Journal Club

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Inflammatory-Mediated Neuron-Glia Communication Modulates ALS Pathophysiology

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Review of Dutta et al.

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease. It is characterized by the deposition of aggregated proteins and predominantly affects motor neurons and the motor cortex. Although ALS is a genetically heterogeneous disease, mutations in superoxide dismutase 1 (SOD-1) and transactive response DNA-binding protein 43 (TDP-43), encoded by TARDBP, are the most prevalent. SOD-1 mutations are frequent in familial ALS, whereas TDP-43 is linked to sporadic ALS, with 97% of all sporadic cases exhibiting TDP-43 protein aggregates (Scotter et al., 2015).

Transgenic mice expressing mutant SOD-1 or mutant TDP-43 are the favored animal models for studying ALS *in vivo*, and these well-established models recapitulate many features observed in ALS patients, including TDP-43-related neurodegeneration and a rapid disease progression (Joyce et al., 2011). Mutant SOD-1 and mutant TDP-43 mice also develop protein aggregates resulting in neuroinflammation and motor neuron death, greatly enhancing their relevance in ALS research (Wegorzewska et al., 2009; Gill et al., 2019).

Neuroinflammation is a vital component of ALS pathogenesis. It is mediated

by neuronal interactions with infiltrating peripheral immune cells, such as lymphocytes, and with activated glial cells, including microglia (Komine and Yamanaka, 2015). During the early development of ALS, microglia adopt a so-called M2 activation state, in which they release neuroprotective factors, such as anti-inflammatory cytokines and neurotrophic factors (Suh et al., 2013; Gravel et al., 2016; Zhang et al., 2018). But as the disease progresses, microglia polarize toward an M1 activation state, in which they release pro-inflammatory cytokines and reactive oxygen species, both of which are toxic in motor neurons (Liao et al., 2012; Liu and Wang, 2017). The M1 activation state is induced by NF κ B, a transcription factor that serves as a master regulator of inflammation. NF κ B is upregulated in ALS and is a major trigger of microglial-induced motor neuron death (Swarup et al., 2011; Frakes et al., 2014). Because TDP-43 is a known activator of NF κ B, dysregulation of TDP-43 is suggested to be a key factor driving NF κ B upregulation and neuroinflammation in ALS pathogenesis (Swarup et al., 2011).

To further investigate the links between NF κ B, neuroinflammation, and ALS pathogenesis, a recent article by Dutta et al. (2020) published in *The Journal of Neuroscience* examined the effects of expressing a neuron-specific super-repressor of NF κ B signaling in mice. Specifically, the authors expressed a form of the NF κ B inhibitor I κ B α , which was modified to prevent phosphorylation

and subsequent proteasomal degradation, selectively in neurons in TDP-43 and SOD-1 mouse models of ALS. Importantly, expression of mutant I κ B α (I κ B α -SR) reduced molecular and behavioral hallmarks of ALS pathogenesis. Expression of I κ B α -SR in TDP-43 mutant mice reduced the nuclear translocation of the p65 subunit of NF κ B, thus reducing neuroinflammatory NF κ B signaling. This was associated with reduced cytoplasmic accumulation of TDP-43 in spinal motor neurons compared with TDP-43 mutant controls expressing WT I κ B α . Moreover, spinal cord extracts from single TDP-43 mutant mice exhibited higher levels of insoluble TDP-43 than double-transgenic I κ B α -SR/TDP-43 mice. This suggests that neuron-specific reduction in NF κ B signaling reduced TDP-43 protein aggregation in mice.

Because gliosis is one of the key changes associated with neuroinflammation in the pathophysiology of ALS, Dutta et al. (2020) asked how I κ B α -SR expression in TDP-43 mice affected markers of glial activation, specifically astrocytic GFAP and microglial ionized calcium binding adaptor molecule 1 (Iba1). Significant reductions in both markers were observed relative to control TDP-43 mice, suggesting that the neuronal reduction in NF κ B signaling was sufficient to modulate gliosis. In addition, the authors used immunofluorescent staining to examine astrocytes and microglia, focusing on microglial morphology. Resting microglial cells have a densely branched “ramified” morphology, whereas activated microglia exhibit less branching

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and become amoeboid to facilitate mobility and phagocytosis. Whereas microglia in control TDP-43 mutant mice had relatively few, and short branches in addition to intense Iba1 staining, double-transgenic $I\kappa B\alpha$ -SR;TDP-43 mice had more ramified branch patterns and Iba1 levels similar to WT controls.

