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Microglia in motor neuron disease: Signaling evidence from last 10 years

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Abstract

Motor neuron disease (MND), including amyotrophic lateral sclerosis, spinal muscular atrophy and others, involved the upper or lower motor neurons selective loss, is characterized by neurodegeneration and neuroinflammation, in conjunction with microglia. We summarized that pathways and key mediators are associated with microglia, such as fractalkine signaling, purinergic signaling, NF-κB signaling, p38 MAPK signaling, TREM2-APOE signaling, ROCK signaling, C1q signaling, and Ion channel, which are involved in the activation, proliferation, and inflammation of microglia. This review aims to identify the microglia-related molecular target and explore potential treatment strategies for MND based on that target.

KEYWORDS

ALS, microglia, MND, neuroinflammation, signaling

1 | INTRODUCTION

Motor neuron disease (MND) is a neurodegenerative condition that influence muscle function, such as progressive muscle weakness and wasting, body weight loss, fasciculations, emotional lability, and cognitive dysfunction. A notable characteristic of MND is the selective loss of upper or lower motor neurons (MN). The most common MND are spinal muscular atrophy (SMA), progressive muscular atrophy (PMA), and amyotrophic lateral sclerosis (ALS). Previous studies reported an incidence of MND of 5.69/100,000 person-years (Apolloni, Fabbrizio, Amadio, et al., 2016; Burchardt et al., 2022). There are a variety of pathological conditions within MND, which are associated with neuroinflammation (Papadimitriou et al., 2010). Neuroinflammation is a term coined to describe cellular and molecular processes, which encompass activation of microglia and astrocytes and infiltration of peripheral immune cells. In recent imaging and genetic studies, microglia have been reported to drive neuroinflammation and subsequent pathology (Ashford et al., 2021)

Microglia are macrophage-like cells in the central nervous system (CNS), account for 5%−12% of CNS cells, and regulate homeostasis both in the brain and spinal cord (SC) (Vilhardt et al., 2017). Microglia was recognized to divided two states, the M1 (classical activation) phenotype plays a role in pro-inflammation, and M2 (alternative activation) phenotype acts as an anti-inflammatory agent. M1 microglia express pro-inflammatory molecules that include tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ), and nitric oxide (NO), as well as cell surface markers, CD86 and CD68. On the other hand, IL-4 induces M2 polarization. M2 microglia express different molecules, such as IL-4, arignase1, Ym1, CD206, and IL-10 and show neuroprotective effects (Kobayashi et al., 2013). The dynamic and bidirectional transformation of M1/M2 appears to play a crucial role in neurodegeneration (Xuan et al., 2019). Not only phenotype transformation found in MND, but microgliosis is also found when the intense reaction of CNS microglia to pathogenic insults. This shows an increasing number of activated microglia at the site of the lesion (Vilhardt et al., 2017).

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In recent 10 years, growing evidence shows that microglia in SC and the brain are significantly different in cellular phenotype and biological functions. The effects of microglia in neuromuscular disorders had been reported; however, the related mechanism needs to be further investigated.

Known as the most common disease in MND, ALS is a fatal adult-onset neurodegenerative disease, with the prevalence of 6–7/100,000 in Europe (Bonafede & Mariotti, 2017). Symptoms of ALS include muscle weakness, fasciculations (muscle twitching), paralysis, and atrophy, eventually leading to the loss of voluntary movement and death, with a lifetime risk of 1/500 (Kiernan et al., 2011). Neuroinflammation, accumulation of neurofilaments, protein aggregates, oxidative stress, glutamate excitotoxicity, and mitochondrial dysfunction may be involved in ALS (Bonafede & Mariotti, 2017). A recent study found that microglia expressing superoxide dismutase 1 gene (SOD1) participate in the mediation of MN death (Frakes et al., 2014). SOD1 is one of the most important genes which contributes to the development of ALS in the other gene. SOD1 accounts for 15% of familial ALS (fALS) and 1%–2% of sporadic ALS (sALS). For the past 10 years, different mutation carrier SOD1 mice has been used in ALS studies. SOD1 mice model is mainly the $SOD1^{G93A}$ mouse, followed by the G37R, G85R, and G86R. SOD1 promotor and regulatory elements, increased (1700% for $SOD1^{G93A}$) levels as compared to the endogenous mouse SOD1. In addition, G85R expression like human. G37R model has a significant increase in the activity rather than the endogenous mouse SOD1 and the other models (Philips & Rothstein, 2015). Additionally, SMA is another classical disease in MND. It is common in childhood, and often results in death. It is characterized by selective loss of MN within the anterior horns. The formation of glial bundles is another pathological hallmark of SMA, particularly in the anterior roots and lumbar region where MN degeneration occurs. The deficiency of survival motor neuron (SMN), a 38-kDa protein, has recently been reported as one of the causes of SMA. It has been proved that SMN regulates oxidative stress and inflammatory response in microglia (Ando et al., 2020). Additionally, synaptic dysfunction and elimination has been supposed to be the pathogenic mechanism of SMA (Papadimitriou et al., 2010).

Therefore, the current review summarizes the signaling and mediators related microglia involved in MND. The current review also contributes to better understanding of the specific role of microglia in MND and provides update potential treatment of neuromuscular disorders.

2 | TREM2 SIGNALING POSSIBLY INVOLVES IN MICROGLIAL NEUROINFLAMMATORY RESPONSE DURING MND

Triggering receptor expressed on myeloid cells 2 (TREM2), a membrane-bound protein of the immune system, is expressed exclusively by microglia in the CNS (Xie, Liu, et al., 2022).

