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EXPERT OPINION

Thymosin β4 as a restorative/ regenerative therapy for neurological injury and neurodegenerative diseases

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Thymosin β4 (Tβ4) promotes CNS and peripheral nervous system (PNS) plasticity and neurovascular remodeling leading to neurological recovery in a range of neurological diseases. Treatment of neural injury and neurodegenerative disease 24 h or more post-injury and disease onset with Tβ4 enhances angiogenesis, neurogenesis, neurite and axonal outgrowth, and oligodendrogenesis, and thereby, significantly improves functional and behavioral outcomes. We propose that oligodendrogenesis is a common link by which Tβ4 promotes recovery after neural injury and neurodegenerative disease. The ability to target many diverse restorative processes via multiple molecular pathways that drive oligodendrogenesis and neurovascular remodeling may be mediated by the ability of Tβ4 to alter cellular expression of microRNAs (miRNAs). However, further investigations on the essential role of miRNAs in regulating protein expression and the remarkable exosomal intercellular communication network via exosomes will likely provide insight into mechanisms of action and means to amplify the therapeutic effects of Tβ4.

Keywords: microRNA, oligodendrocyte progenitor cells, thymosin beta 4, tissue plasminogen activator, traumatic brain injury

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Treatment of neurological diseases has traditionally focused on reducing lesions, for example, infarct and cell death after stroke and traumatic brain injury (TBI), and reducing focal lesions in multiple sclerosis (MS). This approach has led to few clinical benefits, essentially, no effective long-term clinically relevant therapeutic approaches. For example in stroke, tissue plasminogen activator (tPA) is employed to thrombolyse the intravascular clot causing the stroke. Yet only ~ 5% of patients receive tPA and, the beneficial effect is modest, with only ~ 30% of those patients receiving tPA showing significant improvements [1]. Similar conditions exist for neural injury after TBI, neurodegeneration, for example, after MS. Likewise in the peripheral nervous system (PNS), for diseases such as peripheral neuropathy, which affects the elderly, diabetic patients and patients on chemotherapy, there is a paucity of therapeutic options. It is therefore time to reconsider how we think about treating neural injury and disease. A fruitful therapeutic approach would be to concentrate efforts on the restorative processes intrinsic to the peripheral and CNS. Thus, we would seek to amplify by pharmacological means endogenous restorative processes present after injury; treatment would primarily target intact tissue to amplify rewiring, and enhance neurovascular remodeling and thereby improve neurological recovery.

Restorative therapies, designed to remodel the nervous system and thereby to promote neurological recovery have multiple benefits compared with neuroprotective strategies. A major benefit is that the treatment may be applied well after the onset

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of injury or the onset of clinical symptoms for degenerative diseases. Neuroprotection by targeting the damaged tissue must be applied before the target tissue undergoes irreversible processes leading to cell death. The neuroprotection therapeutic window is measured at time scales of minutes or at most a few hours post-damage [2]. Thus, treatment must be delivered to the site of injury to reverse molecular cascades leading to necrosis and apoptosis. The ability to deliver therapy to these affected tissues faces a further complication of compromised perfusion, abnormalities or lack of blood flow to systemically deliver drug to reverse injury. In contrast, neurorestorative agents that target intact tissue have a therapeutic window, within the time span of days and weeks, since there is no race against rapidly dying cells. Likewise, tissue perfusion and blood flow to the target tissues are likely not compromised by the injury and therefore treatments whether cell-based or pharmaceutical-based may be effectively delivered to the site of injury.

The therapeutic approach to stimulate endogenous neurorestorative processes has shown promise for the treatment of stroke and TBI. Some of the very initial work on neurorestorative therapy for the treatment of neural injury and disease was performed using cell-based therapy. Stem/progenitor cells stimulate a set of highly interactive processes, such as angiogenesis, neurogenesis, oligodendrogenesis, synaptogenesis and axonal outgrowth, which in-concert orchestrate neurological recovery [3,4]. Cell-based therapy, for example, stimulates the generation of angiogenic factors that promote vascular remodeling, and the activated and newly formed endothelial cells act as a source of trophic factors, that also enhance neurogenesis within the subventricular zone (SVZ) and neurite outgrowth. The newly formed cells migrate from the SVZ to the region of injury, interact with activated vasculature and differentiate into neurons and oligodendrocytes (OLGs) [3-5]. This restorative tapestry of events creates a supportive environment for neurovascular plasticity, neurite outgrowth and myelination of axons. In addition, the stimulated and newly generated cells release trophic factors that facilitate remodeling throughout the nervous system. Thus, treatment of stroke and TBI with cell-based or pharmacological-based restorative therapy promotes cortical spinal track remodeling and plasticity in the spinal cord and contralateral and ipsilateral hemispheres, which drive neurological recovery [3,6].

Thymosin β4 (Tβ4) promotes remodeling of the CNS/PNS post-neural injury and thereby improves neurological recovery [7-10]. Tβ4 expressed in nearly all mammalian cells is a secreted peptide containing 43-amino acid, which sequesters monomer G-actin. Tβ4 is a pleiotropic peptide that impacts and amplifies multifaceted restorative processes. We and others have employed Tβ4 to treat a variety of neurological injuries and degenerative diseases post-onset of neurological symptoms, including stroke, TBI, intracerebral hemorrhage and diabetic peripheral neuropathy [7-11]. During the chronic stage after neural injury or clinical onset of symptoms, Tβ4 amplifies neurovascular remodeling that promotes neurological benefit [10].