Dutta et al. (2020) also observed an increase in autophagy in the spinal cord of mice expressing both $I\kappa B\alpha$ -SR and mutant TDP-43 compared with mice expressing mutant TDP-43 alone. This is notable because dysregulation of autophagy is thought to contribute to ALS progression. Mutated TDP-43 and SOD-1 have been implicated in propagating ALS pathogenesis through the induction of aberrant autophagy, a cellular process involved in packaging organelles and protein aggregates destined for lysosomal degradation. TDP-43 and SOD-1 transcriptionally regulate and influence several proteins involved in autophagy (Bose et al., 2011; Lee et al., 2015; Ying et al., 2016; Massenzio et al., 2018). Furthermore, mutated forms of TDP-43 upregulate autophagy markers in ALS patient tissues, whereas mutated SOD-1 proteins have been linked to an increase in autophagic vacuoles in mouse motor neurons (Ding et al., 2015; Wang et al., 2015; Xie et al., 2015). In addition, deletion of Beclin-1, an important autophagy regulator in mutant SOD-1 mice, was reported to impair autophagy, increase motor neuron loss, escalate SOD-1 aggregation, and upregulate microglial activation relative to that seen in control mice. Thus, impaired autophagy appears to exacerbate ALS pathogenesis (Tokuda et al., 2016). Together, the results presented by Dutta et al. (2020) indicate that inhibition of neuronal NF κ B signaling can significantly attenuate neuroinflammatory glial activation, stimulate autophagy, and reduce TDP-43 protein aggregates typically observed during the pathogenesis of ALS.

One factor that might explain both the reduction in microglial activation and the increase in autophagy is disruption in fractalkine signaling, a critical intercellular communication pathway modulated by NF κ B that becomes dysregulated in numerous brain disorders, including ALS (Harland et al., 2020; Pawelec et al., 2020). Fractalkine is a chemokine that is constitutively expressed by neurons, whereas its receptor is solely expressed by microglia (Harland et al., 2020). Fractalkine signaling has been identified as an integral means of mediating neuron-microglia crosstalk, which is

a process critical for the maintenance of synaptic function, information processing, and neuronal health (Sheridan and Murphy, 2013). Although the exact role of fractalkine within the CNS is not completely understood, multiple studies have suggested that fractalkine signaling has an anti-inflammatory effect by modulating microglial polarization toward the neuroprotective M2 phenotype (Liao et al., 2012; Zhang et al., 2018). Importantly, abnormal neuron-glia communication has previously been observed in a mouse model of ALS, with these mice expressing lower fractalkine levels accompanied by increased polarization of microglia toward a neurotoxic M1 phenotype (Zhang et al., 2018). Treatment with pro-inflammatory lipopolysaccharide, a well-established NF κ B activator, significantly reduces brain expression of fractalkine, leading to increased inflammatory microglial activation (Harland et al., 2020). Moreover, these effects were specific for the neuronal fractalkine ligand, with viral knockdown of neuronal fractalkine increasing inflammatory microglial activation (Harland et al., 2020). This suggests that excessive inflammation evident in ALS may precipitate aberrant fractalkine-mediated neuron-glia signaling, stimulating harmful microglial polarization, causing excessive neuroinflammation and motor neuron death.

Furthermore, fractalkine signaling has been shown to regulate autophagy, which is crucial for clearing protein aggregates in the cytoplasm of spinal neurons (Hebron et al., 2014). Interestingly, in transgenic ALS mouse models, loss of fractalkine signaling disrupted the autophagy-lysosome degradation pathway, and this was accompanied by abnormal protein accumulation (Liu et al., 2019). Additionally, loss of fractalkine signaling resulted in faster disease progression and significantly shorter lifespan in transgenic ALS mice, further indicating the importance of this signaling pathway in the pathophysiology of ALS (Liu et al., 2019).

Understanding how dysregulated NF κ B signaling is involved in ALS and other neurodegenerative diseases is critical for identifying potential therapeutic strategies. With a focus on the TDP-43 mouse model, using specific suppression of neuronal NF κ B, Dutta et al. (2020) have provided strong evidence that activation of this signaling pathway in neurons contributes to harmful neuroinflammatory processes resulting in toxic proteinopathy and motor neuron death. Increased motor neuron death was observed along with increased

gliosis, including excessive M1 microglial polarization, and this was alleviated with neuron-specific suppression of NF κ B signaling. This suggests that crosstalk between neurons and microglia is critical in ALS pathophysiology. Moreover, reductions in neuronal NF κ B signaling increased autophagy activity, suggesting that neuroinflammatory tone contributes to neuronal protein detoxification efficiency, which is critical for maintaining neuronal health. Previous work has shown that neuron-glia communication via chemokine mediators, such as fractalkine, plays an important role in restoring homeostatic microglial function and maintaining autophagy. However, the interaction between inflammatory signaling, neuron-glia communication, and ALS pathogenesis is complex and requires much further characterization of the neuroinflammatory mechanisms involved in the early potentiation of ALS motor neuron death.

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