Multiple sclerosis is linked to MAPK<sub>ERK</sub> overactivity in microglia

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Abstract
Reassessment of published observations in patients with multiple sclerosis (MS) suggests a microglial malfunction due to inappropriate (over)activity of the mitogen-activated protein kinase pathway ERK (MAPK<sub>ERK</sub>). These observations regard biochemistry as well as epigenetics, and all indicate involvement of this pathway. Recent preclinical research on neurodegeneration already pointed towards a role of MAPK pathways, in particular MAPK<sub>ERK</sub>. This is important as microglia with overactive MAPK have been identified to disturb local oligodendrocytes which can lead to locoregional demyelination, hallmark of MS. This constitutes a new concept on pathophysiology of MS, besides the prevailing view, i.e., autoimmunity. Acknowledged risk factors for MS, such as EBV infection, hypovitaminosis D, and smoking, all downregulate MAPK<sub>ERK</sub> negative feedback phosphatases that normally regulate MAPK<sub>ERK</sub> activity. Consequently, these factors may contribute to inappropriate MAPK<sub>ERK</sub> overactivity, and thereby to neurodegeneration. Also, MAPK<sub>ERK</sub> overactivity in microglia, as a factor in the pathophysiology of MS, could explain ongoing neurodegeneration in MS patients despite optimized immunosuppressive or immunomodulatory treatment. Currently, for these patients with progressive disease, no effective treatment exists. In such refractory MS, targeting the cause of overactive MAPK<sub>ERK</sub> in microglia merits further investigation as this phenomenon may imply a novel treatment approach.

Keywords MAPK<sub>ERK</sub> · Multiple sclerosis · DUSP6 · LMP-1 · Microglia · Demyelination

Introduction
More than 160 years after Jean-Martin Charcot’s description of MS, the pathophysiology of this neurodegenerative disease is still rather enigmatic. The paradigm most adhered to is that MS is caused by autoimmune reactant against central nervous system (CNS)-antigens. This concept is supported by clinical benefits of immune suppression and immunomodulation, and these approaches represent the mainstay of contemporary MS treatment. However, this concept does not explain why many patients eventually deteriorate neurologically despite optimized immunomodulation. Such condition is common in patients with progressive MS, but often this befalls also MS patients that initially responded favorably to immunosuppression but eventually become refractory to this approach. As this shortcoming of treatment of refractory MS constitutes a remarkable dichotomy in the disease (effectively treatable MS versus refractory MS), this contrast propels the quest for further understanding the mechanisms behind the disease and, by this, the search for effective treatment with regard to this pathophysiology. In this review, literature that points to overactivity of mitogen-activated protein kinases (MAPK) in MS, in particular MAPK<sub>ERK</sub>, is summarized. It appears that overactivity of MAPK<sub>ERK</sub> in MS microglia can lead to locoregional inflammation within the CNS besides dysfunction of regional oligodendrocytes.

In view of the overall pathogenic complexity, several mechanisms have been considered to contribute to this devastating neurodegenerative disease. The interpretation of data on MS presented here is novel and signifies another mechanism that can explain and unify phenotypic characteristics of MS.
Preclinical indications on involvement of MAPK in neurodegeneration

TAK1 in microglia

In 2013 Goldmann and co-workers demonstrated that microglia-endogenous TGFβ-activated kinase-1 (TAK1) is a key component in the regulation of CNS inflammation [1]. In mice with induced experimental autoimmune encephalopathy (EAE, a model for MS), depleting TAK1 in microglia ameliorated clinical disease manifestations, reduced CNS inflammation, and diminished axonal and myelin damage (see Fig. 1a and b). Depleted TAK1 function in microglia inhibited NF-κB signaling via the mitogen-activated protein kinase (MAPK) pathways JNK, p38, and ERK (MAPK^JNK, MAPK^p38, and MAPK^ERK, respectively). In the context of the pathophysiology of MS, these observations draw attention to these very pathways. This in particular since activation of these pathways in microglia induces cytokine release into the microenvironment that can lead to local inflammation in the CNS [2–4]. This work by Goldmann et al. focused on a role of MAPK pathways in a murine model of MS, EAE.