It is well known that potential ligands for TREM2 include lipids, apolipoprotein E (APOE) and $A\beta$[5, 6, 45]. Yet, recent studies in vitro found that extracellular human TDP-43 (hTDP-43) was detected in iPSC neurons, indicating that TDP-43 may serve as a potential TREM2 ligand. TREM2 directly interacts with TDP-43 to activate TREM2 signaling (Xie, Zhao, et al., 2022). Upon binding to TDP-34, TREM2 transduces downstream signaling through its adapter protein DNAX activating protein of 12 kDa (DAP12) and TYRO protein kinase-binding protein (TYROBP). Phosphorylated DAP12 recruits spleen tyrosine kinase (SYK) to activate its downstream signaling molecules, including mitogen-activated protein kinase (MAPK) and others (Xie, Zhao, et al., 2022).

Recent studies found that TREM2 interaction with TDP-43 affects microglial molecular expressions. TREM2 is known to stimulate myeloid activation and phagocytosis, inhibits the transcription of pro-inflammatory cytokines, and promotes microglial survival, proliferation, migration, and phagocytosis (Ashford et al., 2021; Cooper-Knock et al., 2017; Xie, Liu, et al., 2022). Aggravating CNS inflammation is a typical feature in the pathogenic mechanism of ALS. Both in vitro and in vivo studies indicated an anti-inflammatory role for TREM2. In vitro knockdown of TREM2 impairs IL-4-induced anti-inflammatory response of primary microglia and enhances the expression of pro-inflammatory mediators including iNOS, TNF-α, IL-1β, and IL-6 following lipopolysaccharide (LPS) treatment (Xie, Zhao, et al., 2022). TREM2 regulates inflammatory responses, which may provide a treatment option for ALS. However, it is still under debate whether TREM2 variants are risk factors for ALS. A previous study with a large population showed that TREM2 variant P. R47H was more common within patients with ALS, which is thought to be a critical risk factor for ALS. In addition, TREM2 expression was higher in spinal cord samples from patients with ALS and in $SOD1^{G93A}$ mice, supporting the conclusion that TREM2 is dysregulated in the disease (Cady et al., 2014). Notably, all these studies focused on TREM2 variant R47H, while other variants are largely understudied (Krasemann et al., 2017; Maniatis et al., 2019; Xie, Liu, et al., 2022). It has been reported that TREM2 deficiency facilitates motor impairments. TREM2 in microglia could sense degenerating neurons and myelin debris in ALS. TREM2-deficient microglia lose their ability to phagocytose TDP-43 inclusions, suggesting a protective role for TDP-43. The results of this study indicate that TDP-43 protects microglia through a molecular mechanism (Xie, Zhao, et al., 2022).

APOE regulates a subset of microglia, which exhibit a common disease-associated phenotype. Deletion of APOE in phagocytic microglia showed cell-autonomous regulation of the phenotypic switch. TREM2 induced a phenotypic

switch associated with MND, activated APOE signaling, and subsequently suppressed the homeostatic regulation of microglial phenotype. Furthermore, the activation of antibody-dependent TREM2 on microglia increases the density of oligodendrocyte precursors in the demyelinated area and promotes the formation of mature oligodendrocytes, thereby promoting remyelination sheath formation and axon integrity (Cignarella et al., 2020). In ALS and Alzheimer's disease (AD) mice models, TREM2 induced APOE signaling, and targeting this pathway prevented neuronal degeneration in microglia (Krasemann et al., 2017). Results indicate that TREM2 may be a potential target for promoting myelin re-differentiation in microglia (Krasemann et al., 2017). A recent study has found that TREM2 has a key role in regulating disease-associated microglial gene expression in both human AD and in mouse models of AD using single nuclear RNA sequencing, supporting the role of TREM2 in microglial gene expression regulation (Krasemann et al., 2017). However, microglial gene expression differed greatly in humans compared to mouse models of AD, indicating a species-specific role for TREM2 in microglial regulation. Furthermore, while TREM2 is highly associated with microglial activation and regulation, the expression of TREM2 by human microglia has recently been questioned. TREM2 immunohistochemistry on human cortical tissue found that TREM2 was overwhelmingly expressed by infiltrating monocytes, with little expression was observed in native microglia (Fahrenhold et al., 2018).

In a recent study, a data-driven system approach directly linked microglia to motor neuron pathology progression and found that, as with AD, soluble TREM2 levels were higher in early phase of ALS (Suárez-Calvet, Araque Caballero, et al., 2016; Suárez-Calvet, Kleinberger, et al., 2016). Compared with late phase, soluble TREM2 was three times higher in early phase. Furthermore, in late phase, soluble TREM2 levels showed a significant positive correlation with disease duration. Reversely, no significant correlation was found in early phase. It was supposed that the acute elevation of TREM2 expression in early phase may reflect an initial immune response to deposition of pathological aggregates, which declines over time, and the expression of TREM2 in late phase may reflect a sustained neuroprotective microglial response (Cooper-Knock et al., 2017). As a result, TREM2 provides a broad therapeutic target for ALS.

3 | PURINERGIC SIGNALING MODULATES NEUROPATHIC PAIN AND AUTOPHAGY BY ESTABLISH MICROGLIAL BEHAVIOR

In the CNS, the purinergic system serves as a crucial regulatory pathway as well as a basic signaling system that develops the behavior of microglia. Purinergic signaling consists of ADO, ATP, ADP, AMP, purinergic ionotropic receptors (P2X), purine nucleotide receptors (P2Y), P1, P2 receptors, and extracellular enzymes involved in ATP secretion (Braun et al., 2000). The role of purinergic signals in inflammation and immune response may be more relevant and common than originally thought (Michelet et al., 2018). P2X7R is the low-affinity purinergic receptor in microglia. ATP is the main trigger of pro-inflammatory action in inflammation (Ferrari et al., 2006). Extracellular nucleotides play a critical role in neuron–microglia communication through purinergic P2X and P2Y receptors expressed in microglia. The microglial purinergic receptor is essential for neuropathic pain. After nerve damage, spinal microglia increase purinergic receptors. Hyperexcitability in SDH neurons involves microglial purinergic signaling (Tsuda & Inoue, 2016).