An important common thread by which Tβ4 weaves neurological recovery in multiple disease and injury states is by stimulating OLGs, OLG progenitor cells (OPCs) and myelination. OLGs and their equivalent in the PNS, Schwann cells, play vital roles in neurological recovery. OPCs and mature OLGs/Schwann cells secrete factors and act as stem-like and progenitor cells that foster recovery. The OLGs are the only myelinating cells in the CNS, and Schwann cells are the parallel myelinating cells in the PNS. Myelination or the loss thereof adversely affects neurological function. Diabetes, MS, TBI and stroke evoke demyelination and white matter damage, which result in neurological deficits. OLGs also maintain the structure of the CNS and their abundant presence in brain retards cerebral atrophy [5]. Thus, Tβ4 that promotes generation and differentiation of OPCs and thereby increases OLGs and myelination contributes to the improvement of neurological recovery found in multiple animal models of neurological injury and neurodegenerative disease [7,9,10]. Although the focus of this editorial is on the effect of Tβ4 on oligodendrogenesis as the common medium for multi-neurological disease deficits, we do not exclude major neurorestorative effects of Tβ4 in activating an array of molecular pathways, and we also affirm Tβ4 as a potent anti-inflammatory agent in mediating neurological recovery. These multifaceted Tβ4-mediated events are likely complementary, interdependent and additive, if not synergistic. We and others have demonstrated that Tβ4 concurrently stimulates the expression of a variety of molecular mediators of neurovascular plasticity; this includes the angiopoietin/Tie2, PI3k/Akt, and epidermal growth factor pathways, among others.

How does a pleiotropic agent such as Tβ4 promote oligodendrogenesis and impact multiple pathways of neurological recovery? To obtain insight into the Tβ4-mediated activation of multiple molecular pathways, we investigated the effects of Tβ4 on microRNAs (miRNAs), small 22 – 25 nt, noncoding RNAs that have the ability to concurrently affect the translation of many proteins [12]. We found that Tβ4 prominently stimulates miRNA-146a in embryonic OPCs and in an OPC-related cell line, and promotes the generation and differentiation of OPCs and OLGs, which may serve as a common therapeutic mechanism [13]. We are, however, acutely aware that miRNA-146a is not alone in driving neurological recovery, and there are interwoven webs of miRNAs affected by Tβ4 that enhance neurological recovery. As an illustration of principle, we focus on miR-146a as mediating the restorative effects of Tβ4 on neurorecovery, particularly in regard to oligodendrogenesis.

What is missing from this analysis of how an agent such as Tβ4 mediates neurovascular remodeling in many disease states via miRNAs, is how the Tβ4-mediated miRNA instructions are communicated among cells. There is a major emerging literature that exosomes mediate this intercellular communication. Exosomes are small (~ 30 – 100 nm) lipid particles, which contain RNAs, proteins, miRNAs and membrane receptors. They form a quantized means to communicate and to

protect the transfer of proteins, and gene-regulating instruction between cells [14]. Exosomes, nanoparticle size vesicles that mediate intracellular communication, may be affected by Tβ4, and this effect of Tβ4 on exosomes and the cargo of the exosomes have not been addressed in the literature, and may be a fertile area of future research in understanding the broad affect of Tβ4 on neurological recovery in many diseases. Elucidation of this vital medium of intercellular communication and the potential role of Tβ4 in modulating this communication may lead to promising therapies for neurological and other diseases.

Tβ4 has substantial potential for clinical translation for the treatment of neurological disease and injury. Safety of Tβ4 has been demonstrated in human trials [15]. Preclinical studies, as described, provide robust data demonstrating efficacy at reasonable doses and clinically applicable time windows of treatment. Mechanisms of neurorestorative therapeutic activity have been well characterized. Thus, the evident safety of Tβ4 and the varied and multiple preclinical studies neurorestorative satisfy necessary conditions and provide ample justification to move Tβ4 into clinical trials for the treatment of neurological disease and injury.

Expert opinion

Tβ4 has a broad net of restorative effects on neurological injury and degeneration in the CNS and PNS. Tβ4 has a remarkable capacity to promote CNS and PNS plasticity and neurovascular remodeling leading to neurological recovery in a range of neurological diseases. Treatment of neural injury and neurodegenerative disease 24 h or more post-injury and disease onset with Tβ4 enhances neurovascular plasticity, for example, angiogenesis, neurogenesis, neurite and axonal outgrowth, and oligodendrogenesis, the focus of this editorial, and thereby, significantly improves functional and behavioral outcomes. The effect of Tβ4 on OLG/OPCs is common to all the neurological disease and injury states we have tested, stroke, TBI, experimental autoimmune encephalomyelitis/MS and diabetic peripheral neuropathy, and we propose, oligodendrogenesis may be a common link by which Tβ4 promotes recovery. The ability to target many diverse restorative processes via multiple molecular pathways that drive oligodendrogenesis and neurovascular remodeling may be mediated by the ability of Tβ4 to alter cellular expression of miRNAs. Here, we have focused on miR-146a, with no intention to minimize or neglect the potential effects of Tβ4 on other miRNAs, and we also have not addressed the issues of intercellular communication via exosomes. However, given the essential role of miRNAs in regulating protein expression and the remarkable intercellular communication network mediated by exosomes, particularly the miRNA-based genetic instructions for protein expression, will provide insight into mechanisms of action and means to amplify the therapeutic effects of Tβ4.

Declaration of interest

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Notice of correction

Please note the sentence "This paper is part of a supplemental issue, sponsored by SciClone" was added to the declaration of interest section after initial online publication of this article (23rd January 2015).