. Furthermore, during T-cell differentiation, the level of CD44, a ubiquitous multistructural and multifunctional cell surface adhesion molecule involved in cell-cell and cell-matrix interactions, is chronically elevated and confers signals to inflammatory cytokine genes [28, 29].

Some inflammatory cytokines, including TNFα, IFN-γ, IL-4, and IL-17, have been confirmed to be closely linked with MS. IL-17 is known to play a crucial role in the early phase of MS, whereas IFN-γ seems to be involved both in the early phase and in subsequent relapses of the disease [11]. IL-4 recruits regulatory T cells and induces clinical recovery in experimental autoimmune encephalomyelitis (EAE), whereas

Foxp3-positive cells act as novel regulatory cells to exert a significant influence on self-reactive CD4+ T-cell regulation during the progression of MS [30–35]. These cells and molecules are then reactivated and interact to promote the production of inflammatory cytokines; furthermore, activated PAD2/4 converts myelin basic protein (MBP) arginine residues into citrullines [36]. However, IL-4/Foxp3 and the anti-inflammatory cytokine SHP-1 fail to modulate the expansion and function of conventional T cells, thereby protecting against the disease [4, 7, 8].

All of these factors interact to cause various neurological deficits that stem from myelin damage and axonal degeneration. Oligodendrocyte precursor cells (OPCs) can differentiate into mature oligodendrocytes, which wrap the axon and form myelin. Proliferating OPCs migrate to lesion sites, where they are capable of acute remyelination but are unable to completely repair or restore immune system-mediated myelin damage. Immune attacks affect the oligodendrocyte survival and proteolytic processing of myelin basic protein in NAWM [6, 16, 37]. Various permanent clinical neurological disabilities such as cognitive dysfunction, fatigue, bowel/bladder abnormalities, and neuropathic pain were associated with myelin deficiency in MS [24].

Together, many lines of evidence have shed light on MS pathogenesis, but it remains unknown how these genes are regulated. However, over the last few decades, increasing evidence has illuminated the role of epigenetics in MS. Vitamin D deficiency, smoking, and Epstein-Barr virus have been shown to contribute to the risk of developing MS through epigenetic mechanisms. Epigenetic modifications, including DNA methylation, histone modification, chromatin remodeling, and noncoding RNA regulation, have been reported to regulate gene expression and participate in gene-environment interactions in the etiology of MS [18–21]. In recent years, DNA methylation—one of the first epigenetic modifications described—has been suggested to participate in the detailed regulatory mechanisms underlying abnormal gene expression in MS [18–20].

DNA Methylation

DNA methylation is an important regulator of MS pathogenesis [38, 39]. DNA methylation is achieved via DNA methyltransferase (Dnmts), which utilizes methyl groups provided by S-adenosyl methionine (SAM) to methylate the fifth carbon atom of cytosine, which is then converted to 5-methylcytosine (5mC) [40]. The Dnmts family includes Dnmt1, Dnmt2, and Dnmt3. Dnmt1 maintains DNA methylation after replication, Dnmt2 methylates aspartic acid residues of cytosine-38 in the anticodon loop of tRNA, and Dnmt3 (collectively Dnmt3a, 3b, and 3L) triggers the de novo establishment of DNA methylation [41, 42]. After the transfer of a carbon, methyl-binding domain proteins (MBDs) mediate the suppression of gene transcription after DNA methylation. In general, MBDs recruit histone deacetylases (HDACs) and co-repressors (Co-rep) of Dnmts to form transcription repressor complexes that silence or inhibit gene expression [43, 44]. There are six members in the MBD family, consisting of MBD1–4, methyl-CpG-binding protein 2 (MeCP2), and Kaiso [45, 46]. At present, MeCP2 is known to have two biologically active isoforms called MeCP2E1 and MeCP2E2 [47]. Conversely, demethylation is the process by which a methyl group (CH3) is removed from 5mC, so that DNA that has been previously methylated can once again express protein. The ten-eleven translocation (TET) family of methylcytosine dioxygenases, including TET1-3, is capable of catalyzing the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) to promote DNA demethylation [48], as shown in Fig. 1.

