Epigenetic insights into multiple sclerosis disease progression

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Multiple sclerosis (MS), a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system, is today a leading cause of unpredictable lifelong disability in young adults. The treatment of patients in progressive stages remains highly challenging, alluding to our limited understanding of the underlying pathological processes. In this review, we provide insights into the mechanisms underpinning MS progression from a perspective of epigenetics, that refers to stable and mitotically heritable, yet reversible, changes in the genome activity and gene expression. We first recapitulate findings from epigenetic studies examining the brain tissue of progressive MS patients, which support a contribution of DNA and histone modifications in impaired oligodendrocyte differentiation, defective myelination/remyelination and sustained neuro-axonal vulnerability. We next explore possibilities for identifying factors affecting progression using easily accessible tissues such as blood by comparing epigenetic signatures in peripheral immune cells and brain tissue. Despite minor overlap at individual methylation sites, nearly 30% of altered genes reported in peripheral immune cells of progressive MS patients were found in brain tissue, jointly converging on alterations of neuronal functions. We further speculate about the mechanisms underlying shared epigenetic patterns between blood and brain, which likely imply the influence of internal (genetic control) and/or external (e.g. smoking and ageing) factors imprinting a common signature in both compartments. Overall, we propose that epigenetics might shed light on clinically relevant mechanisms involved in disease progression and open new avenues for the treatment of progressive MS patients in the future.

Keywords: DNA methylation, epigenetics, histone modifications, multiple sclerosis, nervous system diseases, neurodegenerative diseases.

Introduction

Multiple sclerosis (MS), a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS), is today a leading cause of unpredictable lifelong disability in young adults. The progressive form of disease, characterized by constant worsening of disability, remains the greatest challenge in the care of patients with MS. Recent development of advanced molecular and analytical tools has enabled researchers to tackle some of the challenges of studying the brain by taking advantage of the epigenetic marks. Epigenetics, which literally means ‘on top of’ the genes, refers to stable and heritable changes in gene expression that do not require changes in the genetic code. By integrating internal (genes) and external (environment) signals, epigenetic mechanisms orchestrate the cellular adaptation in a context-specific and time-dependent manner (Fig. 1a).

Epigenetic mechanisms rely on the stable deposition of biochemical modifications onto the genome, such as DNA methylation and histone post-translational modifications (PTMs), which exert regulatory function on chromatin conformation and transcription (Fig. 1b). The covalent addition of a methyl group to cytosines (5mC) in mammals is most often established and maintained by the DNA methyltransferases (DNMTs) 3A/3B and DNMT1, respectively, in a CpG dinucleotide context [15]. The functional impact of DNA modifications, for example on gene expression, genome stability and X-chromosome inactivation, is highly dependent on the genomic context [16]. In association with CpG-rich promoter regions, 5mC is a well-known repressive mark, whereas DNA methylation in gene
bodies promotes transcription [16, 17]. Active demethylation is carried out by the ten–eleven translocation family of proteins (TETs) [18]. Importantly, 5-hydroxymethyl cytosine (5hmC), a key intermediate in the process of demethylation, is particularly abundant in the brain tissue especially in neurons [19] and has a distinct functional effect compared with 5mC [20, 21]. The degree of chromatin compaction is further determined by PTMs at the N-terminal tails of the 8 core histones forming the nucleosome unit around which the DNA is wrapped. The deposition and removal of the
most known histone PTMs, that is acetylation and methylation, are coordinated by histone acetyltransferases/deacetylases (HATs/HDCAs) and methylases/demethylases (e.g. HMTs/KDMs), respectively [22]. Overall, DNA and histone modifications jointly determine the level of compaction of the chromatin and its accessibility to the transcriptional machinery, with some of these changes persisting through cell divisions long after the original triggers.

Emerging evidence supports the hypothesis of epigenetic mechanisms as mediators of aetiological MS factors on altered cellular functions underpinning disease development [23–29]. This review aims to provide current insights into the mechanisms underlying MS progression from an epigenetic perspective. Assimilating the emerging findings from epigenetics might also aid in appreciating the potential for future developments in the care of progressive MS patients.

**Etiopathology of multiple sclerosis**

MS is characterized by autoimmune demyelination and subsequent neuro-axonal loss. Insults to the protective myelin sheath insulating axons lead to defective conduction of electrical impulses causing neurological symptoms. Sustained myelin and neuro-axonal damage nested in a toxic pro-inflammatory milieu ultimately result in degeneration of oligodendrocytes (OLs), the myelin-producing cells in the CNS, and neurons. More than 2.3 million people worldwide live with an unpredictable life-long disability inflicted by MS, ranging from sensory, visual and motor functions to fatigue and cognitive impairment. The multifaceted nature of disease results in highly heterogeneous clinical manifestations and disease development as well as variable response to treatments. Overall, the majority of patients with MS are women diagnosed with a relapsing–remitting form of MS (RRMS) at around 30 years of age (Fig. 2a) [1]. This early stage of disease presents with recurring neurological episodes, triggered by infiltration of immune cells into the CNS and ensuing damage to the tissue (Fig. 2b), followed by full or partial recovery. After around a decade from diagnosis, more than half of RRMS patients transition to a secondary progressive (SPMS) stage characterized by continuous worsening of disability without amelioration between isolated relapses (Fig. 2a) [1]. In addition, approximately 10-15% of individuals affected by MS manifest a primary progressive (PPMS) form of disease with a gradual deterioration without improvement already from the onset.

The susceptibility to develop MS is conferred by an intricate combination of genetic and environmental/lifestyle factors [2]. Genetic variants in the locus encoding the HLA class II genes carry the largest risk for developing MS [3, 4]. This risk is dominated by the HLA-DRB1*15:01 allele, which increases the probability of developing MS by ~ threefold in populations of northern European origin, with additional influences and complex interactions existing within the extended locus [5, 6]. The risk of developing disease is further considerably increased by influences from environmental/lifestyle factors such as Epstein–Barr virus infection, smoking, low sun exposure and vitamin D levels, and increased body mass index (BMI) in adolescent years [2]. The associations with the HLA class II genes that encode molecules on the surface of antigen-presenting cells (APC), involved in presenting antigenic peptides to the CD4+ T helper cells, strongly suggest the importance of the interaction between APCs and T cells in the aetiology of MS. Additionally, a large fraction of recently identified genetic associations supports a substantial role of dysregulated adaptive immunity in triggering MS [4, 6–8]. These findings translate into the efficacy of current disease modifying therapies in RRMS, with drugs targeting adaptive immune reactions exerting the most pronounced therapeutical efficacy [9, 10].

