

# Motor Neuron Disease

Subjects: **Clinical Neurology**

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Motor neuron disease (MND) is a group of fatal neurodegenerative diseases with no effective treatment, which have the shared characteristic of the progressive loss of upper and/or lower motor neurons. Disease onset is insidious, with patients gradually losing control of their voluntary muscles, resulting in relatively late diagnosis. Due to the nature of the disease and the lack of effective treatment, MND patients usually die within 2 to 3 years following diagnosis, primarily because of the loss of respiratory function. The only drug approved in Australia, Riluzole, only prolongs the median life expectancy by 2 to 3 months. New and effective treatments are therefore urgently needed.

receptors

Eph

EphA4

motor neurons

motor neuron death

neurodegenerative disease

motor neuron disease

pathogenesis

## 1. Introduction

It is widely accepted that MND is a complex disease, with both genetic and environmental factors co-contributing to its pathogenesis [1][2][3]. Although the details of its pathogenesis are unclear, motor neuron cell death is regarded as the hallmark. Preventing this death is the primary therapeutic aim [4][5][6]. Here we discuss the importance of EphA4 signalling in motor neuron cell death, the mechanisms of signalling and the potential for ameliorating MND by blocking its signalling.

EphA4 belongs to the A subgroup of Eph receptors and is well known as a pan-receptor that can widely bind, albeit with varying affinities, to all ephrin ligands, including five glycosyl phosphatidylinositol (GPI)-linked cell membrane-bound type A ephrins and three transmembrane type-B ephrins [7][8][9]. This striking feature indicates that EphA4 interaction with both A- and B-type ephrins regulates both normal and pathophysiological functions [10]. This suggests that blocking EphA4 signalling could be more efficacious than blocking other Ephs or ephrins. Well established as a guidance molecule involved in the development of the corticospinal tract [11][12], EphA4 has also been shown to inhibit axon regeneration following spinal cord injury (SCI) [13][14][15]. The fact that EphA4 represses the axonal regrowth of motor neurons after SCI suggested that it may also contribute to the differential vulnerability of motor neurons in MND through a similar mechanism [16][17]. In support of this view, emerging research has identified that EphA4 is indeed associated with MND pathogenesis [16][17][18][19][20].

In two zebrafish models of MND, overexpressing human mutant SOD1 and TDP-43, knockdown of the zebrafish parologue of EphA4, Rtk2, rescued both mutant SOD1-induced axonopathy and axonal outgrowth defects caused

by the mutant TDP-43. The same study also examined the effect of EphA4 in rodent SOD1 G93A models of MND. EphA4  $+$ / $-$  mice were crossed with SOD1 G93A mice to obtain EphA4  $+$ / $-$  SOD1 G93A mice. Compared with EphA4  $++$  SOD1 G93A controls, the heterozygous deletion of EphA4 significantly increased motor performance and survival. The administration of EphA4-blocking peptide to SOD1 G93A rats also delayed disease onset and enhanced survival. Finally, in MND patients, lower levels of expression of EphA4 mRNA in whole blood samples correlated with prolonged disease progression [16]. This study showed for the first time that EphA4 is involved in the disease progression of MND, and inhibiting EphA4 expression or activation can affect disease progression, making it an attractive target for MND therapies. Subsequently, a novel isoform of full-length EphA4 (EphA4-FL) was identified in mice and humans, EphA4-N, which contained the extracellular domains and transmembrane domain of EphA4-FL. EphA4-N was alternatively transcribed from the EphA4-FL gene, successfully translated into a functional protein, and was able to function as an endogenous dominant-negative inhibitor in terms of its repressive effect on EphA4-FL signalling in vitro. It has been shown that there was a lower level of expression of the inhibitory EphA4-N in human MND patients, compared to healthy controls, allowing more aggressive signalling by EphA4-FL. In SOD1 G93A mice, there was an increase in EphA4-FL expression in the pre-symptomatic phase, indicating that EphA4-FL signalling was dominant in the early pathogenesis of MND [18]. In previous studies, we generated a wildtype EphA4-Fc (a recombinant fusion protein derived from the extracellular domain of wildtype EphA4 and the Fc domain of human IgG) which effectively blocks EphA4-ephrin interaction in vitro and demonstrated that it improves functional performance in mice and rats after SCI by increasing the number of axons reaching and crossing the lesion site compared with saline-treated controls [13][14][18].