DNA Methylation in MS

In previous studies, MBD2 has been found to regulate autoimmunity through T-bet/H2.0-like homeobox (Hlx) proteins. T-bet/Hlx are two Th1-specific transcription factors known to play a role in EAE, an animal model of MS [49]. T-bet regulates the expression of the hallmark Th1 cytokine, which correlates with IFN-γ expression in Th1 and NK cells. Furthermore, Hlx gene expression drives IFN-g expression in cooperation with T-bet [50, 51]. In addition, MeCP2, a member of the MBD family, could play a role in remyelination and/or myelin repair in conjunction with brain-derived neurotrophic factor (BDNF) in the treatment of MS [24]. In the PBMCs of MS patients, Dnmt1, TET2, and 5hmC expression is significantly downregulated, and aberrant methylation of their target genes' promoters has been observed [52]. Aberrant methylation and demethylation at these loci has also been in MS. In the section below and in Table 1, we summarize these findings and how they contribute to the risk or pathogenesis of MS.

Hypermethylation of HLA-DRB1 and no Methylation Variation of HLA-DRB5 in CD4+ T Cells

CD4+ and CD8+ T-cell surface molecules play a role in T-cell recognition and activation by binding to their respective class II and class I major histocompatibility complex (MHC) ligands on antigen-presenting cells (APCs) [23]. Human leukocyte antigen (HLA), a human MHC, includes the class I loci A, B, and C; the class II loci DR, DP, DQ, etc.; and various class III loci. In particular, the MS-related MHC class II has been finely mapped to the extended haplotype HLA-DQA1*0102, HLA-DQB1*0602, HLA-DRB1*1501, and