Both progressive forms of MS disease, that is PPMS and SPMS, share common features typified by gradual and inexorable increase in disability without remission. Disease progression in MS is commonly ascribed primarily to CNS-restricted neuroinflammatory and neurodegenerative processes, as opposed to the predominant peripheral immune contribution characterizing RRMS. The highly variable nature of disease progression renders its diagnosis retrospective, that is after monitoring disease worsening, and its prognosis unpredictable. Overall, robust biomarkers are lacking and efficient treatments are scarce, impeding proper care of progressive MS patients [11]. The current challenge of treating progressive MS patients alludes to the insufficient understanding of the mechanisms behind disease progression. In that regard, the different idiosyncratic subtypes of the same disease might rather reflect a continuum of processes affecting the highly context-savvy immune and nervous tissues (Fig. 2a). The CNS is
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Fig. 2  Progressive course of multiple sclerosis. (a) Natural history of multiple sclerosis (MS). The X-axis depicts time in years and a conversion from relapsing-remitting MS to secondary progressive MS. Orange bars indicate inflammatory episodes detected by brain Magnetic Resonance Imaging (MRI) as gadolinium (Gd)-enhancing lesions, whilst light violet line indicates new T2-hyperintense brain lesions. Dark violet line depicts brain atrophy that starts early and gradually progresses over time. (b) Overview of the cellular players in the MS pathogenesis involving meningeal accumulation of peripheral T and B cells, oligodendrocyte and neuron damage, and astroglial activation. (c) Putative mechanisms implicated in MS progression. Chronic demyelination and subsequent neuro-axonal injury result from exposure to a toxic microenvironment partly mediated by immune cells (peripheral or CNS-compartmentalized) and CNS-resident cells (microglia and astrocytes), releasing inflammatory mediators, reactive oxygen and iron species. The loss of structural and trophic support from defective myelinating oligodendrocytes leads to increase energy demands for nerve-impulse conduction. Proper electrical activity is crippled by mitochondrial injury and break of ion homeostasis. Dysfunctional mitochondria, reflected by impaired axonal transport, susceptibility to oxidative damage and mitochondrial DNA mutations, induces energy failure and enhances oxidative stress. Ion channel imbalance, including excess glutamate, promote cytoplasmic Ca\textsuperscript{2+} accumulation and excitotoxicity. These changes collectively promote brain atrophy.
the ultimate site that mediates the influence of proximal (CNS-resident) and distal (CNS-infiltrating) cellular sources of damage (Fig. 2b), with remnants of prior autoimmune attacks likely impinging the whole brain. Brain atrophy is the strongest predictor of disability, its effect becoming conspicuous later in life when CNS resources are likely exhausted from enduring damage superimposed by ageing processes. Commonly implicated mechanisms of MS progression involve, amongst others, CNS-compartmentalized immune reactions, microglia dysregulation, chronic oxidative stress, accumulation of mitochondrial damage in neurons/axons, altered ion channel activity and glutamate excitotoxicity (Fig. 2c) (reviewed by [11–14]). It is noteworthy that signals between the inflammatory cells and the target tissue are likely bidirectional, making the mechanisms underlying MS progression tightly intertwined. However, a limited ability, due to the inaccessibility of the brain, to study the mechanisms underlying neuroinflammation and neurodegeneration in situ has so far hampered our understanding and care of patients affected with progressive MS.

Mechanistic insights from epigenetic studies of the progressive MS brain

Epigenetic landscapes are highly context dependent insofar as epigenetic marks are inherently specific to distinct cell types and to a given time. These key concepts anchor the following section on the overview of the studies performed in the CNS tissue from patients with MS, using candidate-gene approaches and more recently genome-wide profiling of epigenetic marks. An overview of the genome-wide studies highlights different experimental designs and findings (recapitulated in Table 1). Briefly, the first genome-wide study has profiled DNA methylation changes in normal-appearing white matter (NAWM) from patients with MS in comparison with white matter (WM) from controls without MS [23]. Another study has investigated DNA methylation changes in demyelinated hippocampi in comparison with intact demyelinated hippocampi MS patients with MS [30]. Finally, the only cell type-specific study to date has examined DNA methylation changes in subcortical WM neurons from patients with MS compared to controls, further disentangling true methylation (5mC) from hydroxymethylation (5hmC) changes [31]. Functional pathways of progressive MS emerging from epigenetic studies, both using direct profiling of epigenetic patterns in human tissue and interfering with epigenetic enzymatic machinery in experimental models, are discussed in the following sections.

Epigenetic changes associated with white matter vulnerability

Epigenetic mechanisms underlying myelin destabilization and immunogenicity

Loss of myelin is a hallmark of MS and an important contributor to sustained damage and disease progression. Structural changes in the myelin have been proposed as one of the mechanisms underpinning autoaggressive immune response in MS. Increased levels of citrullinated (deaminated) myelin basic protein (MBP), catalysed by peptidyl arginine deiminases such as PADI2 and PADI4, have been observed in NAWM of patients with MS compared to WM from controls [32, 33]. In rodents, enhanced deaminated MBP isoform leads to destabilization of myelin sheaths and focal demyelination concomitant to astrogliosis and macrophage activation, indicative of exposure to potentially immunodominant epitopes [34]. The hypothesis of creation of neoantigens from citrullinated myelin is further supported by epitope spreading of autoantibody responses to native and citrullinated myelin epitopes in animal model of MS [35]. Elevated citrullinated MBP isoform in the white matter of patients with MS was associated with increased levels of PADI2 and PADI4 enzymes, PADI2 showing the highest enzymatic activity against MBP [33, 36]. Abnormally high PADI2 levels in the NAWM of patients with MS were further correlated with reduced PADI2 promoter methylation, compared with control individuals or patients affected with other neurological diseases [32]. Interestingly, a methylation-dependent upregulation of PADI2 gene has also been observed in peripheral blood mononuclear cells (PBMCs) from RRMS patients, suggesting immune priming against citrullinated MBP in the periphery towards break of tolerance [37]. Additionally, elevated nuclear levels of PADI enzymes, specifically PADI4, in the NAWM of patients with MS could be linked to aberrant citrullination of its preferential substrate histone H3 [36]. PADI4 translocation into the nucleus was correlated to increased levels of a pro-inflammatory cytokine TNF, potentially derived from astrocytes. Functional investigation in an MS-like model as well as a transgenic models of demyelination in rodents confirmed that accumulation of nuclear PADI4 and histone H3 citrullination was attributed to increased TNF, prior to and during clinical signs of demyelination [34, 36].
Interestingly, upregulation of \textit{PADI4} has also been observed in PBMCs of patients with MS compared to their unaffected relatives [38]. \textit{In vitro} investigation implicated PADI4-mediated histone citrullination in the derepression of \textit{TNF} and \textit{IL8} cytokine genes and reactivation of human endogenous retroviruses (HERVs), which are augmented in MS PBMCs as well. These data suggest the