BRAF^V600E in microglia

Recently, Mass and co-workers showed that the induction of MAPK^ERK pathway overactivity in mouse microglial cells resulted in neurodegeneration [5]. Expression of BRAF^V600E, a mutated form of the gene BRAF, which encodes the oncogenic B-Raf kinase, leads to substantial overactivity of the MAPK^ERK pathway, a phenomenon widely acknowledged in today’s clinical oncology and hemato-oncology practice [6]. Mass et al. investigated neurodegeneration that occurs in the context of neurohistiocytosis, as this disease can harbor BRAF^V600E [7].

Mass et al. introduced this BRAF^V600E expression in erythro-myeloid progenitor cells that give rise to microglia, the tissue-resident macrophages of the CNS. The ensuing modification of MAPK^ERK overexpression in mouse microglia cells within the CNS resulted in late-onset neurodegeneration (see Fig. 2a and b) with progressive hindlimb paresis. Notably, the very induction of MAPK^ERK overexpression in murine microglia also resulted in clinical and histopathological deviations in vivo: amoeboid microglia, GFAP-positive astrogliosis, expression of the PDGFα receptor as well as VLA4 and CD11a, deposition of amyloid precursor protein, and synaptic loss. Phenomena included localized demyelination and, finally, neural death. These histopathological features are identical to those found in human MS lesions. Importantly, Mass et al. observed that treatment of mice with BRAF^V600E-overexpressing microglia with the BRAF^V600E inhibitor PLX4720 mitigated disease progression as well as prevented histopathological aberrations (see Fig. 2c).

The observations in mice illustrate that inducing MAPK^ERK pathway overactivity leads to late-onset progressive paresis and histopathology that resembles MS. These findings gain even more weight by reciprocity: the demonstration that blocking of MAPK^ERK pathway overactivation, by BRAF^V600E inhibition, mitigated the progression of neurodegeneration.

Combination of the reports by Goldmann [1] and Mass [5], both based on a mouse model of neurodegeneration, with the abundant data on biochemical and epigenetic signals in MS in man (refer to Tables 1 and 2), points to a likely pivotal role of...
MAPK<sup>ERK</sup> pathway overactivity in microglia in the neurodegenerative pathology of MS.

### Association of MAPK overactivity with MS

Separate observations on several biochemical phenomena in MS hint at the involvement of, in particular, the MAPK<sup>ERK</sup> pathway. These observations include the Wnt/β-catenin pathway, sphingosine 1-phosphate (S1P), mitogen- and stress-activated kinase-1 (MSK1), melanocortin 1 receptor (MC1r), microphthalmia-associated transcription factor (MITF), carbamoyl-phosphate synthetase (CAD), and vascular cell adhesion molecule 1 (VCAM1). All are mechanistically linked to MAPK<sup>ERK</sup> as well as to MS. These and several other associations between MS and the MAPK<sup>ERK</sup> pathway are described concisely in Table 1. The attenuation of MS disease after inhibition of the MAPK<sup>ERK</sup> pathway and associated factors can be considered to affirm the involvement of this pathway in MS.

Besides, certain microRNAs (miRNA) have been detected in deviant expression levels in patients with MS, when compared to non-MS individuals. These miRNAs play a role in controlling of—or responding to—the activation status of the MAPK<sup>ERK</sup> pathway (Table 2). The relationship of these MS-associated miRNAs with MAPK<sup>ERK</sup> activity also suggests a particular relevance of MAPK<sup>ERK</sup> activity in MS.

In short, the similarities between the role of MAPK activity in microglia in causing mouse neurodegeneration and observations in human disease MS pinpoint overactive MAPK<sup>ERK</sup> as primary involved process. In line, the occurrence of MS-associated miRNAs appears associated with MAPK<sup>ERK</sup> activity.