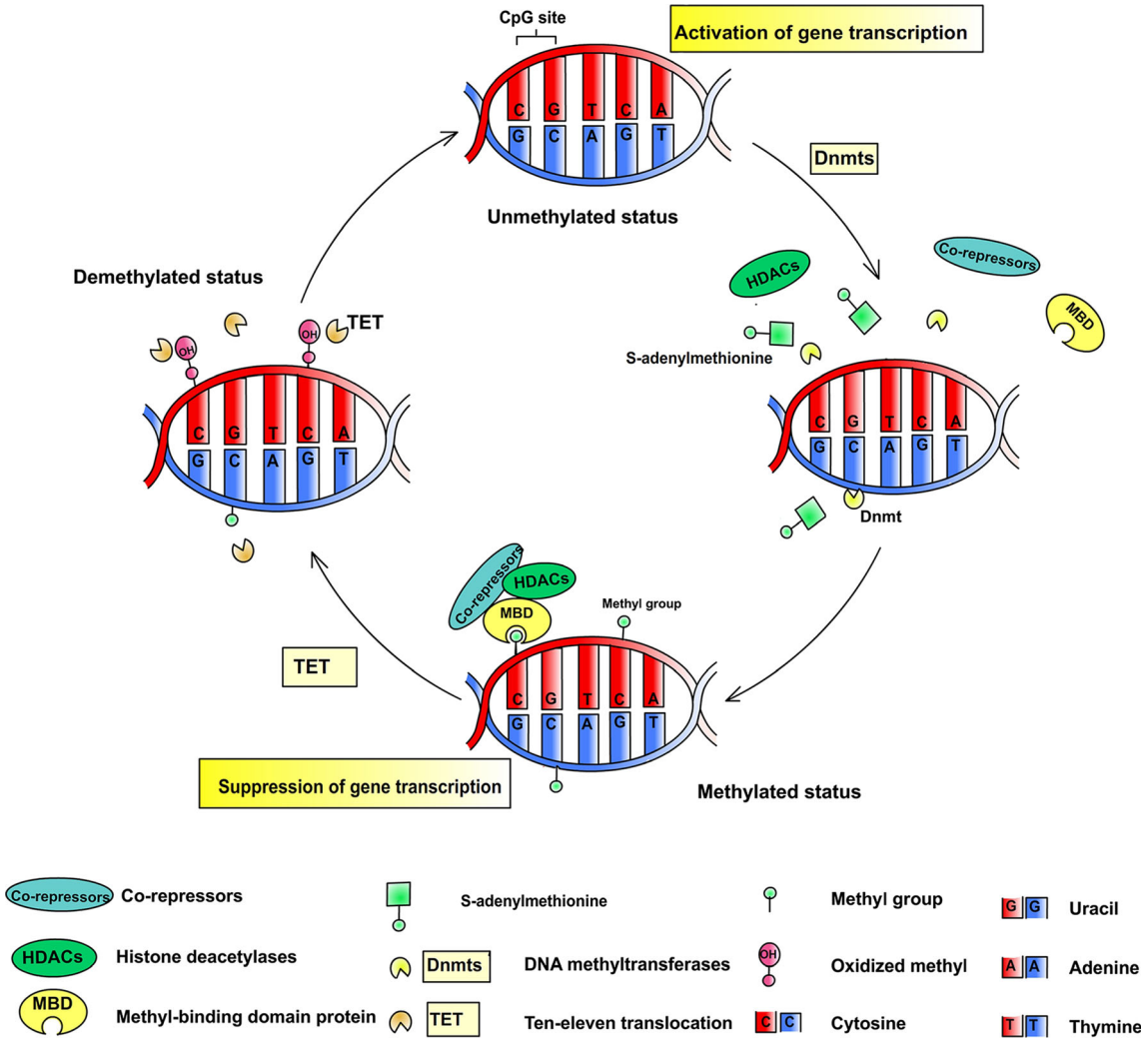

Fig. 1 Schematic: The regulatory mechanisms of DNA methylation in gene transcriptional expression. DNA methyltransferases (Dnmts) utilize methyl groups provided by S-adenosyl methionine (SAM) to methylate the fifth carbon atom of cytosine, which is converted to 5mC. In mammals, the methylation of cytosine nucleotides at CpG sites within promoter regions generally leads to transcriptional inhibition and gene silencing by preventing the transcription of the associated gene. However, TET could catalyze the oxidation of 5mC to 5hmC, thereby promoting DNA demethylation so that transcription can resume

HLA-DRB5*0101 [87]. HLA-DRB1 and HLA-DRB5 are HLA class II beta chain paralogues and play a central role in the immune system by presenting peptides derived from extracellular proteins. HLA-DRB1 may increase the risk of MS, whereas HLA-DRB5 could attenuate MS severity [74, 75, 57, 88].

In previous studies, no significant changes in DNA methylation at HLA-DRB1 (particularly HLA-DRB1*1501) and HLA-DRB5 genes were found in the peripheral blood of MS patients [76]. In addition, there was no effect on the methylation of CpG at the HLA-DRB1 locus in CD8+ T cells, indicating that HLA-DRB1 in CD8+ T cells does not contribute to the risk of MS [59]. However, recent evidence has demonstrated an association between DNA methylation at the HLA-DRB1 locus in CD4+ T cells and MS risk. Beyond HLA, many of these hits have been implicated previously in MS risk, lending credibility to these findings. Most notably, nine CpGs showing increased methylation in MS patients mapped to a 20-kb region within the T-cell receptor α (TCRα), which plays a key role in immune recognition [58]. These results suggest that CpG hypermethylation at this gene may have a reduced effect on its expression and may also be involved in immune deregulation in MS.

Genome-Wide DNA Methylation in CD8+ T Cells

Previous neuropathologic studies demonstrate that CD8+ T cells are the most numerous inflammatory infiltrate in MS lesions at all stages of lesion development [89]. And they

Table 1 A summary of the changes, effect of methylated changes in EAE mice, and proposed effect in MS patients