Behavioral studies have shown that P2Y13R/P2Y12R activation in microglia induces the development of neuronal hyperexcitability (P. W. Liu et al., 2017). P2Y13 receptor (P2Y13R) is highly and stably expressed by microglia in the brain (Pannell et al., 2016) but remains the least characterized from a functional point of view (Zeng et al., 2014). Both P2Y13R and P2Y12R have been related to microglia in the spinal cord, with the production and secretion of inflammatory cytokines, such as TNF-α IL-1β and IL-6 (P. W. Liu et al., 2017). P2Y13R and P2Y12R triggered the paracrine signaling in the inflammatory process in vitro (Calovi et al., 2019; Quintas et al., 2018). In vivo studies have reported the upregulation of P2X4R in spinal microglia due to nicotinic antagonist treatment in hyperalgesia models (X. Zhang et al., 2017) and neuropathic pain models (Jurga et al., 2017). The pharmacological blockade of P2X4R reduces pain-related behaviors of animals (Calovi et al., 2019). Purinergic signaling initiates the intracellular Rho-associated coiled-coil containing protein kinase (ROCK) pathway, associated with microglial contraction, migration, or death. ROCK pathway regulates the activation of p38 mitogen-activated protein kinase (p38 MAPK) simultaneously, which is a critical biomarker during the onset of neuropathic pain (Tatsumi et al., 2015). Studies have confirmed that P2X7R stands out as a leading participant and a promising target for innovative drugs (Adinolfi et al., 2018). A recent study in vitro co-cultured the N9 microglial line with a glioma cell line has found could upregulate the expression of the P2Y14 receptor (P2Y14R), which suggests a new role for P2Y14R in microglia–glioma communication (Calovi et al., 2019; Curet & Watters, 2018).

ATP released by microglia also represents a significant paracrine and autocrine signal (Calovi et al., 2019). Autophagy abnormalities have been involved in chronic neurodegenerative conditions, such as ALS. Evidence proved that P2X7 plays a dual role in autophagy in the $SOD1^{G93A}$ model (Apolloni, Fabbrizio, Amadio, et al., 2016; Apolloni, Fabbrizio, Parisi, et al., 2016). On the one hand, initial activation of P2X7 might have a positive effect on

microglia through stimulating autophagy; conversely, persistently engagement of P2X7 might, in turn, inhibit autophagy. P2X7 activates microglia through autophagic flux modulation, and it was a new mechanism discovered in recent years. Recent evidence showed that inhibition of P2X7 by using the antagonist A-804598 in SOD1^{G93A} mice leads to suppressing SQSTM1/p62 upregulation in the lumbar spinal cord, thus confirming P2X7 as an in vivo modulator of the ALS pathological mechanism of autophagy (Fabbrizio, Amadio, Apolloni et al., 2017).

In both ALS patients and mouse models, it is known that microglia shift from a beneficial M2 phenotype in the early stage of the disease to a harmful M1 phenotype, which is more pronounced when the disease develops rapidly and motor neuron damage increases (Beers et al., 2011; Henkel et al., 2009; Hooten et al., 2015; Zhao et al., 2013). Apolloni et al. suggested that the short-term P2X7-dependent autophagy activation proved in vitro in SOD1^{G93A} microglia may be related to the beneficial effect of P2X7 activation in the early stage of the disease (Fabbrizio et al., 2017).

4 | ROCK SIGNALING MEDIATES THE CHANGE OF MICROGLIAL PHENOTYPE IN ALS

Rho/ROCK pathway, including ROCK1 and ROCK2, is involved in various physiological activities, such as cytoskeletal rearrangement, contraction, cell migration, phagocytosis, adhesion, stress fibers formation, inflammation, and angiogenesis (Wang et al., 2013). ROCK1 is dominant in the liver, lungs, testes, blood and immune system, whereas ROCK2 is dominant in the brain and muscles. Furthermore, ROCK pathway is the major regulator of microglial activity. In microglia, the ROCK pathway maintains stress fibers and focal adhesions, which proved to be involved in the release of inflammatory cytokines (NO, IL-1β, IL-6, TNF-α, etc.) and mediation of the microglial phenotype (Barcia et al., 2012; Borrajo et al., 2014; Yan et al., 2012). These inflammatory cytokines may be potential pathogenic factors of ALS (Ding et al., 2010). Patients with sporadic ALS showed an increase of ROCK2 protein, most prominent in skeletal muscle tissue (Conti et al., 2014). ROCK pathway is related to neuroinflammation and is closely related to the progression of ALS, including the activation of microglia in diseased tissues in human patients with ALS (Philips & Rothstein, 2014).

The microglial cells are the first cell type to be activated in ALS, while ROCK is increased in microglial cells (Conti et al., 2014; Evans et al., 2013). In ALS mouse models, microgliosis occurs as well in pre-symptomatic as in symptomatic SOD1^{G93A} mice and SOD1^{G37R} mice. The SOD1^{G93A} mice microglial phenotyping suggests a predominance of M2-type in early onset SOD1^{G93A} mice and of a rather classically activated M1-type at the end stage (Tönges et al., 2014). The expression of inflammatory phenotype of microglia is often concerned in the treatment of ALS. Further experiments also confirmed this view. In both microglia types, wildtype and mice SOD1^{G93A} microglia, strong pro-inflammatory cytokines and chemokines were released after stimulation with LPS. This inflammatory response could be effectively attenuated when ROCK inhibitor Fasudil is used to intervene these microglia (Roser et al., 2017; Tönges et al., 2014). This underlines that ROCK is considerably involved in the modulation of microglial cell function. Furthermore, treatment with the isoquinoline-type ROCK-inhibitor Fasudil reduced the microglial release of the pro-inflammatory factors NO, IL-1β, IL-6, and TNF-α (Ding et al., 2010). However, microglia numbers increased with Fasudil treatment. In addition, an in vitro activation analysis of primary microglia demonstrated that Fasudil changed the release of cytokines in a way that was anti-inflammatory. Thus, it can be postulated that Fasudil shifts the microglial phenotype toward M2 in SOD1 mice (Tönges et al., 2014).

