TARGETED DELIVERY OF THE NgR-Fc PROTEIN TO PROMOTE NEUROREPAIR IN A MODEL OF MULTIPLE SCLEROSIS

Sining Ye[1], Paschalis Theotokis[2], Danica Nheu[1], Olivia Ellen[1], Padmanabhan Ramanujam[1], Jae Young Lee[3], Michael F Azari[1], Steven Petratos[1]

[1] Department of Neuroscience, Central Clinical School, Monash University, Prahran, Victoria, Australia
[2] B', Department of Neurology, Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Thessaloniki, Greece
[3] ToolGen Inc., Gasan Digital-Ro, Geumcheon, Seoul, Korea

Summary

Multiple sclerosis (MS) is an autoimmune-mediated inflammatory demyelinating and degenerative disease occurring in the central nervous system (CNS). There are no current therapeutics available to treat patients with progressive MS. Hence, mechanisms that govern CNS neuroprotection and repair need to be elucidated to provide novel targeted therapeutics to reverse permanent CNS damage. Our laboratory designed a novel method of delivering the NgR (310) ecto-myc-Fc fusion protein by incorporating the DNA construct into a lentiviral vector and transducing donor hematopoietic stem cells (HSCs) ex vivo, followed by their transplantation in recipient mice to target inflammatory demyelinating lesions that ensue during the MOG35-55-induced experimental autoimmune encephalomyelitis (EAE) mouse model. The aim of this study was to investigate the potential therapeutic effects of lesion-specific delivery of the NgR (310) ecto-Fc protein, following the lineage differentiation of the transplanted HSCs, demonstrating neuroprotection and neurorepair during the course of EAE.

Key words: Nogo A, experimental autoimmune encephalomyelitis, Nogo Receptor, NgR-Fc, haematopoietic stem cells, remyelination, neurorepair, multiple sclerosis

Introduction

Multiple Sclerosis (MS) is an autoimmune disease with neurodegeneration characterized by inflammation and demyelination within the central nervous system (CNS). It impacts an individual's quality of life substantially and places a heavy burden on the public health system, with women more commonly diagnosed at the prime of their lives with onset of symptoms occurring between the ages of 20-40-year-olds [1]. The cause remains elusive, however, the presence of a heterogeneous array of symptoms involving motor, sensory, visual and autonomic systems contribute to it being the most common cause of non-traumatic neurological disability in young adults [2]. The unique symptoms arise from the development of multiple lesions across the CNS; thus, no individual may experience the exact symptoms at a specific stage of the disease course. The major effectors in the pathogenesis and sequelae of MS are infiltrating activated macrophages and endogenous microglia [3]. Due to the leaky blood-brain barrier (BBB) during active MS, monocytic-derived macrophages from the periphery may infiltrate the CNS and along with endogenous microglia, transition into a proinflammatory phenotype, actively contributing to the proinflammatory propagation of demyelination and eventually axonal damage [4]. Moreover, the activation of astrocytes and eventual dropout of mature oligodendrocytes, along with the pathological modifications of the cellular milieu all play a part in the expansion of lesion burden, promulgating neurodegenerative change over time [5, 6].

The heterogeneity of the disease poses a challenge for designing effective therapeutics that target multiple cellular and extracellular reactive changes within the brains of individuals living with MS, especially when the disease progresses. Currently, the treatments available are either immunomodulatory or immunosuppressive, limited to reducing the relapse rate for patients only. As the disease progresses, there exists no effective treatment to halt the progression towards neurodegeneration and elicit neurorepair. Limitations for effective neurorepair, have been suggested partially due to inhibitory factors in the MS lesion milieu exerted through the deposition of substantive myelin-associated inhibitory factors (MAIFs), with the most potent being the integral myelin protein, Nogo-A [7, 8]. Nogo-A, a neurite outgrowth inhibitor, is localized on the surface of oligodendrocytes and myelin sheaths [9]. It exerts

