PERSPECTIVE

Neuroprotective effects of vascular endothelial growth factor A in the experimental autoimmune encephalomyelitis model of multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by multiple demyelinating plaques in the white matter. For decades, the focus of MS research has been on inflammation-mediated demyelination of the white matter. However, recent studies suggest that neurodegeneration is not only an early event but also the primary cause of chronic disability in MS (Popescu and Lucchinetti, 2012). It is well documented that axon transection and loss occurs early in the CNS of MS patients. A number of studies also reveal significant neuron loss in the CNS gray matter of MS patients, including the cerebral cortex, cerebellum, hippocampus, thalamus, and spinal cord. Moreover, magnetic resonance image studies show that progressive brain atrophy in MS patients correlates well with disability. Interestingly, both axon degeneration and neuron loss occur early in the CNS of animals undergoing experimental autoimmune encephalomyelitis (EAE), the primary animal model used in MS research (Stanojlovic et al., 2016). While it is generally believed that inflammation is responsible for neurodegeneration in MS and EAE, the mechanisms governing the viability of neurons and axons in these diseases remain largely unknown (Friese et al., 2014).

Vascular endothelial growth factor A (VEGF-A) was originally identified as an endothelial cell specific growth factor, which stimulates angiogenesis and increases the permeability of blood vessels. Several lines of evidence have suggested that VEGF-A plays a role in various inflammatory diseases by enhancing angiogenesis and vascular permeability. Interestingly, recent studies show that VEGF-A also exerts direct actions on neurons and axons, and acts as a neurotrophic factor in the CNS under normal and disease conditions (Ruiz de Almodovar et al., 2009). The presence of VEGF-A in the CNS increases neuron survival and facilitates neurogenesis in various neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, spinocerebellar ataxia, and stroke. Moreover, there is evidence to suggest that VEGF-A is involved in the development of MS and EAE (Girolamo et al., 2014). Therefore, it is important to understand the effects of VEGF-A on neurodegeneration in MS and EAE.

VEGF-A exerts its function through several receptors, including VEGF receptor 1 (VEGFR1), VEGFR2, Neuropilin 1, and Neuropilin 2. It is believed that VEGF-A exerts direct actions on neurons and axons by binding to VEGFR2, resulting in autophosphorylation of the receptor and subsequent activation of its downstream signaling pathways (Carmeliet and Ruiz de Almodovar, 2013). In the last few years, a number of highly selective inhibitors of VEGFR2 have been identified. Among them, SU5416 (Z-3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone) is a potent VEGFR2 inhibitor and the first inhibitor to enter clinical trials for treatment of human diseases. Importantly, previous studies show that treatment with a low dose of SU5416 (10 mg/kg) attenuates the VEGF-A/VEGFR2 signaling in neurons and aggravates neuron death in mouse models of brain injury (Shimotake et al., 2010). Therefore, we sought to explore the role of the VEGF-A/VEGFR2 signaling in neurodegeneration in EAE mice by using SU5416.

While all major CNS cell types express VEGF-A, including neurons, astrocytes, oligodendroglia, microglia, and endothelial cells, previous data concerning the expression of VEGF-A in MS and EAE are contradictory. Some studies suggest that VEGF-A level is elevated in MS and EAE and that the elevated level of VEGF-A is associated with enhanced inflammation. In contrast, other studies show a decreased level of VEGF-A in these diseases (Girolamo et al., 2014). Therefore, we first measured the protein level of VEGF-A in the CNS of the well-characterized MOG35-55 EAE model using the highly sensitive and reproducible enzyme-linked immunosorbent assay (ELISA). When young adult female C57BL/6J mice are immunized with MOG35-55 peptide, the mice display neurological signs of disease starting as early as post-immunization day (PID) 12, reach the peak of disease around PID 19, and started recovering from EAE at around PID 22 (Lin et al., 2014). We found that VEGF-A level was not changed in the spinal cord of EAE mice at the onset of disease, but was significantly, although moderately, reduced at both the acute phase and chronic phase of EAE, compared to naïve mice (Stanojlovic et al., 2016). Nevertheless, we found that neither VEGFR2 level nor phosphorylated VEGFR2 level were significantly altered in lower motor neurons in the lumbar spinal cord of EAE mice compared to naïve mice (Stanojlovic et al., 2016).