SOD1^{G93A} mice exhibited increased ROCK activity, leading to increased phosphorylated adducin levels, elevated PTEN activation, and decreased Akt activity. In ALS, animal studies using ROCK inhibitors were limited to the transgenic SOD1^{G93A} mouse model. Oral Fasudil slows disease progression, extends survival time, and reduces motor neuron loss by decreasing decreased PTEN activation, increasing PI3K, Akt signaling, and inhibiting ROCK (Takata et al., 2013). Additionally, treatment of ROCK-inhibitor Y-27632 on male SOD1^{G93A} mice is improved to motor function (Günther et al., 2014). Fasudil and Y-27632 are not completely selective for ROCK, and inhibition of other kinases may also contribute to their effects. Despite these limitations, the biological efficacy of these two molecules has been demonstrated.

5 | p38 MAPK SIGNALING PARTICIPATES IN MEDIATING THE MICROGLIAL PHENOTYPE IN ALS

The p38 MAPK is activated by a variety of stimuli, including oxidative stress, excitotoxicity, and inflammatory cytokines. In turn, this can result in neurodegeneration by phosphorylating cytoskeletal proteins and activating cytokines and NO (Tortarolo et al., 2003). A previous study has observed that p38 MAPK is associated with the pathogenesis of ALS. Immunostaining for phosphorylated p38 MAPK in lumbar spinal cord sections of SOD1^{G93A} mice at the presymptomatic and early stages of disease showed an increased labeling in motor neurons that colocalized with phosphorylated neurofilaments in vacuolized perikarya and neurites. Colocalization with GFAP and CD11b immunostaining showed

that as the disease advanced, activated p38 MAPK also accumulated in hypertrophic astrocytes and reactive microglia (Tortarolo et al., 2003). The study shows that the loss of MN in ALS is accompanied by a significant activation of p38 MAPK. This activation becomes apparent at disease onset. The data indicate that most of the activation of p38 MAPK is accounted for by the robust upregulation in the proliferating microglia, and the increase of phospho-p38 MAPK almost exclusively occurs in the ventral part of the spinal cord. SB203580, a p38 MAPK inhibitor, inhibited mutant SOD1-induced apoptosis of MN and LPS-induced activation of microglia (Dewil et al., 2007). Several in vivo studies have successively found that bee venom (BV) and Melittin (the components of bee venom) have anti-inflammatory effects on ALS and that microglia may be involved (Yang et al., 2010; Yang et al., 2011). BV-treated mutant hSOD1 transgenic mice showed a decrease in the expression levels of microglia marker and phospho-p38 MAPK in the spinal cord and brainstem. Melittin is known to have anti-inflammatory and anti-arthritic effects. Melittin-treated animals (injected at the ''ZuSanLi'' (ST36) acupoint in the hSOD1^{G93A} animal model) showed a decrease in the number of microglia and in the expression level of phospho-p38 in the spinal cord and brainstem. It appears that microglia may be involved in mediating the anti-inflammatory effect of BV on ALS. A recent study showed that miR-467f and miR-466q modulate the pro-inflammatory phenotype of activated N9 microglia and of primary microglia acutely isolated from late symptomatic SOD1 mice, a murine ALS model, by downregulating TNF and IL-1β expression. In addition, miR-467f and miR-466q both inhibit the activation of MAPK signaling pathway by inhibiting the expression of their target genes, Map3k8 and Mk2, which dampen the pro-inflammatory phenotype of microglia. This suggests that mesenchymal stromal/stem cells-derived exosomes may affect neuroinflammation by acting on microglia via specific immunomodulatory miRNAs (Giunti et al., 2021). Moreover, in vitro, two types of microglial cells (BV2 and primary microglial cells) were used as models of neuroinflammation. A study reported that steppogenin isolated from Cudrania tricuspidata exhibited potent anti-neuroinflammatory effects in BV2 and primary microglial cells. This might be ascribed to steppogenin's ability to inactivate the nuclear factor-κB (NF-κB), and p38 MAPK pathways, to inhibit the protein expression of iNOS, COX-2, and pro-inflammatory cytokines, and to suppress the production of NO and PGE2 (Kim et al., 2017).

In conclusion, as ALS progresses, p38 MAPK is activated, and microglia show an inflammatory phenotype. At present, in vitro and in vivo experiments have revealed that anti-inflammatory chemicals (such as Steppogenin and melittin) can exert anti-inflammatory effects on microglia phenotype in the process of ALS disease by inhibiting the activation of p38 and the production of pro-inflammatory mediators and cytokines. However, the above research has only gone through the laboratory phase. These chemicals have not been developed into drugs for clinical use, and further exploration should be conducted in this direction in the future.