More recently, to reduce glycosylation, we mutated both the human and mouse EphA4-Fc (mEphA4-Fc) at three glycosylation sites, N235, N340 and N408. This resulted in significantly prolonging the half-life of human mEphA4-Fc from less than 24 h to 31.1 h in healthy Wistar rats following a single intravenous dose, while maintaining comparable binding and blocking ability to the ephrin ligands [21]. Results of the toxicokinetic analysis of human mEphA4-Fc cells in healthy Sprague-Dawley rats following 5 $\times$  weekly repeat intravenous dosing showed that the terminal elimination half-life ranged from 52.8 h to 77.5 h. More importantly, using this glycosylation mutant of mouse mEphA4-Fc in the SOD1 G93A model significantly improved motor performance, including rotarod and hind-limb grip strength tests [17]. Briefly, SOD1 G93A mice were treated with either the mouse mEphA4-Fc or a saline control. Functional behavioural tests were monitored on a weekly basis from week 8 to the end of disease. The balance and motor coordination of mice were assessed by means of the rotarod test, whereas hind-limb grip strength was also monitored. The SOD1 G93A mice receiving the mEphA4-Fc treatment exhibited improved performance in the rotarod test compared to control SOD1 G93A mice from week 17 to week 23, with the differences at weeks 19–21 being statistically significant. Consistent with this result, mEphA4-Fc-treated SOD1 G93A mice also showed better hind-limb grip strength from week 8 to week 22 compared with the vehicle control group, with the differences at weeks 9 and 18–21 reaching statistical significance. Given the substantial loss of induced motor function in this model, these results suggest that mEphA4-Fc is a promising therapeutic treatment for MND and EphA4 activation is involved in the disease pathogenesis [17].

## 2. Direct Regulation of Motor Neuron Death by EphA4

So far, we have observed the directly negative effect of EphA4 activation on motor neuron survival upon both in vitro and in vivo MND backgrounds ( **Figure 1** , **Figure 2** and **Figure 3** and [17]). These are confirmatory evidence to support the promotive effect of EphA4 activation and the protective effect of EphA4 deletion or inhibition on MND progression. Although EphA4 activation has been directly or indirectly involved in different types of cell death, such as NIH 3T3 cells, glioblastoma multiform tumoral cells, retinal ganglion cells and endothelial cells [22][23][24][25], this was the first time that the novel effect of EphA4 on motor neuron survival in MND had been reported. More investigations are required to explicate the underlying mechanisms. In the following review, three possible mechanisms are concisely discussed.

To date, more than 10 different types of cell death have been identified, and four major types among them are apoptosis, necrosis, autophagy and entosis [26]. Although motor neuron death in MND is likely to be multifactorial, the final demise of these cells is more likely to occur via a programmed, energy-dependent cell death pathway resembling apoptosis [27]. It is widely accepted that typical morphological changes which occur during the cell apoptosis process include the release of apoptotic bodies, plasma membrane blebbing and nuclear condensation [28]. Molecules regulating these processes thus played important roles in regulating cell apoptosis [29][30][31][32][33][34]. One of the critical regulators of apoptotic cell membrane blebbing is Rho-associated coiled-coil-containing protein kinase (ROCK), which is also part of the EphA4 downstream signalling pathway [20][24][35][36]. ROCK activation has been shown to contribute to membrane blebbing during the eosinophil peroxidase-induced death of lung epithelial cells in vitro [29]. Similarly, in a human umbilical vein endothelial cell (EC) culture system, the application of combretastatin A-4-phosphate (CA-4-P), a tumor vascular-targeting agent, significantly enhanced ROCK signalling activation and its subsequent myosin light chain (MLC) phosphorylation, which were shown to be responsible for the cell membrane blebbing, loss of cell adherence and decreased viability of ECs due to reorganisation of the actomyosin cytoskeleton. These results suggest that this mechanism may underlie the effect of the CA-4-P treatment in promoting tumor EC death and leading to the shutdown of blood flow in tumors in vivo [30].