Data indicate that the presence of VEGF-A in the CNS facilitates CNS inflammation in EAE mice by increasing the permeability of the blood-brain barrier (BBB) through activation of VEGFR2 in endothelial cells (Girolamo et al., 2014). Our goal was to dissect the effects of the VEGF-A/VEGFR2 signaling on neurodegeneration during EAE. As described above, inflammation contributes to neurodegeneration in MS and EAE. Thus, to achieve our goal, we must minimize the impact of SU5416 treatment on inflammation. Interestingly, a number of studies show that the impact of the VEGF-A/VEGFR2 signaling on CNS inflammation is determined by the timing of activation. A previous study reports that treatment with a high dose of SU5416 (50 mg/kg) during the acute phase of EAE attenuates inflammation in the CNS; however, the same treatment during the chronic phase of EAE does not alter inflammation (Roscoe et al., 2009). Moreover, evidence suggests that neurons are more responsive to the alteration of the VEGF-A/VEGFR2 signaling than endothelial cells in the CNS. Additionally, it is known that BBB breakdown and inflammatory cell infiltration occur in the CNS of EAE mice well before the onset of clinical symptoms (Lin et al., 2013). Therefore, we decided to use a low dose of SU5416 (20 mg/kg) and started the treatment on the day after EAE disease onset. Importantly, we found that treatment with the low dose of SU5416 (20 mg/kg) starting after EAE onset did not significantly alter EAE disease severity or inflammation in the CNS (Stanojlovic et al., 2016).

A previous *in vitro* study shows that oligodendrocyte progenitor cells (OPCs) express VEGFR2 and that VEGF-A enhances OPC migration by activating VEGFR2. Nevertheless, there is no evidence that the VEGF-A/VEGFR2 signaling regulates the viability or function of mature oligodendrocytes under physiological or pathological conditions (Girolamo et al., 2014). It is well documented that oligodendrocyte death and myelin damage are caused by inflammation during EAE. Since treatment with the low dose of SU5416 (20 mg/kg) starting after EAE onset did not significantly alter inflammation in the CNS, it is not surprising

that we found a minimal effect of SU5416 treatment on oligodendrocyte death and demyelination in the CNS of EAE mice. On the other hand, in accordance with the previous studies (Shimotake et al., 2010), we found that treatment with the low dose of SU5416 (20 mg/kg) starting after EAE onset impaired VEGFR2 activation in neurons in the CNS of EAE mice. Importantly, quantitative immunohistochemistry analysis showed that SU5416 treatment exacerbated lower motor neuron loss and axon loss in the lumbar spinal cord of EAE mice. Nevertheless, SU5416 treatment had no effect on Purkinje neuron loss in the cerebellum or upper motor neuron loss in the layer V of the cerebral cortex during EAE (Stanojlovic et al., 2016). These data suggest that activation of the VEGF-A/VEGFR2 signaling protects lower motor neurons and axons in the spinal cord against inflammation during EAE, but does not influences the viability of other neuron-types, such as Purkinje neurons or upper motor neurons.

Evidence suggests that VEGF-A is an essential neurotrophic factor for lower motor neurons in the spinal cord under physiological conditions, and that activation of the VEGF-A/VEGFR2 signaling enhances lower motor neuron survival under pathological conditions (Carmeliet and Ruiz de Almodovar, 2013). In agreement with these studies, our results revealed the neuroprotective effects of the VEGF-A/VEGFR2 signaling on lower motor neurons and axons in the spinal cord during EAE. This study implies that therapeutic strategies that activate the VEGF-A/VEGFR2 signaling in neurons may be beneficial to lower motor neurons and axons in the spinal cord of MS patients.

Although our results derived from a pharmacological approach, namely SU5416 treatment, provide the first evidence that the VEGF-A/VEGFR2 signaling is involved in regulating the viability of neurons and axons in animal model of MS, the approach has several inherent shortcomings. First, it is impossible to rule out the involvement of other CNS cell types in the protective effects of the VEGF-A/VEGFR2 signaling on lower motor neurons and axons in the spinal cord during EAE. All major CNS cell types express VEGFR2, including neurons, astrocytes, oligodendroglia, microglia, and endothelial cells, and all these cells produce some types of neurotrophic factors. There is a possibility that SU5416 treatment suppresses the production of neurotrophic factors in non-neuronal cells *via* blockage of VEGFR2, resulting in exacerbation of neurodegeneration in EAE mice. Second, we cannot exclude the contribution of the alteration of infiltration of inflammatory cells or expression of inflammatory mediators to the exacerbated neurodegeneration in EAE mice treated with SU5416. The molecular mechanisms by which inflammation causes neurodegeneration in MS and EAE remain largely unknown. Many inflammatory cells and inflammatory mediators are considered as a double-edged sword in neurodegenerative diseases. SU5416 treatment may induce a subtle change in infiltration of inflammatory cells or expression of inflammatory mediators *via* blockage of VEGFR2 in endothelial cells or inflammatory cells, resulting in exacerbation of neurodegeneration in EAE mice. Third, it is unclear how SU5416 treatment exacerbates lower motor neuron loss and axon loss in the spinal cord during EAE, but does not affect the viability of Purkinje neurons or upper motor neurons. Does the VEGF-A/VEGFR2 signaling selectively function in certain neuron types during EAE? Clearly, the protective role of the VEGF-A/VEGFR2 signaling on neurons in MS warrants further investigation. A genetic mouse model that allows for activation or inactivation of VEGFR2 selectively in neurons would be an ideal model to overcome the shortcomings mentioned above.