6 | FRACTALKINE SIGNALING INFLUENCES THE PROGRESSION OF ALS THROUGH THE CHANGE OF MICROGLIAL PHENOTYPE

Fractalkine is a chemokine with a cysteine signature motif -Cys-X-X-X-Cys- at the N-terminal end and is the only known representative of the δ-chemokine family. CX3CL1 was first discovered in 1997 and named neurotactin and fractalkine, with the latter name currently in use. It shows high expression in the brain, where it is synthesized by neurons and has neuroprotective functions (Jung et al., 2000; Maciejewski-Lenoir et al., 1999; Noda et al., 2011; Zujovic et al., 2000). As the only member of the chemokine CXC subfamily, CX3CL1 binds to its own receptor in microglia (CX3CR1). It is a soluble factor and regulates microglia activation in CNS (Harrison et al., 1998; Lyons et al., 2009; Mecca et al., 2018).

Fractalkine signaling mediates the activity of microglia and participates in neuronal protection by regulating phagocytic activity. Within ALS, SOD1^{G93A}/CX3CR1$-/-$ mice exhibit accelerated disease progression and exacerbated neuronal death and show more neuronal cell loss (Boillée et al., 2006; Cardona et al., 2006). This indicates a protective role of fractalkine signaling in ALS. The functional variant of human CX3CR1 gene is associated with shorter survival time in patients with ALS and has been identified as the most relevant genetic factor reported so far (Lopez-Lopez et al., 2014).

Fractalkine signaling shows dynamic changes in microglia during motor neuron loss in ALS mouse model. For example, it increases during microglia proliferation (J. Zhang et al., 2018). In addition, fractalkine induces microglial chemotaxis and proliferation, that are membrane bound, while soluble fractalkine has been shown to attenuate microglial neurotoxicity in neurodegenerative disease (Bhaskar et al., 2010). Further studies found that patients with ALS who carried one or two allele of the variant CX3CR1-Ile249 showed a faster progression rate of the symptoms and decreased survival time, when compared to CX3CR1-Val249-Thr280 (wildtype) carriers (Calvo et al., 2018). SOD1^{G93A} microglia induced motor neuron death in an NF-κB-dependent mechanism as one of the manifestations of ALS is the death of MN. Other research shows that the disruption of fractalkine signaling indicated NF-κB activation and induces to pro-inflammatory (M1) microglia phenotype that causes MN death (C. Liu et al., 2019). So, the activation of CX3CR1 may serve as an anti-inflammatory signal, and CX3CL1 delayed microglial activation might be mediated through CX3CR1 in ALS mice

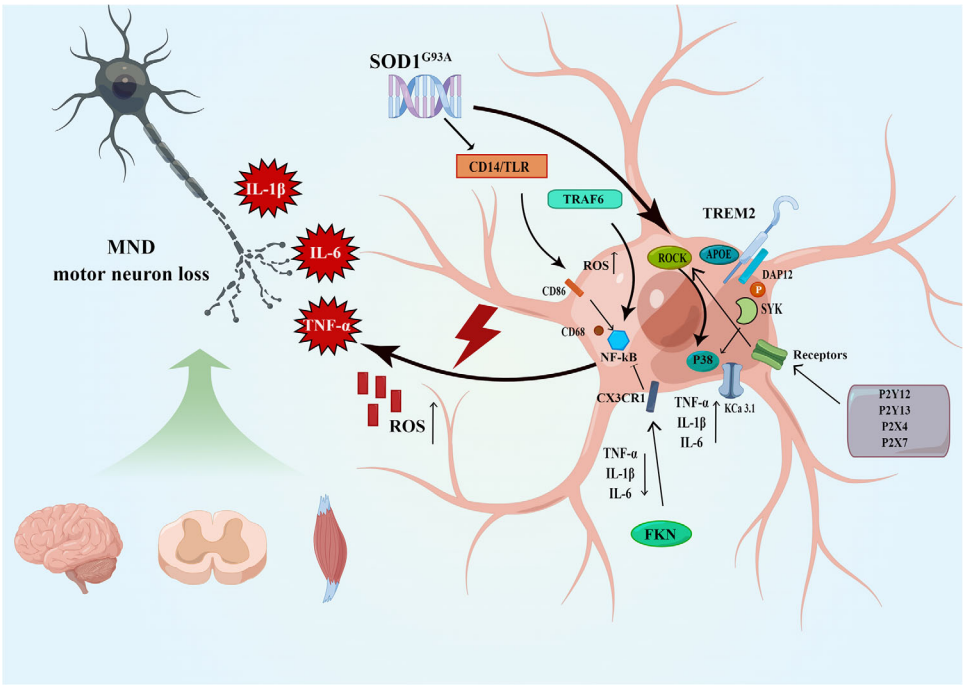

FIGURE 1 The signaling and relevant mediators involved in motor neuron disease (MND) related to microglia

(Frakes et al., 2014). Fractalkine signaling has a potential effect on anti-inflammation and neuroprotection via NF- κB inhibition in ALS. Therefore, increasing CX3CL1 or inhibiting this reduction of CX3CL1 and maintaining effective communication between CX3CL1 and CX3CR1 may be a new therapeutic target for ALS treatment. At present, the potential therapeutic role of CX3CL1 has been proved in animal experiments, but it still needs to be further verified.

Further study on the molecular mechanism of fractalkine signaling regulating microglia activation may provide useful information for determining the value of fractalkine signaling axis as a therapeutic target for ALS.

7 | NF-κB SIGNALING REGULATES MICROGLIAL INFLAMMATORY RESPONSES IN MND

NF-κB is consisted of p65/p50 heterodimer, a major regulator of inflammation. Classical NF-κB signaling drives gene expression of pro-inflammatory cytokines, chemokines, enzymes, and adhesion molecules, and these factors were upregulated in the ALS process (Frakes et al., 2014; Matsye et al., 2017). Therefore, the intracellular pathway of NF-κB signaling is involved in physiological processes of the nervous system such as regulation of apoptosis, neurite outgrowth, and synaptic plasticity (Arumugam et al., 2018). It was proved that NF-κB signaling is activated in the spinal cord in several MND, such as ALS and SMA (Frakes et al., 2014).