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|c|}
\hline
Ref. & Tissue & Cells & Cohort (F/M) & Comparison & Methods & Findings \\
\hline
[23] & Frontal NAWM & Bulk & DC: 28 MS (3 RRMS, 17 SPMS, 7 PPMS, 17 /11), 19 NNC (7/12); VC: 10 MS (SPMS, 7/3), 20 NNC (14/6) & MS vs. NNC & BS-450K, EpiTYPER; mixed (5mC/5hmC) & 220 hypomethylated DMRs (1235 CpGs) and 319 hypermethylated DMRs (1292 CpGs) at oligodendrocyte-specific genes (\textit{BCL2L2, HAGHL, NDRG1, CTSZ, LGMN}) Correlation with expression change of a fraction of corresponding genes. \\
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[30] & Hippocampi & Bulk & 8 myelinated MS (6 SPMS, 2 PPMS, 5/3), 7 demyelinated MS (6 SPMS, 1 PPMS, 5/2) & Demyelinated vs. myelinated & BS-450K, ELISA, IHC, RT-qPCR; mixed (5mC/5hmC) & 144 DMPs (75 genes) in demyelinated vs. myelinated: 62 hyper, 82 hypo (\Delta\beta> 20\%) in astrocytic and neuronal genes. TSS-DMPs at AKNA, EBPL, HERC6, SFPR1, NHLH2, PLCH1, TMEM132B and WDR81 correlated with gene expression changes. \\
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[31] & Subcortical WM neuronal nuclei & Cohort 1: 5 SPMS (4/1), 5 NNC (1/4), cohort 2: 10 SPMS (7/3), 7 NNC (3/4), IF: 7 PMS (6/1), 8 NNC (3/5) & MS vs. NNC & BS/oxBS-450K, MSRE-GSRE, IF; mixed (5mC/5hmC), 5mC and 5hmC & COHORT 2: 2811 5mC-DMPs and 1534 5hmC-DhmPs with predominant hypo-5mC and hyper-5hmC colocalizing at gene bodies of genes involved in axonal guidance and synaptic integrity. Correlation with decreased gene expression. Meta-analysis: CREB remains the top altered pathway and associates with reduced P-CREB in NAWM neurons compared with WM neurons from NNC. \\
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\caption{Genome-wide DNA methylation studies in postmortem brain tissue from progressive multiple sclerosis patients}
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5mC, DNA methylation; 5hmC DNA hydroxymethylation; 450K, Illumina Infinium Human450 Beadchip; BS/oxBS, bisulphite and oxidative BS treatment; DC, discovery cohort; DMP, differentially methylation positions; DMR, differentially methylation region; IF, immunofluorescence; MS, Multiple Sclerosis; MSRE-GSRE, methyl- and glucosyl-sensitive restriction enzymes; RRMS, relapsing-remitting MS; PPMS, primary progressive MS; Ref., reference; SPMS, secondary progressive MS; NAWM, normal-appearing white matter; NNC, non-neurological controls; VC, validation cohort; and WM, white matter.
possibility of pervasive effects of PADI enzymes, acting across different cellular players in MS pathogenesis. Given that PADI2 is able to upregulate PADI4 and that subsequent PADI4-mediated H3 citrullination is responsible for oligodendrocyte apoptosis [34], elevated PADI levels and their respective epigenetic changes in the white matter might represent an early change preceding demyelination. Consistent with that, treatment with PADI inhibitor ameliorated disease severity and promoted remyelination in four independent models of neurodegenerative/demyelinating conditions, supposedly recapitulating different aspects of MS pathology [39-41]. In the autoimmune MS-like paradigm, reversal of white matter protein hypercitrullination suppressed T-cell autoreactivity and T-cell infiltration especially when PADI inhibitor was administered prior to disease onset.

Surprisingly, genome-wide profiling of DNA methylation changes in MS hippocampi following demyelination [30] or in MS NAWM compared with control WM [23] could not identify alterations of PADI genes. Similarly, no transcriptional difference of PADI genes could be found in transcriptome analysis of MS NAWM compared with control white matter [23]. In contrast, recent study in mice demonstrated that PADI2-mediated citrullination is necessary for proper oligodendrocyte lineage progression and myelination [42]. Thus, additional investigations that can shed a light on the potential involvement of epigenetic dysregulation leading to a PADI-dependent demyelination are warranted.

**Histone modifications and impaired oligodendrocyte differentiation**

During demyelinating episodes, resident neural stem cells (NSCs) and oligodendrocyte progenitor cells (OPCs), recruited to the site of injury, participate in tissue repair by producing soluble mediators and differentiating into myelin-producing OLs [43, 44]. However, in progressive MS stages these mechanisms fail to fully repair and restore inflammation-induced damage. *In vitro* and *in vivo* experimental models have established an important role of histone acetylation, albeit highly context- and stage-dependent, in regulating neuroglial differentiation, OPC plasticity and OL myelinating capacity [45-54]. In line with this, changes in SIRT1, a member of the HDAC class III family, have been observed in the brain and PBMCs of patients with MS [55]. Moreover, decreased levels of H3 acetylation have been detected in oligodendrocytes located in active demyelinating and remyelinating areas of lesions compared with the adjacent periplaque white matter [56]. The same study, however, reported a contrasting finding with the increased H3 acetylation in frontal NAWM of chronic MS patients as well as aged individuals. Elevated histone acetylation was coherent with an upregulation of HAT family members in a subgroup of female patients with MS and this effect correlated with disease duration. Mechanistic exploration associated increased H3 acetylation at the TCF7L2 gene, a key player in Wnt signalling-mediated inhibition of OL differentiation [50], and its transcriptional activation. Overall, this suggests that deacetylation of H3 histone occurring in early MS lesions might shift to a hyper-acetylation and subsequent repression of OL differentiation in NAWM of patients, especially women with long disease duration. Despite evident high context dependency, the histone acetylation paradigm has been utilized to treat demyelinating disease models *in vivo* using HDAC inhibitors with the treatment efficacy being linked, at least in part, to increased capacity of OL differentiation and myelin gene expression [49, 54, 57]. Inhibition of HDAC activity was also beneficial in ischaemia-induced white matter damage *in vivo* [58]. The suggested mechanism involves HDAC inhibitor-induced promotion of oligodendrogenesis via the brain-derived neurotrophic factor (BDNF)-Trk signalling pathway, which has been shown to control OPC proliferation and development [59, 60].

**Epigenetic mechanisms underlying oligodendrocyte functions and myelin repair**

Recent study, using oligodendrocyte dating, suggests that, unlike in rodents, progenitor cells have a limited role in generating myelinating OLs in progressive MS and that pre-existing differentiated OLs might be the primary source of new myelin [61]. Indeed, analysis of oligodendrocyte heterogeneity in the MS brain tissue identified mature OLs with a capacity to increase transcriptional myelination programmes [62]. Genome-wide profiling of DNA methylation changes in MS NAWM identified subtle but numerous changes converging to oligodendrocyte state and function [23]. Despite using bulk NAWM tissue, results were proposed to arise primarily from oligodendrocytes, with hypermethylated genes relating to oligodendrocyte function whilst hypomethylated genes associated with immune processes. Oligodendrocyte-specific genes such as MBP, SOX8 and GJB1 were found to be hypermethylated in NAWM of patients with MS. Interestingly, improvement in
clinical symptoms following the treatment of MS-like disease in rodents with HDAC inhibitor and thyroid hormone T3 was also associated with an increased expression of myelin-related genes, that is Sox8, Mbp, CNPase, Mog, Mag and Plp [54]. This supports the hypothesis of epigenetic dysregulation of OL genes during CNS inflammation via the combined action exerted by the two epigenetic modalities, DNA methylation and histone deacetylation, known to be tightly interconnected [63, 64]. Genes regulating OL survival (e.g. NDRG1 and BCL2L2) were also found hypermethylated and displayed concordant lower levels of expression in MS NAWM [23]. The fact that these changes are detected in normal-appearing WM suggests that the impairment in OL function occurs prior to myelin damage potentially due to exposure to diffuse mediators in the tissue.