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### From MAPK<sup>ERK</sup> to demyelination, hallmark of MS

There is a direct causal relation between clinical manifestations in MS with the pathological hallmarks (inflammation, neurodegeneration, and demyelination). Therefore, when considering MAPK<sup>ERK</sup> overactivity in microglia as a common thread in MS, a negative influence by these affected microglia on oligodendrocytes regarding myelination substantiates linkage between these cell types. In fact, such crosstalk between microglia and adjacent oligodendrocytes has been reported in 2014 by Peferoen and colleagues [3]. Earlier, it was found that affected microglia have a detrimental effect on adjacent oligodendrocytes [63]. In line, oligodendrocytes appeared particularly susceptible to microglia-emitted factors in reaction to MAPK<sup>ERK</sup> activity [64]. Microglial MAPK<sup>ERK</sup>-induced cytokines include IL-1β and TNF-α [65, 66], and these cytokines damage locoregional oligodendrocytes resulting in hypomyelination. Taken the essential role of oligodendrocytes in the trophic support of axons any insult to oligodendrocytes will also impact on axonal physiology [67, 68].

Together, microglia can influence oligodendrocytes in an unfavorable fashion, and this can explain local demyelination in response to regional MAPK<sup>ERK</sup>-overexpressing microglia. Moreover, MAPK<sup>ERK</sup> activation in microglia has been identified to lead to emission of various pro-inflammatory mediators [69] further contributing to sclerosis of affected tissue within the CNS.

### On MS phenotypes

The individual MS patient’s disease status is usually described as one of four recognized phenotypes [70]. These phenotypes,
or actual course descriptions, are designated clinically isolated syndrome (CIS), relapsing remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS). All phenotypes are defined by several parameters including disease history, actual relapse rate, and disease progression status [71].

One peculiar distinction between these MS phenotypes is the varying benefit from anti-inflammatory and immunomodulatory treatment. While clinically relevant responses to these therapies can be observed in RRMS, in progressive MS phenotypes and/or when there is a longer period of time after diagnosis, these treatment modalities show modest or no beneficial effect anymore. This is a meaningful distinction as it implies that MS neurodegeneration is most probably caused by other factors than influenceable inflammation alone.

It was proposed that the different MS phenotypes may be variations on a central theme [72]. Conceptually, symptoms and pathology of progressive MS are caused by neurodegeneration leading to dysfunctional axon-myelin units, while relapses are due to immune hyper-reactivity against antigens released from degenerating units. Histopathological evidence of MS pathology preceding autoimmune neuropathology includes dysfunctional axon-myelin units [73] and nodules of reactive microglia surrounding degenerating axons [74].

It may thus well be that the neuroinflammation is an effect that is superimposed on or occurs in parallel to the consequences of overactive MAPK<sup>ERK</sup> in microglia. Both mechanisms can be explained by microglia-endogenous enzymes downstream TAK1 [1]. Disturbances of downstream TAK1 can lead to both overactive MAPK<sup>ERK</sup> and overactive MAPK<sup>P38</sup> [75]. Activated MAPK<sup>P38</sup> is classically associated...
miRNA-146a Analysis of miRNA in CSF and active lesions in patients with MS show upregulation of miR-146a and miR-146b. miR-146b expression is regulated via different MAP kinase pathways. miR-146b signaling pathway activation is upregulated as a consequence of SPRY2 due to higher expression of this microRNA.

miRNA-221 miR-221-3p is found in higher levels in blood of MS patients. Its expression may relate to neurogenesis in the context of MAPK ERK signaling. miR-221 is identified to target directly DUSP6. The miR-145 appears up-regulated in MS, in PBMC as well as in MS lesions.

miRNA-145 Dual-specificity phosphatase 6 (DUSP6, or MKP3) is a cytoplasmic phosphatase with high specificity for MAPK ERK. The miR-145 is identified to target directly DUSP6. The miR-145 appears up-regulated in MS, in PBMC as well as in MS lesions. p53 expression is higher in MS lesions, and this p53 can lead to miR-145 upregulation. By this, DUSP6 can be targeted which leads to lower negative feedback on MAPK ERK.