Among the disease subtypes of ALS, family history of the disease and sporadic patients both have significant striking hallmarks, neuroinflammation, characterized by extensive astrogliosis, microglial activation, and peripheral immune infiltration at neurodegenerative sites (Frakes et al., 2014). Activated microglia in the vicinity of diseased or injured CNS tissue produce cytokines and chemokines, enhancing the inflammatory response through recruitment and activation of immune cells, such as IL-1β and TNF-α (Matsye et al., 2017). In this process, the NF-κB pathway plays a pro-inflammatory role and mediates microglia phenotypic transformation and the induction of neurotoxicity. Recently, immunohistochemical studies showed that NF-κB is highly induced in microglia of sporadic patients, and those with mutation in the gene optineurin, a negative regulator of TNF-α, induced NF-κB activation that has been shown to cause ALS (Matsye et al., 2017). The mutation of SOD1, as the classical biomarker of ALS pathology process, causes progressive MN degeneration, further aggravating the disease process. In vitro studies have shown that murine astrocytes and microglia expressing mutant SOD1 and human astrocytes from sporadic and familial patients with ALS can induce MN death (Frakes et al., 2014). NF-κB was activated by the G93A-mutated SOD1 protein via CD14 and TLR pathways in ALS pathogenesis. When inflammatory mediators bind their respective receptors, a signaling cascade is initiated that leads to phosphorylation and activation of IKKb (a subunit of the inhibitor of IkB kinase [IKK] complex). Activated IKKb phosphorylates the IkB inhibitory protein IkBa, targeting it for ubiquitination and

TABLE 1 Related signaling and ion channels affect motor neuron disease (MND) by regulating microglia

Pathway	Neuron-derived mediators	Influence on microglia	Effect on MND	References
TREM2-APOE pathway	↑ TREM2 ↓ IL-1β ↓ TNF-α ↓ iNOS ↓ IL-6 (+)APOE	Expressed by microglia, promotes microglial survival, proliferation, migration, and phagocytosis and affect microglial molecular expressions	Regulating inflammatory of MND	[Benoit et al., 2012-Calvo et al., 2018]
Purinergic signaling	↑ ATP ↑ P2X7 (+) P2X7R	Activate microglia	Plays a crucial role in neuropathic pain	[Cooper-Knock et al., 2017-Ferrari et al., 2006]
ROCK signaling	↑ TNF-α ↑ IL-1β ↑ IL-6	Proved to be involved in the release of inflammatory cytokines	Affect ALS by inducing the release of inflammatory factors	[Garaschuk & Verkhratsky, 2019-Kiernan et al., 2011]
p38 MAPK signaling	↑ iNOS ↑ COX2 ↑ NO ↑ PEG2	Promote the pro-inflammatory phenotype of microglia	Affect ALS by promoting the pro-inflammatory phenotype of microglia	[Kobayashi et al., 2013-Liao et al., 2012]
Fractalkine signaling	↓ TACE ↑ TNF-α ↓ IL-1β ↑ IL-6 ↑ CCL21 (+) CX3CR1	Activates the proliferation and migration of microglia, facilitated by TACE or CatS, transduces inhibitory signals that ameliorate microglial activation and mediated neuron–microglia interaction	Rapidly respond to the incoming signals	[Maciejewski-Lenoir et al., 1999-Pannell et al., 2016]
NF-κB signaling	↑ NF-κB ↑ TNF-α ↑ IL-1β ↑ IFN-γ ↑ iNOS ↑ COX2 ↑ IL-6 ↑ NO ↑ CD68 ↑ CD86	Promotes convergent phenotype of microglia via neuroinflammation	Plays a pro-inflammatory role and mediates microglia phenotypic transformation and the induction of neurotoxicity in MND	[Ashford et al., 2021, Papadimitriou et al., 2010-Quintas et al., 2018]
C1q classical component	↓ TNF-α ↓ IL-1β↓ IL-6	Leads to microglia-mediated synaptic elimination, a general anti-inflammatory role of C1q	MN is incapable to maintain high-frequency discharge and may induce SMA	[Suárez-Calvet et al., 2016-Tsuda & Inoue, 2016]
KCa 3.1	(+) Ca^{2+} (+) K^+ (+) ATP ↑ TNF-α	Expressed by microglia, activate pro-inflammatory phenotype of microglia, and regulate cell migration and phagocytic activity	Reduce ALS-associated neuroinflammation and to protect MNs from degeneration	[Xie et al., 2022-Yang et al., 2010]

Abbreviations: ALS, amyotrophic lateral sclerosis; IFN-γ, interferon-γ; IL-1β, interleukin-1β; MN, motor neurons; NO, nitric oxide; SMA, spinal muscular atrophy; TNF-α, tumor necrosis factor α; TREM2-APOE, triggering receptor expressed on myeloid cells 2-apolipoprotein E.

proteasomal degradation and subsequent release of NF-κB (p65/p50) to the nucleus, and this led to the activation of NF-κB (Frakes et al., 2014; Matsye et al., 2017). Activated NF-κB pathway induces a shift in the M1 phenotype of microglia. Mutant SOD1 microglia exhibit increased expression of many proinflammatory genes. This makes the M1 microglia express proinflammatory molecules that include tumor necrosis TNF-α, IL-1β, IFN-γ, iNOS, COX2, IL-6, and NO as well as cell surface markers, CD86 and CD68 (Frakes et al., 2014; Kobayashi et al., 2013). Additionally, study in wild-type (WT) mice showed that constitutive activation of NF-κB selectively in myeloid cells in WT mice creates the pathological features of ALS in the spinal cord (i.e., gliosis and MN death) (Frakes et al., 2014). This result once again shows that NF-κB activation is the mechanism by which $SOD1^{G93A}$ microglia induce MN death.