Gene	Methylated state	Research object	Expression	Effect of methylated changes in EAE mice and proposed effect in MS patients	Reference
MBD2	–	EAE mice	Increase	MBD2 regulates TH17 differentiation by controlling the homeostasis of the T-bet/Hlx axis, which may correlate with IFN-γ and IFN-g expression during the inflammation process.	[49–51]
IFN-γ/IL-17a	Demethylation	EAE mice	Increase	This change in CD44+ encephalitogenic T cells induced the differentiation of naive T helper cells into Th2 cells and activated inflammation process.	[53, 54–56]
IL-4/Foxp3	Hypermethylation		Decrease	This change decreased the production of Th1 cells, macrophages, and IFN-γ to render protection against EAE in CD44+ encephalitogenic T cells.	[29, 54]
MeCP2	–	EAE mice	Increase	The increase of MeCP2 inhibits remyelination and/or myelin repair by regulating BDNF in EAE mice, suggesting a role of MeCP2 through inhibition of BDNF-induced myelin repair in MS.	[24]
TET2 5hmC Dnmt1	–	MS patients	Decrease	Changes in peripheral blood mononuclear cells (PBMCs) lead to aberrant methylation of some target genes, which contributes to the risk of MS.	[52]
HLA-DRB1	Hypermethylation	MS patients	Decrease	HLA-DRB1 changes in CD4+ T cells affect the presentation peptides which derived from extracellular proteins.	[57, 58]
	No change		No change	No change was found in the peripheral blood and CD8+ T cells of MS patients.	[59]
HLA-DRB5	No change	MS patients	Increase	No significant change of DNA methylation in HLA-DRB5 gene was found in the peripheral blood. But the HLA-DRB5 (especially DRB5*0101) could interact with HLA-DRB1 restricts MBP-specific T cells.	[74, 76]
DNHD1 and others	Hypermethylation	MS patients	Decrease	The change may affect the function and movement of CD8+ T cells by numerous cellular processes, cis-elements, and transcription factor networks, which result in increased vulnerability to MS.	[38, 59, 60, 61]
BCL2L2 HAGHL NDRG1	Hypermethylation	MS patients	Decrease	The change would affect the regulation of neuronal and oligodendrocyte survival in normal-appearing white matter (NAWM).	[15, 16, 62, 63]
LGMN CTSZ	Hypomethylation		Increase	The change may impair the neuronal survival and generate immunogenic peptides in NAWM.	[16, 37, 64, 65]
PAD2	Demethylation	MS patients	Increase	Increased the synthesis of PAD2 protein results in the loss of myelin stability due to increased citrullinated MBP.	[66–68]
SHP-1	Hypermethylation	MS patients	Decrease	This change increased leukocyte-mediated inflammation by inhibiting the negative regulation of proinflammatory signaling.	[69–72]
MHC2TA	No change	MS patients	No change	The methylation of MHC2TA does not contribute to MS risk.	[92]

have also been demonstrated to be important regulators of blood-brain barrier permeability [90]. Recent genome-wide DNA methylation studies revealed that CD8+ T cells have distinct DNA methylation profiles [59]. These lines of evidence have showed that DNA hypermethylation at many genes in CpG sites, including dynein heavy chain domain 1 (DNHD1), adenomatosis polyposis coli 2 (APC2), and other genes that control the movement of immune cells by affecting microtubule stability in MS patients, indicates a role for DNA methylation in CD8+ T cells of MS patients [59, 60, 61]. Another study has reported the layered genetic control of DNA methylation and gene expression by a long-range methylation quantitative trait loci (meQTLs) method, which could increase vulnerability to MS [38]. Although many differentially methylated CpGs are associated with MS, there was no major CpG effect at the MS risk gene HLA-DRB1 locus in CD8+ T cells. Moreover, significant differences in DNA methylation were not detected at individual CpG sites [59].

Combined with the evidence of these methylated changes, the process of DNA methylation remodeling might accompany the changes of function and movement of the CD8+ T cells via numerous cellular processes, cis-elements, and transcription factor networks [78]. Further studies which focus on cellular processes and molecular networks targeted by DNA methylation were needed to be explored in MS.

Demethylation of IFN-γ/IL-17a and Hypermethylation of IL-4/Foxp3 in CD44+ Encephalitogenic T Cells

CD44+ encephalitogenic T cells play a crucial role in the differentiation of Th cells. Studies have shown that CD44 expression is chronically elevated during demyelination in

MS [29, 54]. IFN-γ is a dimerized soluble cytokine that belongs to the type II class of interferons, and its aberrant expression is associated with MS [79, 80, 55]. Furthermore, IL-17a, a proinflammatory cytokine produced by activated T cells, and IL-4, a cytokine that induces the differentiation of naive T helper cells (Th0 cells) into Th2 cells, could decrease the production of Th1 cells, macrophages, and IFN-γ [53, 56]. Foxp3 also plays an essential role in maintaining the stability of the Treg lineage and in directly modulating the expansion and function of conventional T cells [77]. Thus, IFN-γ/IL-17a plays a role in inducing the inflammation of MS, while IL-4/Foxp3 could protect against the disease.