In summary, using a well-characterized, selective VEGFR2 inhibitor, our recent study indicates the neuroprotective role of the VEGF-A/VEGFR2 signaling in the EAE model of MS. Nevertheless, our mouse model system has its limits; additional studies are essential and necessary to verify the cell autonomous role of the VEGF-A/VEGFR2 signaling in neurons in the development of MS and EAE. Additionally, there are many other open questions regarding the role of VEGF-A in MS and EAE: 1) cellular sources of VEGF-A; 2) the mechanisms regulating the production of VEGF-A; 3) the effects of the VEGF-A/VEGFR2 signaling on other CNS cell types.

We thank Sarrabeth Stone for critically reading the manuscript. Wensheng Lin is supported by grants from the National Institutes of Health (NS073132 and NS094151) and the National Multiple Sclerosis Society (RG4813-A-2 and RG5239-A-3).

Wensheng Lin*
Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA; Institute for Translational Neuroscience, University of Minnesota, Minneapolis, MN, USA
***Correspondence to:** *Wensheng Lin, M.D., Ph.D., linw@umn.edu.*
***Accepted:** *2017-01-14*
***orcid:** *0000-0003-4931-7642 (Wensheng Lin)*

***doi:** *10.4103/1673-5374.198982*
***How to cite this article:** *Lin W (2017) Neuroprotective effects of vascular endothelial growth factor A in the experimental autoimmune encephalomyelitis model of multiple sclerosis. Neural Regen Res 12(1):70-71.*

References

Carmeliet P, Ruiz de Almodovar C (2013) VEGF ligands and receptors: implications in neurodevelopment and neurodegeneration. Cell Mol Life Sci 70:1763-1778.

Friese MA, Schattling B, Fugger L (2014) Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. Nat Rev Neurol 10:225-238.

Girolamo F, Coppola C, Ribatti D, Trojano M (2014) Angiogenesis in multiple sclerosis and experimental autoimmune encephalomyelitis. Acta Neuropathol Commun 2:84.

Lin W, Lin Y, Li J, Fenstermaker AG, Way SW, Clayton B, Jamison S, Harding HP, Ron D, Popko B (2013) Oligodendrocyte-specific activation of PERK signaling protects mice against experimental autoimmune encephalomyelitis. J Neurosci 33:5980-5991.

Lin Y, Huang G, Jamison S, Li J, Harding HP, Ron D, Lin W (2014) PERK activation preserves the viability and function of remyelinating oligodendrocytes in immune-mediated demyelinating diseases. Am J Pathol 184:507-519.

Popescu BF, Lucchinetti CF (2012) Pathology of demyelinating diseases. Annu Rev Pathol 7:185-217.

Roscoe WA, Welsh ME, Carter DE, Karlik SJ (2009) VEGF and angiogenesis in acute and chronic MOG((35-55)) peptide induced EAE. J Neuroimmunol 209:6-15.

Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P (2009) Role and therapeutic potential of VEGF in the nervous system. Physiol Rev 89:607-648.

Shimotake J, Derugin N, Wendland M, Vexler ZS, Ferriero DM (2010) Vascular endothelial growth factor receptor-2 inhibition promotes cell death and limits endothelial cell proliferation in a neonatal rodent model of stroke. Stroke 41:343-349.

Stanojlovic M, Pang X, Lin Y, Stone S, Cvetanovic M, Lin W (2016) inhibition of vascular endothelial growth factor receptor 2 exacerbates loss of lower motor neurons and axons during experimental autoimmune encephalomyelitis. PLoS One 11:e0160158.