Based on this finding, NF-κB is a promising therapeutic target for ALS. A previous study showed that inhibition of NF-κB selectively in microglia rescues MN survival in vivo and in vitro. Heterozygous inhibition of NF-κB in microglia substantially delayed disease progression in the severe $SOD1^{G93A}$ model. One of the most extended extensions in this severe model reported extending survival in the ALS mice delaying disease progression by 47%. NF-κB inhibition in microglia leads to a marked reduction in prototypic inflammatory markers (Frakes et al., 2014). Minocycline as a commonly used, strong inhibitor of microglial activation, this drug is a useful tool to investigate the mechanisms underlying microglial polarization and the pathogeneses of many diseases accompanied by microglial activation (Kobayashi et al., 2013). In mice model of ALS, minocycline intervention inhibits microglia polarization. Minocycline selectively inhibited the microglial polarization into M1, but not M2, and it can inhibit the expression of cell surface markers of M1-polarized microglia CD86 and CD68 as well as the production of inflammatory cytokines (IL-1β, TNF-α and IFN-γ) in vivo and in vitro (Kobayashi et al., 2013). It has been reported that minocycline partially suppresses the production of inflammatory molecules (IL-6, TNF-α, and IL-1β) induced by LPS in peripheral monocytes through inhibition of the nuclear translocation of NF-κB. Minocycline may work in the early disease phase by suppressing the microglia activation polarized to M1, leading to the suppression of the pathogenesis of disease (Kobayashi et al., 2013). Therefore, using minocycline could have potential for improving the survival of patients with ALS.

The canonical NF-κB activation regulates neuronal and motoneuron cell survival, and evidence are emerging about its role in neurodegenerative disorders and neuronal injury. NF-κB not only regulates the pathogenesis of ALS but also plays a significant role in the pathogenesis of SMA. In the pathogenesis of SMA, the deficiency of SMN protein in microglia promotes oxidative stress, thereby activating the NF-κB pathway and regulating neuroinflammation in microglia.

In addition, a previous study has observed an increased production of TNF-α in SMN-depleted RAW264.7, which is consistent with previous reports of SMN-deficient BV2 cells. The elevated expression of TNF-α might contribute to motor neuron degeneration in SMA pathology. Regarding TNF-α, the neuronal apoptosis inhibitory protein (NAIP) gene is known to be related to the SMA gene, which is deficient in 50% of SMA type I patients. NAIP functions as a blocker against TNF-α induced-cell death in neuronal cells. As such, the loss of NAIP in SMA may accelerate the vulnerability of MN to TNF-α. Therefore, regulation of inflammation in microglia could be an effective therapeutic target for SMA (Ando et al., 2020).

8 | C1q OF THE CLASSIC COMPLEMENT SYSTEM CASCADES ARE RELATED TO SMA

C1q, formed by the three C1q α-, β- and γ-chains (encoded by the C1qa/b/c genes), represents the initiating component of the classic complement pathway (C1→C4/C2/C3→C5) that is typically triggered by C1q recognizing an antibody-coated target, whereas C3 is central to the classic and the alternative complement pathway (C3→C3/B→C5) that is triggered by spontaneous C3 proteolysis (Alexander et al., 2008; Stephan et al., 2013). Microglia is the major source of C1q in developing spinal cord (Vukojicic et al., 2019). In vitro studies indicated that, with the absence of other complement components, C1q modulated phagocytosis by monocytic cells and enhanced clearance of apoptotic neurons by microglia, acting as a direct opsonization agent for phagocytes (Färber et al., 2009; Fraser et al., 2009; Fraser et al., 2010). C1q also modulates subsequent cytokine production by activated microglia, monocytes, and macrophages, reducing specific pro-inflammatory signals. This suggested a general anti-inflammatory role of C1q by combining rapid clearance of apoptotic cells, followed by reducing deleterious inflammation (Benoit et al., 2012; Fraser et al., 2009; Fraser et al., 2010).

It has been proved that C1q is involved in the refinement of spinal sensory-motor circuits during normal development process. Additionally, C1q has been identified to be involved in the dysfunction and selective elimination of proprioceptive synapses on spinal MN in SMA mice (Vukojicic et al., 2019). MN adopt activation of the classical complement via direct or indirect contacts with spinal interneuron circuits. It triggers C1q tags vulnerable synapses pathway within spinal motor circuits, leading to microglia mediated synaptic elimination (Ferreira-Pinto et al., 2018; Hong et al., 2016). SMN deficiency in proprioceptive synapses decreases the release of presynaptic glutamate onto MN, resulting in the reduction of MN firing ability. MN is incapable of maintaining high-frequency discharge, leading to defects of muscle contraction and limb motor (Vukojicic et al., 2019).

Additionally, in vivo experiments found that pharmacological inhibition of C1q or synapses deposition by anti-C1q antibody confers both structural and functional rescue of synapses in SMA mice. These results suggested that inhibition of C1q may be a potential therapeutic target (Vukojicic et al., 2019). Thus, the classical complement pathway plays critical roles in the refinement of developing motor circuits, while its aberrant activation contributes to motor neuron disease (Lui et al., 2016).

Although both ALS and SMA show a reduction of nerve synapse, the cause of synapse reduction is different. Previous studies have considered that C1q was relevant to

neurotoxicity in the pathogenesis of ALS. However, recent studies indicated that C1q induction and complement pathways have no effect on the toxicity of ALS via microglia. In vitro study, C1q deletion showed no influence on the global neuroinflammatory response in $SOD1^{G37R}$ mice, and this result has been confirmed by immunohistochemical staining for activated microglia (Iba1/Aif1 and Mac2/Lgals3) and glial fibrillary acidic protein (GFAP) in affected lumbar spinal cords. Similarly, C1q did not change the time course of overall microglial activation (which initiated before onset) in $SOD1^{G37R}$ ALS mice (Davalos et al., 2005; Lobsiger et al., 2013).