this effect by binding with high affinity to Nogo-66 receptor 1 (NgR1), which can also bind other MAIFs such as myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) [9]. The expression of Nogo-A and NgR1 has been found upregulated in many CNS disorders, that can include MS, spinal cord injury (SCI) and brain injury, stroke, glaucoma to name a few [10]. It has been established that absence or blockage of Nogo-A may limit and protect the progression of the animal model of MS, namely experimental autoimmune encephalomyelitis (EAE) [11, 12]. Furthermore, there are suggestions that Nogo-A inhibition may shift activated macrophage and microglia from pro-inflammatory to anti-inflammatory phenotypes, promoting repair [12]. Thus, limiting the effects of Nogo-A may be a potential target to overcome the barriers to neurorepair. Designing an effective therapy that can target Nogo-A must also take into consideration that it must be able to traverse the BBB, allowing access to lesion sites within the CNS. Hence, investigations utilizing novel means of delivering antagonizing biologics such as the NgR1-Fc fusion protein may well prove to be an excellent neuroprotective or even reparative measure. The use of a NgR1-Fc fusion protein has had promising results in preclinical studies in spinal cord injury (SCI) and stroke, however, clinically effective measures of delivery across the BBB still pose a major challenge [13]. The possibility of targeting Nogo-A and its cognate receptor, NgR1, as a potential therapeutic along with hematopoietic stem cell (HSC)-based delivery methods to overcome these limitations has been investigated in our laboratory.

Blocking NgR1 signalling and how novel is the treatment?

There are several methods that can be used to block NgR1 signalling, such as humanised antibodies, fusion proteins, peptides, and pharmacological blockers [14]. Blocking of the signalling pathways can be done by blocking either the receptor (NgR1) or blocking the ligand (MAIFs). An example of blocking the receptor is the conditional deletion of NgR1 ($ngr1^{-/-}$) via the cre-lox system in EAE mice reduced axonal damage in the optic nerves of mice even in the presence of neuroinflammatory lesions [8]. In the non-human primate model of spinal cord injury (SCI), administration of NgR1-Fc increased the axon density compared to the control group [15]. On the other hand, an example of blocking the ligand is through therapeutic antibodies directed against Nogo-A in EAE rats to promote recovery and remyelination [16]. Recently the humanised anti-Nogo-A-antibody ATI355 entered a phase I clinical trial to treat SCI [17]. In a study conducted by *Tsai et al.*, giving adult rats with stroke anti-Nogo-A-antibody (11C7) ameliorates the impairment of the forelimbs [18]. In the study of optic nerve lesion, knocking down NgR1 or neutralising NogoA leads to more regeneration of the nerve but the growth rarely exceed 2 mm [19].

NgR (310) ecto-Fc fusion protein

In order to facilitate myelin debris uptake and promote remyelination, NgR (310) ecto-Fc fusion protein is constructed. The fusion protein consists of the soluble portion of NgR1 containing the ligand-binding domain, which binds to MAIF to limit the inhibition of axonal neurite outgrowth [20]. Combining this soluble portion with Fc region of immunoglobulin G (IgG) enhances the binding to activated monocytes, increasing the myelin debris clearance [21].

Delivering NgR (310) ecto-Fc fusion protein using HSCs

The delivery of fusion protein to the CNS is often hindered by the presence of a blood-brain barrier [22] but immune cells such as T cells and macrophages can pass through the BBB [23]. HSCs, as mentioned before, are capable of differentiating into these immune cells. HSCs can also be used as vehicles for drug delivery [24] and they can be modified using a lentiviral vector to express the gene of interest [25-27]. Consequently, HSCs can be used to deliver the NgR(310)ecto-Fc fusion protein to the lesion sites, which will promote the myelin debris clearance by macrophage [20].

Conclusion

The hallmarks of multiple sclerosis are inflammation in the central nervous system and demyelination of neurons, leading to axonal injury as well as neurological decline. The cause of this disease is still yet to be determined but peripheral immune cells and glial cells have been shown to contribute to the pathophysiological aspect of multiple sclerosis. Currently, there is no curative treatment for clinically diagnosed patients and the available therapies have little to no efficacy on patients with the progressive forms of the disease. Since haematopoietic stem cells are able to traverse the BBB and NgR (310) ecto-Fc fusion protein has been shown to increase the myelin debris clearance and remyelination, combining both therapies might provide a better therapeutic avenue for MS patients.

References

[1] Ahmad H, Palmer JA, Campbell JA, Mei I, Taylor B. Health Economic Impact of Multiple Sclerosis in Australia in 2017. Australia: Multiple Sclerosis Research Australia; 2017 August 2018. Contract No.: 1.