Epigenome-wide profiling of hippocampi following demyelination unravelled changes occurring during abnormalities of the myelin, including dysregulation of DNA methylation enzymatic machinery [30]. The majority of annotated genes harbouring DNA methylation changes in demyelinated hippocampi were, however, assigned to multiple human brain cell types, whilst a 14%, 12%, 9% and 5% were uniquely associated with microglia, neurons, astrocytes and oligodendrocytes, respectively [30]. Many (57%, 43/75) genes with methylation changes also displayed altered expression in demyelinated compared with intact hippocampi in MS [65]. Amongst the epigenetically dysregulated genes broadly assigned to oligodendrocytes, isoforms of Neurofascin (NFASC), an axo-glial cell adhesion molecule attaching myelin to axons, has been strongly associated with chronic inflammatory demyelinating neuropathies, including MS [66]. The Secreted Frizzled-Related Protein 1 (SFRP1) gene, encoding a Wnt antagonist, has been shown to contribute to oligodendrocyte differentiation and myelin production [67]. H$_2$O$_2$-induced glioma cell apoptosis [68], and amyloidogenic cognitive decline [69], with beneficial or deleterious effect depending on the context. Other demyelination-associated changes, assigned to astrocytes, neurons and microglia, converge to various processes involved in cell adhesion and synaptic connectivity (such as SDK1/2, AJAP1), metabolism and oxidative stress (e.g. AHR, PON1 and CPXMS genes) as well as neuroinflammatory processes (with TGFBI gene, amongst others). Interestingly, some of the identified genes (13%, 10/75) were also found dysregulated in NAWM compared with control WM [23]. Amongst them, genes showing anti-correlated promoter DNA methylation changes and transcript levels, EBPL, PLCH1 and TMEM132B display the same direction of change in both studies, suggesting that some alterations observed in the NAWM of patients with MS might occur prior to myelin damage and persist during demyelination.

Epigenetic changes associated with neuronal vulnerability and neurodegeneration

Epigenetic changes associated with neuronal vulnerability and neurodegeneration

Epigenetic alterations in CREB-mediated neuro-axonal impairment

The vulnerability of neurons/axons residing in inflammatory milieu has important implications for their function and survival and consequently for disease progression. By investigating genome-wide methylation patterns in neuronal nuclei, we showed that 5hmC accounts for a substantial fraction of DNA methylation in neurons and that discriminating 5mC from 5hmC enables the discovery of changes that might otherwise be missed or misinterpreted using conventional bisulphite-based methodology [31]. We observed wide-spread loss of true 5mC and gain of 5hmC in postmortem neurons from patients with MS compared to non-neurological controls. These 5mC changes predominantly occurred in gene bodies and associated with reduced transcriptional activity, likely reflecting poor functional state of MS neurons in the terminal stage of the disease. Interestingly, a subset of cortical neurons particularly vulnerable to degeneration in MS, identified in recent transcriptomic investigation of postmortem single nuclei, displayed downregulation of several genes that we found associated with methylation changes (e.g. ROBO1, GRM6, GRIA4, FARS2 or KCNB2) [70]. As one important role of DNA methylation is to retain the transposable elements silent, decreased 5mC could lead to the release of expression of HERVs, the evolutionarily youngest transposable elements, which has been detected in MS tissues [71, 72]. Although the role of HERVs in MS is primarily ascribed to their ability to induce inflammation [73, 74], methylation-mediated expression of HERVs has been associated with postnatal neurodegeneration [75] and direct cytotoxic effects of HERVs have been implicated in several neurodegenerative diseases [76]. In addition to these global changes, key players in neuronal homeostasis such as axonal guidance (e.g. multiple ephrin/ephrin receptors, semaphorin andplexin genes) and synaptic plasticity (e.g. multiple ion channels
and genes regulating GABA/glutamate activity) displayed methylation changes in MS neurons compared with neurons from control individuals.

MS neuronal methylome changes converged on the cAMP response element-binding (CREB) signalling pathway that was highly enriched with multiple members exhibiting methylation changes [31]. Moreover, several genes in CREB-signalling demonstrated altered DNA methylation and expression in the bulk brain MS tissue [23, 77]. In line with this, neurons exhibiting particular vulnerability to degeneration in MS brain display drastic and specific downregulation of a plethora of CREB-related genes (amongst them CREB2L3 and multiple CAMKs) [70]. Functional investigation by immunostaining of the active form of CREB, that is phosphorylated CREB, revealed considerably reduced numbers of neurons with active CREB in NAWM from patients with MS compared to WM of controls [31]. Significantly decreased phosphorylated CREB, but not total CREB, was also found in demyelinated MS hippocampi [65]. The CREB factor has important functions in controlling neurogenesis and neurodegeneration [78–81], including genes involved in metabolism, cell survival and growth factors, and dysregulation of this pathway has been implicated in many neuropathological conditions [82]. In line with this, studies of chromatin occupancy have revealed a large number of genes with critical roles in neuronal development, synaptic plasticity and neuronal survival to be regulated by the CREB binding [83, 84]. Signalling pathways downstream ion channels and receptors for growth factors and neurotransmitters converge on CREB through several CREB-activating protein kinases; thus, lower CREB-signalling in MS neurons may indicate decreased cellular activity, which agrees with the proposed reduced transcriptional activity [31]. The BDNF is an important neurotrophic and pro-survival factor that increases CREB activation [85], whilst phosphorylated CREB in turn binds to a promoter of BDNF and regulates its transcription thereby controlling neurotrophic responses [86]. Accordingly, neuroprotective effects of BDNF in an inflammatory environment are mediated by CREB [87]. Patients with MS exhibit overall lower levels of BDNF [88], and BDNF molecule has been found around MS lesions [89]. Expression of BDNF is regulated by promoter methylation and its interaction with transcriptional repressor, MeCP2, which recruits HDACs to the locus [90, 91]. Although changes in BDNF methylation or expression have not been reported in the brain tissue [23, 26, 30, 65, 77], methylation and expression changes in another BDNF regulator, BDNF-antisense long noncoding RNA (BDNF-AS), have been found in susceptible neurons of patients with MS [31, 70] and methylation of BDNF gene has been suggested as a potential prognostic factor of disease progression [92]. Collectively, these data suggest the importance of interplay between epigenetic mechanisms in controlling neuronal function and survival.