miRNA-30d miR-30d is found enriched in feces of patients with untreated MS. Synthetic miR-30d given orally ameliorates the effects of experimental autoimmune encephalomyelitis (EAE, model of MS) in mice.

miRNA-101 MicroRNA-101 participates in the regulation of MAPKs as it targets MAPK Phosphatase-1 (MKP-1). As negative feedback control enzyme system, MKP-1 also dephosphorylates MAPK ERK besides MAPK S38.

miRNA-145 is identified to target directly DUSP6. miRNA-145 is identified to target directly DUSP6. The miR-145 appears up-regulated in MS, in PBMC as well as in MS lesions. p53 expression is higher in MS lesions, and this p53 can lead to miR-145 upregulation. By this, DUSP6 can be targeted which leads to lower negative feedback on MAPK ERK.

miRNA-146a Analysis of miR-146a and miR-146b shows upregulation in MS. Transcription of miR-146a and miR-146b appears upregulated via different MAP kinase pathways. miR-146b expression is regulated via different MAP kinase pathways. miR-146b signaling pathway activation is upregulated as a consequence of SPRY2 due to higher expression of this microRNA.

miRNA-21 MicroRNA-21 is upregulated in CSF, and also found in brain white matter lesions in patients with MS. Transcription of miR-214a and miR-214b appears upregulated via different MAP kinase pathways. miR-146b signaling pathway activation is upregulated as a consequence of SPRY2 due to higher expression of this microRNA.

Table 2

| Parameter   | Association                                                                 | References |
|-------------|-----------------------------------------------------------------------------|------------|
| miRNA-21    | MicroRNA-21 is upregulated in CSF, and also found in brain white matter lesions in patients with MS. Its expression may relate to neurogenesis in the context of MAPK ERK signaling. | [36–38]   |
| miRNA-30d   | miR-30d is found enriched in feces of patients with untreated MS. Synthetic miR-30d given orally ameliorates the effects of experimental autoimmune encephalomyelitis (EAE, model of MS) in mice. | [39, 40] |
| miRNA-101   | MicroRNA-101 participates in the regulation of MAPKs as it targets MAPK Phosphatase-1 (MKP-1). As negative feedback control enzyme system, MKP-1 also dephosphorylates MAPK ERK besides MAPK S38. | [41–44] |
| miRNA-145   | Dual-specificity phosphatase 6 (DUSP6, or MKP3) is a cytoplasmic phosphatase with high specificity for MAPK ERK. | [45–49] |
|              | The MAPK ERK activity was found to promote an increase in miR-221. Both are linked to MAPK ERK activity. | [50]      |
| miRNA-219   | miRNA-219 is found downregulated in chronic MS lesions. | [55, 56] |
| miRNA-221   | miR-221-3p is found in higher levels in blood of MS patients. Its expression may relate to neurogenesis in the context of neural regulation. | [57, 58] |
|              | The MAPK ERK activity was found to promote an increase in miR-221. |            |
| miRNA-338   | miRNA-338 is downregulated in chronic MS lesions. | [55]      |
|              | This miRNA inhibits the MAPK ERK signaling pathway: when overexpressed in GBM a lower expression of MEK-2 and ERK-1 was observed. | [59]      |
| miRNA-564   | In patients with MS, miRNA-564 has been identified to be downregulated in T-cells (whether any level of this miRNA is lymphocytogenic or whether it originates from intercellular exchange is not analyzed). | [60–62] |

with inflammation [50, 51], and in the CNS, this may cause damage separate from the neurodegeneration caused by overactive MAPK ERK. Such mechanistic diversity in pathogenesis could explain the divergent clinical courses known from MS disease phenotypes [70].