In MS, CD44 confers signals to the IFN-γ and IL-4 loci during T-cell differentiation, thereby promoting IFN-γ gene expression [28]. Activation of CD44+ encephalitogenic T cells by myelin oligodendrocyte glycoprotein (MOG) peptide led to the demethylation of the IFN-γ/IL-17a promoter region and the hypermethylation of the IL-4/Foxp3 gene promoter. Interestingly, similar activation of CD44-deficient encephalitogenic T cells led to increased hypermethylation of the IFN-γ/IL-17a gene and marked demethylation of the IL-4/Foxp3 gene promoter. These findings reveal that CD44 reciprocally regulates the differentiation of encephalitogenic Th1/Th17 and Th2/Treg T cells via the DNA methylation of IFN-γ/IL-17a and IL-4/Foxp3 gene promoters [29].

Methylation of SHP-1 and no Methylation Variation of MHC2TA Loci in PBMCs

The protein tyrosine phosphatase SHP-1 is a negative regulator of proinflammatory signaling and autoimmune disease [69, 70]. It is expressed in normal oligodendrocytes in human brain white matter, and a deficiency in SHP-1 has been reported in a subset of MS subjects []. Virus-induced DNA methylation of the SHP-1 promoter can silence SHP-1 expression and function in hematopoietic cells in cases of inflammatory disease. Due to SHP-1 deficiency in MS leukocytes, proinflammatory genes are upregulated; increased methylation of the SHP-1 promoter decreases SHP-1 expression, thereby increasing leukocyte-mediated inflammation in MS [72].

The expression of MHC class II molecules is regulated primarily through the MHC class II transactivator (MHC2TA) [91]. MHC2TA, as a non-DNA binding coactivator, could coordinate multiple events that are required for the activation of transcription, including the recruitment of transcription factors and the phosphorylation of RNA polymerase II [92]. It has three independent promoters: promoter I (pI), promoter III (pIII), and promoterIV (pIV). Methylation of promoter IV has been shown to alter the expression of MHC2TA [81, 92]. However, in PBMCs, no methylation or sequence variation in pIV has been found. These results do not support the hypothesis that the methylation of MHC2TA contributes to MS risk, although tissue- and timing-specific epigenetic modifications cannot be ruled out [73].

In addition, although the methylated changes of many genes had been detected as we showed in this review, the methylation of several MS associated genes might need to be explored in the future. For instance, increased levels of human endogenous retrovirus W (HERV-W) related to transcripts were subsequently observed in PBMC and brains from patients with MS as compared with control individuals. And hypomethylation correlating to reactivation of HERV-W in testicular cancer was also observed [82]. However, whether altered methylation pattern of HERV-W would be associated with MS was unknown [83, 93]. Besides, IL-17, which could lead to exaggerated Th17 responses, is associated with EAE [84]. In the study of Ma et al., they observed that T-cell factor-1(TCF-1) mediates an active process to repress IL-17 gene expression via hyperacetylation together with trimethylation of Lys-4 at the IL-17 locus during T-cell development [85]. However, the methylated change of TCF-1 in EAE or MS was still not explored. Thus, further studies should probe into the methylation of those MS- or EAE-associated genes in order to find more DNA methylation evidence for MS.