**Methionine metabolism-dependent epigenetic alterations and mitochondrial defect**

Defects in neuro-axonal metabolism, reflected by reduced levels of N-acetylaspartate (NAA), have been suggested to precede neurodegeneration in MS [93–95]. Changes in the methionine cycle metabolites can influence not only mitochondrial and metabolic processes but also histone and DNA methylation through perturbation of histone and DNA methyltransferase activity in the CNS [96]. Decreased circulating plasma methionine observed in early MS patients has been further proposed as a biomarker of impaired methionine metabolism [96]. Investigation of cortical grey matter revealed reduced levels of methionine metabolites, including the methyl donors S-adenosylmethionine (SAM) and betaine, in MS compared with controls [97]. This is consistent with the genome-wide decrease in true DNA methylation observed in WM neurons from patients compared with neurons from control subjects [31]. Loss of methionine metabolite was associated with decreased levels of the active mark H3K4me3 and impaired axonal metabolic function and mitochondrial respiration, reflected by lower NAA and downregulation of the NDUSF4 gene in neurons. In vitro treatment of neuroblastoma cells could recapitulate the phenotype observed in post-mortem brain and cell exposure to betaine could rescue mitochondrial defect. This effect was further linked to preferential binding of H3K4me3 to genes involved in cellular and mitochondrial metabolism and transcriptional regulation of some of them [97]. Thus, this study highlights a functional link between B12-dependent methionine metabolism and mitochondrial defect in MS.

Interestingly, in addition to the established neurotoxicity of NAA [98], the inability for oligodendrocytes to catabolize neuronal NAA has been associated with compromised myelin lipid synthesis seen in patients with paediatric leukodystrophy
such as Canavan disease [99]. In line with this, levels of sphingomyelin have been found to positively correlate with NAA levels in NAWM of patients with MS [100]. Functional exploration in cultured mouse primary oligodendrocytes showed that neuronal-derived NAA regulates oligodendrocyte differentiation and myelin lipid synthesis potentially through changes in histone methylation [100]. NAA is synthetized and catabolized by key enzymes encoded by NAT8L and ASPA genes, respectively, with defective NAA degradation caused by mutation in ASPA gene leading to Canavan disease. Epigenome-wide profiling studies in the NAWM or demyelinated hippocampus in MS did not report dysregulated NAT8L or ASPA genes. However, methylene profiling in neurons isolated from the WM has detected hypomethylated CpGs in the promoter of ASPA gene in patients with MS compared to controls [31]. The possibility of upregulated ASPA gene in neurons appears surpassing as ASPA expression and subsequent NAA breakdown supposedly occur primarily in oligodendrocytes and astrocytes. However, given the strong interconnection between glutamate and NAA enzymatic cascades, one can speculate that neuronal ASPA regulation could derive from disrupted glutamate homeostasis.

**Shared functional pathways between blood and brain in progressive MS patients**

DNA methylation studies in peripheral blood immune cells of progressive patients are scarce, with to date only one genome-wide study that included SPMS patients [28]. By using the novel analytical nonparametric combination framework (omicsNPC), we have recently reported common methylation changes between patients and healthy controls occurring in four immune cell types sorted from peripheral blood. Remarkably, shared DNA methylation patterns across immune cell types classified methylation changes specific for progressive MS stage (hereafter referred to as the SPMS cluster), comprising an unexpected signature of neuronal and neurodegenerative genes, compared with methylation changes occurring in patients with MS in general (the MS cluster) [28]. Although there are no other studies with SPMS patients that could provide replication, the enrichment of neuronal pathways could be validated in whole blood methylene signatures of SPMS patients from an independent cohort [28]. The aim of this section was to gain more insights into this overlapping pattern and to provide possible explanations and putative underlying mechanisms.

**Overview of the shared changes between the immune and nervous compartments**

To elucidate the extent of the neuronal signature in peripheral immune cells, we compared findings from all three aforementioned genome-wide DNA methylation studies in the CNS, that is bulk NAWM, demyelinated hippocampi and sorted neuronal nuclei, with peripheral immune cells.

An exploratory overview of the affected genes confirmed overlapping changes between nervous and immune cells, with ~28% (136/491) of the annotated genes harbouring methylation changes in the SPMS cluster of immune cells found significantly altered in either neurons or bulk brain tissue (Fig. 3a). A similar proportion, that is 25% (121/476), of overlapping genes could be found between the MS cluster in immune cells and CNS samples. This contrasts with the relatively low number of common affected CpGs (<3%) between the immune and nervous compartments, indicating that differences affect distinct CpGs from the same gene. Unsurprisingly, the most prevailing changes are shared between neuronal and brain tissue. Interestingly, despite the comparable number of overlapping genes between SPMS cluster or MS cluster with CNS cells, gene ontology analysis revealed enrichment of distinct biological functions. Overlapping genes between the SPMS cluster of immune cells and CNS cells are primarily involved in nervous processes such as axonal guidance and synaptic transmission, as well as chromatin remodelling (Fig. 3a). On the other hand, biological functions associated with overlapping genes between the MS cluster of immune cells and CNS cells relate to general functions such as enzymatic activity, metabolism and adhesion. Genes that are shared between the SPMS immune cluster and nervous cells (listed in Table 2) include, amongst others, genes encoding members of glutamate receptor complex (GRIN1, SYNGAP1), cytoskeleton molecules (ANK1, VAV2), downstream signalling molecules such as protein kinases (PRKCs), mediators of Shh/Notch signalling pathways (GLI3, NOTCH4, CUX1) and chromatin/transcription regulators (WHSC1, SMYD3, KDM4B, H2AJ, EIF2C2/AGO2). To further decipher the common signature in the immune and CNS compartments, we examined the directionality of
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Fig. 3  Shared methylome signature from blood and brain cells of progressive multiple sclerosis patients. (a) Top panel: Venn diagrams showing the number of differentially methylated genes overlapping between sorted blood immune cells (grey of the secondary progressive multiple sclerosis (SPMS) (left) or MS (right) cluster, and CNS samples: white matter (WM) neurons 5mC (red), WM neurons BS (top 1000 DMPs from meta-analysis, blue), WM bulk brain (green) and demyelinated hippocampus (yellow). Lower panel: top Gene Ontology terms associated with overlapping genes between SPMS cluster (left) or MS cluster (right) from immune cells and different CNS samples. (b) Heat map of the differentially methylated positions found significant in both immune cells and CNS samples, blue and red shades represent hypomethylation and hypermethylation, respectively, in SPMS patients compared with respective controls. The methylome studies are referenced 1 to 4 as follows: immune cells [28], WM neuron [31], WM bulk brain [23] and demyelinated hippocampus [30]. (c) Example of putative genetically dependent methylation at cg26034531 (LPPR5), with from top to bottom: genomic annotations of the region harbouring cg26034531, with predicted DNase hypersensitivity, transcription factor binding (TF), CpG island (CGI) and chromatin state (only two cell lines depicted); heat map of the methylation Quantitative Trait Locus (meQTL) found significant in both immune cells and brain samples, with blue and red shades representing decreased and increased methylation, respectively, in carriers of the genetic variation; gene expression of LPPR5 transcript across tissues and nervous cell types; expression QTL (eQTL) effect found significant for genetic variations driving meQTL effect, with a forest plot representing normalized effect size (NES). Data were generated using ENCODE/Broad (from UCSC), Blueprint DCC, GTex, Brain xQTL Serve and Brain RNA-Seq portals.