[2] Koch-Henriksen N, Sørensen PS. The changing

demographic pattern of multiple sclerosis epidemiology. The Lancet Neurology. 2010;9(5):520-32.
[3] Brück W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, et al. Monocyte/macrophage differentiation in early multiple sclerosis lesions. Ann Neurol. 1995;38(5):788-96.
[4] Miron VE, Boyd A, Zhao J-W, Yuen TJ, Ruckh JM, Shadrach JL, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci. 2013;16(9):1211-8.
[5] Ponath G, Park C, Pitt D. The Role of Astrocytes in Multiple Sclerosis. Front Immunol. 2018;9:217.
[6] Wolswijk G. Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis. Brain. 2000;123(1):105-15.
[7] Lee JY, Petratos S. Multiple Sclerosis:Does Nogo Play a Role? The Neuroscientist. 2013;19(4):394-408.
[8] Petratos S, Ozturk E, Azari MF, Kenny R, Lee JY, Magee KA, et al. Limiting multiple sclerosis related axonopathy by blocking Nogo receptor and CRMP-2 phosphorylation. Brain : a journal of neurology. 2012;135(Pt 6):1794-818.
[9] Schmandke A, Schmandke A, Schwab ME. Nogo-A: Multiple Roles in CNS Development, Maintenance, and Disease. The Neuroscientist. 2014;20(4):372-86.
[10] Schwab ME, Strittmatter SM. Nogo limits neural plasticity and recovery from injury. Curr Opin Neurobiol. 2014;27:53-60.
[11] Yang Y, Liu Y, Wei P, Peng H, Winger R, Hussain RZ, et al. Silencing Nogo-A promotes functional recovery in demyelinating disease. Annals of neurology. 2010;67(4):498-507.
[12] Alrehaili AA, Lee JY, Bakhuraysah MM, Kim MJ, Aui PM, Magee KA, et al. Nogo receptor expression in microglia/macrophages during experimental autoimmune encephalomyelitis progression. Neural Regen Res. 2018;13(5):896-907.
[13] He HW, Zhang YL, Yu BQ, Ye G, You W, So KF, et al. Soluble Nogo receptor 1 fusion protein protects neural progenitor cells in rats with ischemic stroke. Neural Regen Res. 2019;14(10):1755-64.
[14] Zörner B, Schwab ME. Anti-Nogo on the go: from animal models to a clinical trial. Ann N Y Acad Sci. 2010;1198 Suppl 1:E22-34.
[15] Wang X, Zhou T, Maynard GD, Terse PS, Cafferty WB, Kocsis JD, et al. Nogo receptor decoy promotes recovery and corticospinal growth in non-human primate spinal cord injury. Brain. 2020;143(6):1697-713.
[16] Ineichen BV, Kapitza S, Bleul C, Good N, Plattner PS, Seyedsadr MS, et al. Nogo-A antibodies enhance axonal repair and remyelination in neuroinflammatory and demyelinating pathology. Acta Neuropathol. 2017;134(3):423-40.
[17] Kucher K, Johns D, Maier D, Abel R, Badke A, Baron H, et al. First-in-Man Intrathecal Application of Neurite Growth-Promoting Anti-Nogo-A Antibodies in Acute Spinal Cord Injury. Neurorehabil Neural Repair. 2018;32(6-7):578-89.
[18] Tsai S-Y, Papadopoulos CM, Schwab ME, Kartje GL. Delayed anti-nogo-a therapy improves function after chronic stroke in adult rats. Stroke. 2011;42(1):186-90.
[19] Pernet V, Schwab ME. The role of Nogo-A in axonal plasticity, regrowth and repair. Cell Tissue Res. 2012;349(1):97-104.
[20] Kim MJ, Kang JH, Theotokis P, Grigoriadis N, Petratos S. Can We Design a Nogo Receptor-Dependent Cellular Therapy to Target MS? Cells. 2018;8(1):1.
[21] Kuhlmann T, Brück W. Immunoglobulins induce increased myelin debris clearance by mouse macrophages. Neurosci Lett. 1999;275(3):191-4.
[22] Pardridge WM. Drug transport across the blood-brain barrier. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2012;32(11):1959-72.
[23] Biffi A, De Palma M, Quattrini A, Del Carro U, Amadio S, Visigalli I, et al. Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. J Clin Invest. 2004;113(8):1118-29.
[24] Labusca L, Herea DD, Mashayekhi K. Stem cells as delivery vehicles for regenerative medicine-challenges and perspectives. World J Stem Cells. 2018;10(5):43-56.
[25] Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science. 2013;341(6148):1233151.
[26] Miyoshi H. Gene delivery to hematopoietic stem cells using lentiviral vectors. Methods Mol Biol. 2004;246:429-38.
[27] van Til NP, Wagemaker G. Lentiviral gene transduction of mouse and human hematopoietic stem cells. Methods Mol Biol. 2014;1185:311-9.