To date, synaptic loss and inflammation were considered as the pathological mechanisms of ALS. C1q attenuates the release of pro-inflammatory factors from microglia, and the deletion of C1q might lead to synaptic loss, but not contributing to the neurotoxicity of ALS. Additionally, C1q possibly plays a role in the structural and functional rescue of SMA. However, it was proved that the aberrant activation of C1q contributes to SMA, and pharmacological inhibition of aberrant C1q confers benefit in SMA mice. Therefore, protecting vulnerable synapses by blocking aberrant C1q may represent a viable therapeutic for SMA. Although SMA is caused by gene SMN1, inhibition of C1q and abnormal activation of complement pathway may be the target of future treatment.

9 | CA^{2+}-ACTIVATED K^+ CHANNELS MODULATE MICROGLIA IN MND

Ca^{2+}-activated K^+ channel (KCa3.1), in the CNS, microglia express KCa3.1 channel, the channel will regulate cell migration and phagocytic activity in physiological and pathological conditions (Cocozza et al., 2018). Microglia involved in the control of Ca^{2+} homeostasis and Ca^{2+}-dependent responses to external stimuli, recent research developed a computational model for Ca^{2+} control in microglia, which link ATP-dependent purinoceptor (P2X4 and P2X7) activation to TNF-α production. Microglial responses when ATP binding with purinoceptors at plasma membrane. Purinoceptor activation is strongly related to dramatic changes of intracellular Ca^{2+}- and Ca^{2+}-dependent signaling cascades. P2X4 and P2X7 are the most abundant receptors of the P2X receptors, expressed in microglial membrane (Garaschuk & Verkhratsky, 2019). Furthermore, ATP-dependent TNF-α secretion is strongly correlated with increasing of intracellular Ca^{2+} and p38 phosphorylation (Hide et al., 2000). Under high ATP concentrations and prolonged exposure duration, P2X7 responses predominate over P2X4 and are involved in ATP-dependent TNF-α secretion by microglia. According to distinct channel activation of P2X4 and P2X7, microglia respond differently to low or high levels of ATP, by activating a neurotrophic, or cytokine response, respectively (Chun et al., 2019; Hide et al., 2000).

In both patients with ALS and mouse models, an increasing expression of pro-inflammatory genes was found (Lee et al., 2009; Lu et al., 2016), while anti-inflammatory genes decreased (Lee et al., 2009; Lewis et al., 2014; Liao et al., 2012). In the CNS, microglia express cells KCa3.1channel, and they are involved in regulating cell migration and phagocytic activity, both in physiological and pathological conditions. It was found, by using selective KCa3.1 inhibitor TRAM-34, that chronic inhibition KCa3.1 activity in $hSOD1^{G93A}$ mice restrained the pro-inflammatory (M1) phenotype microglia, increased the number of healthy MNs, and preserved the number of healthy neuromuscular junctions (NMJ) in the tibialis anterior muscle. Furthermore, KCa3.1 inhibition treatment delayed motor symptom appearance, restrained muscle strength and motor coordination, and increased survival in $hSOD1^{G93A}$ mice. These results indicated potential targeting of KCa3.1 to reduce ALS-associated neuroinflammation and to protect MNs from degeneration (Cocozza et al., 2018).

10 | CONCLUSIONS

Microglia is regarded as a homogenous myeloid cell lineage in mammalian CNS and as dynamic cellular mediators of CNS function (Tay et al., 2017). Neurodegeneration and neuroinflammation have been considered as the potential mechanism for MND and are associated with the loss of upper or lower MN (Deschenes et al., 2016). We have summarized seven main signaling and relevant mediators in MND as the following (Figure 1): (1) Pro-inflammation: purinergic signaling, NF-κB signaling, ROCK signaling, p38 MAPK signaling, classic complement system and Ca^{2+}-activated K^+ channels, IL-1β, TNF-α, IFN-γ, iNOS, COX2, IL-6, NO, CD86, CD68, CD14, TLR, ROS, p38 MAPK, ATP, P2X7, P2X7R, P2Y12R, P2Y13R, P2Y14R, NF-κB, NAIP, TDP-43, Ca^{2+}, and K^+; (2) anti-inflammation: fractalkine signaling, TREM2 signaling, TNF-α, IL-1β, IL-6, iNOS, APOE, and CX3CR1.

This review concluded the signaling interaction of microglia in MND (Table 1) and sorted out anti-inflammatory chemicals and drugs targeting microglia via multiple signaling, such as microglia inhibitor (minocycline) and ROCK inhibitor (Fasudil). This review discussed the potential molecular therapeutic target for the research and development of new drug for MND. Although some progress has already been made, it remains to be seen whether these cell mediators, such as TDP-43, CX3CL1, SMN, and expression limit to microglia. Studies of animal models provide a basis for understanding pathogeneses of MND accompanied by microglial activation. Nevertheless, what is not yet clear is that the special effects of these therapeutic target and

whether the effect of the blockade of the related signaling on SOD1^{G93A} mice could be relevant in other fALS, and in sALS.

AUTHOR CONTRIBUTIONS

Liang Kang is the corresponding author on the review. Min-Jia Wang is the first author and responsible for collecting materials and writing first draft of the manuscript. Lu Kang, Yao-Zheng Wang, Bi-Ru Yang, and Yu-Feng Lu helped in organizing the information. Chun Zhang edited the review table and figure. All authors contributed to manuscript revision, read, and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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