shared affected CpGs between CNS cells and CD8+ and CD4+ T cells, CD14+ monocytes and CD19+ B cells (Fig. 3b). Overall, similar variation can be observed in the CNS and at least one immune cell type, particularly CD14+ monocytes. Same direction of changes were observed for all cell types at 8 CpGs, including 5 annotated genes, that is UBE2E2, LPPR5, JMY, PCSK6 and HLA-DMB genes. Opposite pattern of changes was evident at CpGs in GRIN1, TRPV3, GPC6 and EPHA10 genes, all but GPC6 were found hypermethylated in neurons and vice versa in all peripheral immune cells. Thus, comparison of the shared changes between peripheral immune cells and the brain tissue uncovers coinciding alterations of genes implicated in neuronal processes.

Possible mechanisms underlying correspondence of epigenetic signatures between blood and brain

The apparent incongruity of the overlapping patterns between peripheral blood immune cells and the brain tissue of progressive MS patients challenges our categorical thinking of seemingly self-evident intrinsic features displayed by each tissue and cell type. Yet, such phenomenon has been observed in the context of other CNS-related disorders such as Parkinson’s disease [101], schizophrenia [102], traumatic brain injury [103] and stroke [104]. This further raises several hypotheses on the processes underlying such occurrence. One interpretation is that a brain imprint on immune cells occurs when cells are trafficking through the CNS, as observed for regulatory T cells in the case of stroke [104], or when immune cells engage into cell-to-cell contact with the target cells, such as during phagocytosis. The latter hypothesis is supported by recent single-nuclei transcriptomics of the MS brain demonstrating upregulation of OL-specific genes (i.e. MBP, PLP1, ST18), caused by ingestion of myelin-related mRNAs during phagocytosis, together with downregulation of genes involved in neuronal maintenance in myelin-phagocytosing microglia [70]. Recent evidence of reciprocal mechanisms, where nervous cells endorse typical immune gene expression, has been reported in the instance of HLA class II expression by astrocytes, oligodendrocytes and neural stem cells in the context of neuroinflammation [105–107].

Another, not mutually exclusive, explanation implies the influence of an internal and/or external factor imprinting similar epigenetic signature in multiple tissues, with functional consequences being dependent on the affected tissue. Such putative factors are explored in the rest of this section.

Genetic dependency

Shared methylation signature could reflect genetic influence known as methylation quantitative trait locus (meQTL). Akin to the concept of eQTL effect dictating gene expression, meQTL refers to the impact exerted by a specific genetic variant on DNA methylation levels. Putative genetic control of methylation in progressive MS patient is exemplified in Fig. 3c with cg26034531 located in the phospholipid phosphatase related 5 (LPPR5, newly annotated PLPR5) gene, which displays similar hypermethylation in neurons and immune cells (Fig. 3b). The gene region harbouring cg26034531 exhibits promotor-like regulatory features, implying that changes at this CpG might affect
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Methylome studies:
- neuron projection morphogenesis
- transcription regulatory region DNA binding
- regulation of synaptic plasticity
- regulation of neuron differentiation
- Schaffer collateral - CA1 synapse
- positive regulation of excitatory postsynaptic potential catalytic activity acting on a protein
- magnesium ion binding
- cell projection part
- actin cytoskeleton reorganization
- Wnt signalling pathway planar cell polarity pathway
- cellular response to temperature stimulus

Genomic annotation
- chr1 (Mb): 99,469
- LOC100129620
- GO1
- H1-hESC
- HUVEC

meQTL effect
- Brain
- Monocytes
- T cells
- Neutrophils
- eQTLs

Gene expression
- Neurons
- Oligodendrocytes
- Mature astrocytes
- Endothelial cells
- Microglia/macrophages

eQTL effect
- SNP
- NES
- P-value
- eQTL NES (with 95% CI)

Study
- SP versus NNC
- SP versus HC
- SP versus RR

CNS
- SP versus HC
- SP versus RR

CD14
- SP versus HC
- SP versus RR

CD19
- SP versus HC
- SP versus RR

CD8
- SP versus HC
- SP versus RR

CD4
- SP versus HC
- SP versus RR

Methylome studies:
- Immune cells
- WM neuron (SmC)
- WM neuron (BS)
- WM bulk (BS)
- Hippocampus bulk (BS)

Transmembrane receptor protein phosphatase activity
- enzyme binding
- regulation of biological quality
- plasma membrane region
- whole membrane
- Aryl hydrocarbon receptor signalling
- negative regulation of metabolic process
- RNA7-methylguanosine cap binding
- Notch binding
- calcium-independent cell-matrix adhesion

Neuron 5mCNeuron meta-analysis (BS)Bulk (BS)
transcriptional activity of the LPPR5 gene. Moreover, methylation of this CpG is under the control of genetic variants with similar meQTL effect in the brain and in three blood immune cell types [108, 109]. Characterization of LPPR5 expression across tissues and cell types (using GTex and Brain RNAseq databases) indicates that LPPR5 is constitutively expressed in the CNS, particularly in neurons, where it has been shown to promote spine formation during dendritogenesis [110]. Interestingly, out of this 13 SNPs displaying trans-tissue meQTLs, 4 SNPs located upstream LPPR5 and in linkage disequilibrium ($r^2 > 0.4$) exert eQTL effect as well, affecting the levels of expression of one gene only. Notably, the eQTL effect is not only gene-specific but also tissue-specific as these variants predispose for low levels of LPPR5 expression in nerve tissue (Fig. 3c). Occurrence of meQTL and eQTL effect in nervous tissue suggests co-regulation of DNA methylation and expression, a pervasive phenomenon in the human genome [111]. It is thus plausible that trans-tissue meQTL operating primarily in the nervous compartment may be detected in immune cells. However, meQTLs alone cannot account for shared signatures observed in progressive stages that are largely absent from the early disease stage, unless such meQTLs are dependent on triggers occurring later in life, for example meQTLs changing across lifespan [112].