**Causes of MAPK overactivity**

Overactivation of MAPK pathways can be the result of several different mechanisms. Besides activation as result of extracellular receptor tyrosine kinase (RTK)-ligand binding, also intracellular processes can lead to overactivity of this pathway. These include activating mutations in genes encoding proteins that constitute this pathway but with elevated kinase activity. BRAFV600E is one example of such gene mutation-derived protein that leads to substantially higher kinase activity. BRAFV600E is well known for its role in several neoplasms [6]. To date such mutations have not been detected in MS.

MAPK signaling in the cell is controlled by negative feedback systems consisting of dedicated phosphatases (dual-specificity phosphatases (DUSP), also called mitogen-activated protein kinase phosphatases (MKP)). Therefore, an alternative explanation for an overactivated MAPK signaling is failure of this feedback regulation. When these MAPK controlling negative feedback systems fail, MAPK pathway phosphorylation activity becomes uncontrolled, and this results in inadequate higher kinase activity [76]. For instance, miRNA-145 is highly expressed in MS tissue [77, 78]. This miRNA-145 can downregulate DUSP6 [79], and this results in an overactivation of MAPK ERK. Moreover, this overactivation of MAPK ERK in microglia can also be the consequence of other factors related to MS, for instance infection with neurotropic viruses like Epstein Barr virus (EBV). Such infection can result in the pathogenic disturbance of intracellular biochemistry leading to overactivation of MAPK ERK.

**Possible associations of MS risk factors with MAPK ERK overactivity**

Broadly acknowledged risk factors for MS development and progression are low serum vitamin D levels at diagnosis, tobacco smoking, and prior infection with EBV. A common denominator of these factors is that they all negatively affect specific dual-specificity MAP kinase phosphatases (DUSPs).
As these MS risk factors all downregulate DUSPs, this could explain MAPK overactivity.

**Hypovitaminosis D**

Vitamin D supplementation leads to higher DUSP1 levels [80]. As DUSP1 counteracts overactivity of MAPK\(^{P38}\), and to a lesser extent also of MAPK\(^{ERK}\) [80–84], a shortage of vitamin D might result in higher activity of MAPKs, and in particular of MAPK\(^{P38}\). Conversely, as adequate levels of vitamin D facilitate regulation of DUSP1, this can contribute to better controlled MAPK\(^{P38}\) activity and thereby to reduction of MAPK-induced inflammation [85]. Importantly, vitamin D supplementation has been found to consolidate or improve the clinical condition of patients with MS only early after disease onset [86], and this may illustrate that MAPK\(^{P38}\)-related inflammation is clinically relevant primarily in early phases of MS. Refractoriness to immunosuppressive/immunomodulatory measures, observed often in longer existing progressive phenotypes of MS, also reflects MAPK\(^{P38}\) inflammation-independent neurodegeneration in later stages of the disease, as in these patients the neurodegeneration in the context of MS invariably proceeds. The MAPK\(^{P38}\) interference in early stages of MS demonstrates clinical relevance of vitamin D suppletion. In short, hypovitaminosis D seems to diminish activity of DUSP1 in MS, while supplementation of vitamin D shows clinically benefit solely in early stages of MS. These data suggest that in longer existing, progressive stages of MS, other processes are involved that lead to ongoing neurodegeneration. Consequently, in view of the here discussed role of MARK\(^{ERK}\) in MS, also other MAPK phosphatases must be involved, in particular in longer existing or progressive phenotypes of MS, as here classical immune suppression/immune modulation shows infective.

**EBV infection**

Virus infection, in particular infection with Epstein Barr virus (EBV), causes MAPK\(^{ERK}\) overactivity [87], which is probably mediated by downregulation of DUSP6 (MKP-3) and DUSP-8. EBV proteins, including Epstein Barr nuclear antigen-2 (EBNA2) [88] and latent membrane protein-1 (LMP-1) [87], are the most straightforward cause for the MAPK\(^{ERK}\) upregulating properties of EBV. Indeed, EBV infection of microglia can eventually lead to virus latency [89], and the latency-encoded LMP-1 has been proven to substantially downregulate DUSP6 and also DUSP1 [90] resulting in overactivation of MAPK\(^{ERK}\) (see Fig. 3). Furthermore, infection with EBV is presumably needed for reactivation of human endogenous retrovirus (HERV) group K(HML2), to which belongs the in MS encountered HERV-K18 [93]. This HERV-K18 by itself also participates in MAPK\(^{ERK}\) pathway activation effectively [94].