Methylated Changes of Genes Regulating Oligodendrocyte and Neuronal Function in Normal-Appearing White Matter

In order to identify the molecular mechanisms underlying the pathological processes of MS, many studies have studied the gene expression profile in NAWM [94]. Hypermethylation was detected for genes regulating oligodendrocyte and neuronal function, such as Bcl-2-like protein 2 (BCL2L2), which has been shown to regulate neuronal and oligodendrocyte survival [16, 62]. Hydroxyacylglutathione hydrolase-Like (HAGHL) was highly expressed in the brain, and N-myc downstream regulated gene 1 (NDRG1) involved in oligodendrocyte response to stress. Hypermethylation of HAGHL and NDRG1 was also found [15, 16, 63]. Besides, cathepsin z (CTSZ) has been functionally linked to impaired neuronal survival [64, 65]. Legumain (LGMN) can cleave MBP and generate immunogenic peptides [37]. Hypomethylation of CTSZ and LGMN was also detected in these two genes encoding cysteine proteases [16].

PAD2 and PAD4 are two members of the PAD family. Together, they citrullinate the Arg-Gly repeat region of ribosomal protein S2 (RPS2), leading to an increase in the level of citrullinated MBP in MS [95, 66]. However, the lack of methylation of a single arginyl residue of MBP has been implicated in myelin stability in subacute combined degeneration of the spinal cord. The conversion of arginine to citrulline by PAD2/4 has been implicated in demyelination in MS [66, 67]. Moreover, noncovalent inhibitors of PAD enzymes could be potential target agents in cases of increased PAD2 and PAD4 level in the pathogenesis of MS [36, 86]. The methylation of cytosine in the PAD2 promoter in NAWM in MS was decreased to one third of the level in normal white matter. In the peripheral blood of MS patients, the PAD2 promoter region was extensively demethylated, leading to PAD2 gene upregulation []. In the EAE model, the expression of MBP is increased in lymphoid tissue, and the severity of MS has been correlated with the number of citrullinyl residues in MBP [66, 67, 96].

Conclusion and Perspective

This review focuses on DNA methylation involved in MS pathogenesis. Evidence showed the hypermethylation of the HLA-DRB1 gene in CD4+ T cells, the genome-wide DNA methylation in CD8+ T cells, the hypermethylation of IL-4/Foxp3 genes, and the demethylation of IFN-γ/IL-17a in CD44+ encephalitogenic T cells. Studies also showed the hypermethylation of SHP-1 in PBMCs and methylated changes of genes regulating oligodendrocyte and neuronal function in NAWM.

To date, DNA methylation, one of the earliest identified epigenetic modifications, has been shown to silence gene expression, which results in increased risk of MS and is involved in its pathogenesis. In 2015, lymphatic vessels were found in the CNS; gene methylation has been observed in immune cells of the lymphatic system and is known to play a role in the etiology of MS [97]. In particular, immune cells such as autoreactive CD4+ T cells and antibodies have been found in the CNS of MS patients and the EAE model, helping to establish the comprehensive immunopathology of MS and to promote epigenetic research in CNS lymphatic vessels [98–100]. Furthermore, aberrant DNA methylation and demethylation, which has been reported in both postmortem brains and peripheral blood cells of MS patients, could serve as a novel biomarker in MS therapy; several changes in methylation or demethylation are specific in MS [101].

New clinically relevant disease targets and drugs are necessary to improve MS therapy in meaningful ways. Previous research in the field of epigenetic therapeutics for MS involved HDAC inhibitors and lysine acetyl transferase (KAT) inhibitors that are now being used to repress the detrimental effects of MeCP2 [24]. Currently, several drugs related to methylation such as curcumin, the major polyphenolic compound found in curry first discovered two centuries ago, have been shown to inhibit DNA methyltransferase and to modulate immune-mediated inflammation by downregulating inflammatory cytokines [102–104]. These compounds have great potential for the treatment of MS [105]. Furthermore, procainamide, a pharmaceutical antiarrhythmic agent used for the medical treatment of cardiac arrhythmia, and hydralazine, a direct-acting smooth muscle relaxant used to treat hypertension by acting as a vasodilator primarily in arteries and arterioles, are now being tested as Dnmts inhibitors in preclinical studies [106–108]. However, whether these demethylation-related drugs could be used in MS patients and how they target specific methylated genes remains to be explored. In summary, future studies will be most informative if they concentrate on excluding the mechanisms of DNA methylation or demethylation in MS. Elucidating the pathophysiological mechanism of MS will facilitate the development of more effective therapeutic strategies.

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