Beyond meQTL effects, one can speculate that yet unknown genetic predisposition to brain vulnerability in MS and by extension to disease progression, as opposed to immune-related triggers, might be responsible for the observed trans-tissue effect. A new paradigm shedding light on putative distinct genetic predisposition for susceptibility, comprising predominantly immune mechanisms, versus disease outcomes, comprising predominantly target tissue mechanisms, would reconcile the ‘outside-in’ versus ‘inside-out’ debate on the mechanism underpinning MS. Nevertheless, disease outcomes are notoriously challenging to model in MS and attempt to define genetic predisposition to MS severity [113], despite some but conflicting evidence from familial studies, has so far been negative [4, 114]. Future genetic and functional (epi)genomic studies together with functional investigation are required to further aid in

### Table 2. Differentially methylated genes overlapping between immune cells and brain tissue

| Study and Ref. | Shared genes |
|----------------|--------------|
| Neurons (5mC) [31] | WHSC1*, PRDM16*, PRKCZ*, ATP11A*, PTPRN2*, PRKAR1B*, ABR, SBN02, RPS6KA2, PDE6B, MAD1L1, TDP1, KIAA1026, SEC14L1, RNF39, PEL13, C7orf50, SH2D4B, KIRREL3, KCNK4, NOTCH4, ADAMTS10AGAP1, TBCC, SYNGAP1, EML1NL1, KDM4B, SLC9A3, SORCS2, GALNT9, CALB2, LRIG1, SNX9, FAM19A5, SLC22A3, CYP4F22, LRP1, PTPRF, B4GALT3, GPI, SLC35D1, NDUFA7, BUD13, SPSB4, C20orf16, HCN4, B3GNTL1, C12orf56, NRBF2, CTNN, BAIAP2, AFF3, TNIK, CMIP, ZBTB22, ALDH4A1, SCD5, HOXD4, LTB, C10orf14, VWF, ERCC3, MARCH2, WIPF2, RIN3, SPOCK2, RPA1, MICALCL, CYP2W1, ZNF205, C5orf21, TP73, GPR133, NCCRP1, ZFPM1, RBM20, C7orf20, ANKRD11, LOC14581GLI3, VAV2, PRIC285, GAR1, RHPN1, H2AFJ, MYOM2, GRIN1, NNTN1, IQSEC1, ANK1, TRIM71, TMEM201, CDC42BPP, DMRTB1, CDNF, CUX1, TBC1D9, USP44, ODZ4, ITPR3, HLA-DMB |
| Neurons (meta-analysis) [31] | WHSC1*, PRDM16*, PRKCZ*, ATP11A*, PTPRN2*, PRKAR1B*, ABR, SBN02, RPS6KA2, PDE6B, MAD1L1, TDP1, KIAA1026, PCSK6, ITPK1, ADAMTS2, EIF2C2, SCMH1, ROR1, RBM11, SLC7A9 |
| Bulk NAWM [23] | WHSC1*, PRDM16*, PRKCZ*, ATP11A*, PTPRN2*, PRKAR1B*, ABR, SBN02, RPS6KA2, PDE6B, MAD1L1, TDP1, KIAA1026, PCSK6, SEC14L1, RNF39, PEL13, C7orf50, SH2D4B, KIRREL3, KCNK4, NOTCH4, ADAMTS10AGAP1, TBCC, AGPAT1, TNNT3, C9orf139, ABCF2, ZNF414, TRPM2, MBP, RBP3, MT2A, HLA-DMB |
| Bulk hippocampus [30] | SMYD3, LOC10029, EIF2C2, TRPS1, KIAA1026 |

5mC, DNA methylation; NAWM, normal-appearing white matter; and Ref., reference. *overlapping genes that are shared between all studies comparing Multiple Sclerosis patients to non-neurological controls.
deciphering the precise causal variants that influence the severity and progression of MS disease.

**Exposure to smoking**

Both active and passive smoking have been associated with the risk of developing MS that is considerably enhanced by the interaction with the major genetic MS risk factors [115, 116]. Importantly, smoke exposure not only contributes to the susceptibility but also impacts disease progression and neurological disability [117]. The current hypothesis supports the mechanisms underlying smoke exposure to rely on lung irritation and inflammation subsequently promoting peripheral immune deregulation. The impact of smoking is however reversible since risk decreases after 5 years of cessation and returns to the levels of nonsmokers a decade after discontinuance [118]. The effect of smoking is likely mediated by epigenetic mechanisms such as DNA methylation, as established in healthy population [119, 120]. We tested this assumption in patients with MS by examining blood DNA methylation in smokers and nonsmokers [24]. Most of the changes occurred between patients sampled within 5 years from cessation (including current smokers) and those who never smoked and the majority overlapped well-known smoking-related CpGs exhibiting hypomethylation. Differential methylation at the aryl-hydrocarbon receptor repressor (AHRR) gene correlated with higher AHRR transcript levels in PBMCs of MS smokers compared with never-smokers. Globally, smoking-induced changes in methylation were enriched, amongst multiple other functions, with nervous system development and neurological system processes and diseases enriched for smoking CpGs associated with GWAS hits included neurodevelopmental and neurodegenerative diseases [119]. This suggests an association of smoking-induced methylation changes with neurodegenerative processes. Indeed, chronic and second-hand smoking associates with neurocognitive deficits [121] and smoking-induced methylation patterns were found to predict poor cognitive functioning, structural brain integrity and physiological health in older adults [122]. Markedly, whilst there is considerable sharing of smoking-induced methylation changes across different tissues, expression changes were predominantly tissue-specific [123]. This could explain neuronal/neurodegeneration-related functionally overlapping epigenetic signatures between blood immune cells and brain tissues. Interestingly, the intensity of smoking-induced hypomethylation in patients with MS was significantly more pronounced compared with that in matched healthy controls [24]. Thus, it is conceivable that smoking enhances the vulnerability of already-primed cells in MS, partially via epigenetic signatures measurable across multiple tissues, and that such a pervasive deleterious effect contributes to MS progression.

**Inflammaging**

Mounting evidence suggests that accumulation of irreversible disability might be age-dependent. Indeed, age considerably increases the risk of MS progression [124], regardless of initial disease course [125, 126]. Moreover, ageing-related changes, such as age-dependent decline of functional brain recovery capacities [127, 128] and remyelination failure [129], have been reported in the patients with MS. Ageing is defined as a complex and integrated progressive degeneration at a cellular and tissue level, decay of the immune system is further known as immunosenescence. Key features of cellular senescence refer not only to the inability to maintain homeostasis but also to mitochondrial dysfunction, uncontrolled oxidative stress, DNA damage and secretion of pro-inflammatory mediators, amongst others. Whilst cell debris and/or altered endogenous molecules from damaged or dying cells increase with age, their clearance by macrophages/microglia progressively declines concomitant with immunosenescence, further leading to a pro-inflammatory imbalance both locally and systemically. In the early 2000s, the term ‘inflammaging’ has been coined to describe the chronic low-grade inflammation as one mechanism of ageing [130, 131]. The persistence of ageing-related inflammation has been shown to favour susceptibility to age-related diseases [132]. The parallel between progressive MS disease and inflammaging is striking, both phenomena appearing closely interlaced [133]. In line with this, transcriptional dysregulation induced by ageing reveals upregulation of immune-related genes and downregulation of neural-specific genes in the brain [134]. Similarly, age-associated changes in DNA methylation highlight pathways involved in nervous system development, neurogenesis and Neuron differentiation amongst the top enriched terms in adults [135] and neuronal functions in children [136]. Presumably, this tandem inflammaging-neurodegeneration might strongly contribute, simultaneously or sequentially, to both age acceleration and MS-related disease progression. Thus, appearance of shared epigenetic signatures between the blood and brain might
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indicate accelerated ageing in progressive MS, which in turn exerts its neurodegenerative impact on the brain tissue.