In general, these acknowledged risk factors for MS development and progression can all be related to MAPK induced inflammation. The disappointing efficacy of immunosuppression/immunomodulation in progressive MS may imply that neurodegeneration here is driven by other mechanisms than those sensitive to present day anti-MS medicines that are effective often only temporarily and in earlier stages of the disease.

**Conclusions and future directions**

A prominent early pathological feature of MS, that precedes the autoimmune attack, is the presence of microglia nodules, which are composed of activated microglia centering on a degenerating axon [74]. Although the exact induction mechanism of the nodules is not known, the expression of IL-1\(\beta\) indicates MAPK pathway activation [95]. It has been proposed that a proportion of these nodules stimulate the development of inflammatory pathology that is the pathological hallmark of MS [96]. This publication provides a possible explanation for this early aberrant behavior of microglia that disturbs its normal homeostatic functions.
Reassessment of published data reveals that overactivation of MAPK, in particular MAPK\textsuperscript{ERK}, in microglia is unambiguously linked to MS. We posit that as this mechanism is different from other pro-inflammatory stimuli in microglia (e.g., MAPK\textsuperscript{p38} activation), it can explain that MS patients with progressive phenotypes experience ongoing neurodegeneration despite adequate immunosuppression or immunomodulation. Of note, immunosuppression and immunomodulation do affect pro-inflammatory effects of MAPK\textsuperscript{p38}, but these do not affect the mechanisms of MAPK\textsuperscript{ERK} overactivation in microglia. Consequently, neutralization of MAPK\textsuperscript{ERK} overactivity in microglia may be a feasible approach to halt the negative effect of affected microglia on oligodendrocytes and by this achieve attenuation of MS-associated demyelination. The observation that established risk factors for MS all have been found to down-regulate MAPK activity-controlling phosphatases (DUSPs), a possible pathogenic role of MAPKs, in particular the observed MAPK\textsuperscript{ERK} overactivity, is emphasized.

In view of the fact that the MAPK families constitute indispensable and crucial enzymatic pathways for every cell, inhibition of the MAPK\textsuperscript{ERK} pathway is potentially detrimental. This is illustrated by the vast repertoire of severe adverse events observed after the systemic application of anti-neoplastic medicines developed for the inhibition of MAPK\textsuperscript{ERK} overactive disease (e.g., inhibitors of BRAF\textsuperscript{V600E}, MEK, or KRAS\textsuperscript{G12C}).

Neutralization of MAPK\textsuperscript{ERK} overactivity in MS may be achieved by correcting the activity of DUSPs that can be responsible for insufficient negative feedback of the MAP kinases involved. As MAPK\textsuperscript{ERK} overactivity has been found an effect of the EBV latency-encoded LMP-1 [87], this viral protein should be neutralized in order to abrogate the pathological process with seems current in MS. Indeed, higher expression of this LMP-1 has been observed in the brain of patients with MS [94]. Therefore, LMP-1-targeted siRNA [97], RNAi, or possibly LMP-1-directed CRISPR-cas9 [98] may constitute treatment modalities for MS. Finally, patients with MS that show refractory for any contemporary treatment, for instance those suffering from long term progressive phenotypes, could benefit from such approach, as these patients suffer from neurodegeneration unabatedly.

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Author contribution George ten Bosch designed the study, performed the data search, interpreted the data, and wrote the manuscript. Jolande Bolk participated in the literature search and critically weighed interpretation of data. Bert ‘t Hart and Jon Laman critically revised the work.

Declarations

Competing interests The authors declare no competing interests.

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