ROCK and its other substrates have also been reported to regulate the cell death process [31][32][33]. The upregulation of ROCK signalling and the subsequent inhibition of downstream mitogen-activated protein kinases (MAPK) signalling has been shown to promote the loss of cell bipolarity and detachment, resulting in increases in EC death in an EC-fibroblast coculture system in vitro and in xenograft tumors in nude mice growing from a human colorectal cancer cell line in vivo [31]. Another identified ROCK substrate, phosphatase and tensin homologue (PTEN), has been shown to be involved in the protective effect of ROCK inhibition on cardiomyocyte apoptosis [32] and EC survival in the cardiovascular system in vitro [33]. Activation of ROCK signalling is also highly likely to be involved in regulating motor neuron apoptosis in MND. In support of this, Takata and colleagues revealed that inhibition of ROCK by Fasudil or Y-27632 prevented motor neuron death in mouse motor neuron (NSC34) cell cultures and in the lumbar anterior horn in the SOD1 G93A mouse model [34]. Moreover, these two ROCK inhibitors were shown to delay disease onset, prolong the mean survival time and improve functional performance in the SOD1 G93A mouse model [34][37]. A multicentric, double-blind, randomised, placebo-controlled phase 2a clinical trial of Fasudil in 120 MND patients started in early 2019, aiming to assess its safety, tolerability and efficacy (ROCK-ALS trial, NCT03792490, Eudra-CT-Nr. : 2017-003676-31) [38]. In addition, three MND cases have been

reported in which fasudil was compassionately used for treatments from 2017 to 2019, demonstrating good tolerance [39]. However, it is unclear how ROCK signalling is activated/regulated in the pathogenesis of MND.

Considering that ROCK is part of the EphA4 downstream signalling pathway, the correlation of high expression of the EphA4 receptor with rapid disease progression of MND is consistent with the idea that EphA4 mediates this effect through increased ROCK signalling. The EphA4 receptor tyrosine kinase is expressed on the cell surface and exerts diverse effects through the activation of multiple downstream signalling pathways [40][41]. Following the activation of EphA4, the GTPase Rho family, including Rho, Rac1 and Cdc42, is activated, interacting with its downstream effector proteins to exert different effects [15][36][42][43][44]. ROCK is one of the major effectors for Rho, and their interaction induces conformational changes of Rho and activates ROCK [45]. It has been reported that EphA4 negatively affects axon regeneration after SCI primarily through the Rho/ROCK signalling pathway [15], and the administration of Y-27632 has been shown to increase dendritic branching and axonal regeneration [46]. Similarly, the expression of EphA4 is significantly increased following ischemia-reperfusion in vitro and in vivo, and the activation of EphA4 contributes to the disruption of the blood–brain barrier (BBB) post-ischemic brain injury through Rho/ROCK signalling [24]. In the subarachnoid hemorrhage (SAH) rat model, EphA4 activation has also been shown to be responsible for the neuronal apoptosis and BBB breakdown through the ROCK pathway [35]. Moreover, a reduction in EphA4 has been shown to improve the behavioural function of different ischemic stroke animal models via the inhibition of its downstream Rho/ROCK pathway [24][35][36]. Therefore, it is likely that during MND progression, high expression of EphA4 on motor neurons participates in the increasing motor neuron death by activating the Rho/ROCK downstream pathway.

### 3. Conclusions

Research to date suggests that EphA4 dysfunction in motor neurons likely contributes to progressive cell death directly in MND pathology. However, the underlying mechanism remains unclear. Here, we raise three potential mechanisms: EphA4 downstream Rho/ROCK signalling activation, EphA4-mediated d -serine-related NMDAR dysfunction and EphA4-dependent altered Ca<sup>2+</sup> currents. Further investigations are required to determine which mechanism plays the major role in regulating the EphA4-induced effect on motor neuron death in MND. Considering that EphA4 can bind to almost all ephrin ligands, different types of ligand-induced EphA4 activation may exert the same effect on motor neuron survival with different preferences for mechanisms, depending on the tissue- and cell-type specificity. These findings also indicate that mEphA4-Fc is likely to be an effective therapy for MND, due to its ability to competitively bind all EphA4 ligands. The related research into this mechanism could provide insight into combination drug therapies to further improve the therapeutic outcomes for MND patients.

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