Indeed, using the PhenoAge ‘epigenetic clock’, we have recently demonstrated that patients with MS exert higher age acceleration than healthy matched controls [137]. In general ‘epigenetic clocks’, based on blood DNA methylation, are considered amongst the most promising biomarkers of biological age that also correlate with the risk of developing age-related pathologies [138]. The PhenoAge can predict phenotypic age that can be used to evaluate individual’s risk for healthy ageing and ageing-related outcomes such as all-cause mortality, physical functioning or neurodegeneration [139]. Whilst smoking and BMI, known to influence biological ageing and associate with accelerated PhenoAge, also displayed association with age acceleration, the association with MS was independent of these factors [137]. Similarly, following the inflammaging hypothesis in MS, it would be of great interest to explore cellular age acceleration in the target tissue as well. Recent evidence from cross-sectional and longitudinal neuroimaging studies has suggested accelerated brain ageing in patients with MS compared to healthy controls, the higher rate of brain ageing was conditioned to brain atrophy and white matter lesion load [140]. Shorter telomere length, an estimate of biological ageing, in peripheral leucocytes has been shown to associate to decreased brain volume and increased disability over time in MS [141]. Therefore, tackling the intricate relationship between inflammaging and MS progression might open new horizons in the care of patients with MS, as attempts to reverse biological ageing and immunosenescence are starting to emerge [142].

The hypotheses drawn above are neither exhaustive nor exclusive and might jointly coordinate the changes observed in progressive MS patients. They highlight the need for additional studies to link these alterations to perturbed cellular homeostasis underlying MS progression. Nevertheless, the existence of shared epigenetic changes across multiple tissues opens possibilities for studying common-source mechanisms of progression using readily accessible tissues such as blood.

Clinical perspective

Recent technical and analytical advances in the field of epigenetics opened possibilities for deeper understanding of molecular mechanisms underpinning processes occurring during disease progression. Epigenetic studies have already highlighted the importance of the ‘usual suspects’ such as oligodendrocyte death and impairment in their myelinating capacity, changes in the composition of myelin sheaths, mitochondrial energy dysfunction of neurons, axonal and synaptic plasticity in MS progression (Fig. 4). They have also suggested pathways and molecules that may serve as targets for putative reparative and neuroprotective interventions. For example, methylation changes in postmortem neuronal nuclei highlighted impairment of CREB-signalling in MS neurons [31]. Interestingly, RRMS-approved drug, fingolimod, which is a sphingosine 1-phosphate (S1P) receptor modulator, has been demonstrated to promote neuroprotection in experimental models of neurodevelopmental and neurodegenerative diseases through mechanisms often associated with the increase in CREB-signalling [143–145] and BDNF [143, 146]. A potential neuroprotective role of fingolimod in MS has been debatable, not the least due to the challenge of disentangling immunomodulatory vs. neuroprotective effects in the complex chronic neuroinflammatory context and to a failure of the randomized clinical trial in PPMS [147]. Nevertheless, another S1P receptor modulator, that is siponimod, has recently been approved as a first drug demonstrating efficacy in SPMS [148]. Other therapies that are currently investigated in progressive MS [11], such as high-dose biotin and Fluoxetine, believed to reduce axonal degeneration by stimulating energy metabolism, might also act at least in part via the CREB pathway. On the other hand, clemastine is a histamine H1-receptor antagonist discovered to enhance OL differentiation and remyelination using a high-throughput micropillar array screening platform [149]. Two monoclonal antibodies, opicinumab and GNBAC1, have been found to promote OL differentiation by blocking LINGO-1 [150] and the HERV envelope protein [151], respectively. Thus, a number of drugs that are being tested in progressive MS exert putative protective effect by boosting OL differentiation and myelin production [11, 152]; the two other features found dysregulated in MS on the epigenetic level.

In addition to conventional pharmacological medicine, the reversible nature of epigenetic marks portrays epigenetic therapy as a complementary strategy for the care of progressive MS patients. As previously discussed, diverse epigenetic modifiers, such as HDAC inhibitors have been utilized in
preclinical studies with efficacy linked to different aspects of MS-like neuropathology. However, the use of such global epigenetic agents for clinical purpose is rather limited, given the lack of specificity and side effects elicited by broad manipulation of the epigenome. In that regard, novel approaches based on targeted-epigenetic therapy offer the possibility to manipulate epigenetic modifications in a locus-specific manner, without affecting other marks that are essential for proper cell homeostasis. In that respect, recent adaptations of the clustered regulatory interspaced short palindromic repeats (CRISPR)-Cas9 system for epigenome editing may provide an unprecedented molecular platform for remodelling of the chromatin into a transcriptionally permissive or repressive state in an inducible, spatially restricted and durable manner [153, 154]. The CRISPR-dCas9 epimodifier system exploits the concerted action of the catalytic domain of an epigenetic enzyme (targeting DNA methylation or histone PTMs) fused to a deactivated Cas9 endonuclease (dCas9) and the spatial guidance of a programmable single guide RNA. Preclinical investigation supports efficient targeting of neuronal genes, such as BDNF and Klotho found differentially methylated in neurons of patients with MS, in vivo and in vitro [155–157]. Overall, growing body of evidence suggests that, despite current challenges, this versatile system might open previously unmet opportunities to shape long-lasting cellular response. Yet, the clinical applications of the dCas9 tool remain to be fully ascertained, as concerns persist with regards to toxicity and safety, with possible off-target and cross-tissue effects impeding its current use in the context of complex diseases of the CNS. Along with the rapid development of the CRISPR-dCas9 toolkit, emerging delivery approaches, for example nanocarriers, might provide additional tools for tailored tissue specificity targeting.

Conclusions

The complex and unpredictable disease progression leaves MS patients with irreversible gradual disability, and treating progressive MS patients represents one of the most urgent clinical needs. Accumulating evidence from epigenetic studies of progressive MS patients, discussed in this review, advocate for a pivotal role of mechanisms such as impaired myelination/remyelination and sustained neuro-axonal damage in driving disease progression (Fig. 4). Moreover, we addressed the potential to detect some of the functional epigenomic alterations occurring in the CNS of progressive MS patients using easily accessible tissues such as blood. Comparison of epigenetic patterns in immune and CNS cells from patients with MS suggests shared functional imprints converging on genes related to neuronal processes. We further hypothesize that this overlapping pattern likely results from the effect of internal determinants such as genetic variants as well as the influence of external factors such as smoking and ageing. Thus, epigenetic studies provide an additional avenue for the identification of key molecular and cellular processes that can be exploited for future therapeutic interventions.

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Conflict of interest

Authors declare that they have no conflict of interest.
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