Review

Role of GDNF in Spinal Cord Injury Repair

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Abstract: Following an initial mechanical insult, traumatic spinal cord injury (SCI) induces a secondary wave of injury, resulting in a toxic lesion environment inhibitory to axonal regeneration. This review focuses on the glial cell line-derived neurotrophic factor (GDNF) and its application, also in combination with other factors and cell transplantations, for repairing the injured spinal cord. As recent decades of studies strongly suggest combinational treatment approaches hold the greatest therapeutic potential for the central nervous system (CNS) trauma, future directions of combinational therapies will also be discussed.

Keywords: Spinal cord injury, glial cell line-derived neurotrophic factor (GDNF), GFRα-1, cRET, Schwann cells, Astrogliosis, neuroprotection, axonal regeneration, combinational therapies, neurotrauma.

SCI background and need for therapies

Spinal cord injury (SCI) is a devastating chronic condition for which no effective treatments currently exist. Singh, Fehlings et al. [57] conducted a systematic review of global statistics, beginning with 5,874 articles with a final inclusion of 48 articles, reporting worldwide SCI statistics, with the United States having the highest prevalence (906 cases per 1 million people); New Zealand having the highest reported national incidence (49.1 cases of SCI per 1 million people); and Spain (8 cases of SCI per 1 million people) and Fiji (10 cases of SCI per 1 million people) showing the lowest national incidences. The primary cause of SCI cases worldwide is motor vehicle accidents, followed by falls and sports injuries, for most countries [57]. The long-term potential of chronic pain, inflammation, and devastating disabilities that SCI patients endure are compounded by the extensive lifetime costs of care. Approximately 1 - 5 million United States dollars is spent over the lifetime of an SCI patient, depending upon the patient's age and level of injury [NSCISC – National Spinal Cord Injury Statistical Center, 2018]. The national cost in the United States is estimated at more than $400 billion US dollars for current and future healthcare for patients suffering from SCI.

The initial SCI mechanical trauma disrupts local vasculature and leads to a breakdown of the blood-spinal cord barrier [47, 50, 54]. This is followed by secondary wave of injury [55], comprised of hemorrhage, ischemia [59] excitotoxicity, edema, neuronal apoptosis, loss of gray and white matter tissue [60], axonal die-back, chronic inflammation [42], and the formation of a dense astrocytic glial scar surrounding the lesion. During the acute phase after SCI, the astrogliosis is presumed to be a positive regulator in limiting the spread of excitotoxic molecules, thus limiting the lesion area. For decades, the astrocytic glial scar has been considered inhibitory in chronic phases after SCI. However, recent literature supports beneficial axon regeneration in response to the

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astrocytic scar formation [2]. Glial cell line-derived neurotrophic factor (GDNF) has been shown to positively modulate astrogliosis [28, 14, 3], in addition to its known neuroprotective effects, thus making astrocytes a potential therapeutic target in SCI.

Discovery of GDNF family ligands and receptors

The GDNF subfamily of neurotrophic ligands consists of GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN), which bind to the glycosylphophatidylinositol-anchored GFRα receptors 1-4, respectively [68]. The molecular structures of the GDNF family ligands and receptors are nicely detailed by [69], as well as in Figure 1. While ARTN [71-72], NRTN [10, 27, 20], and PSPN [62, 43] have all been shown to be neuroprotective, this mini review focuses specifically on GDNF and its applications for the treatment of SCI.

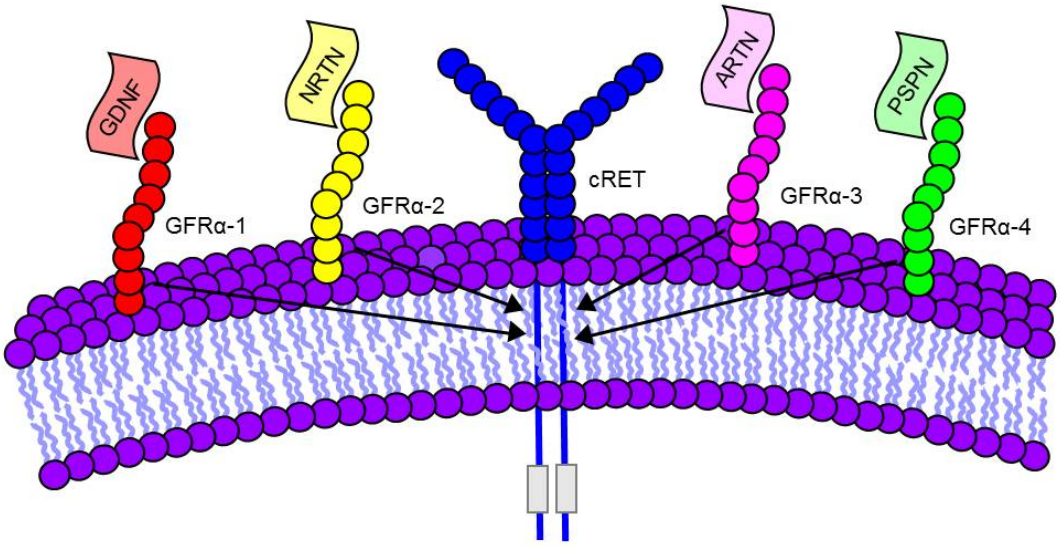

Figure 1: GDNF family of ligands and receptors. GDNF binds to GFRα-1, NRTN binds to GFRα-2, ARTN binds to GFRα-3, and PSPN binds to GFRα-4. GFRα 1-4 bind to cRET co-receptors.

GDNF was first identified as a neurotrophic factor released from glial cells by Engele et al. [19] and Lin et al. [38], in its promotion of the survival of dopaminergic neurons. The GFRα-1 receptor was first reported in Cell in 1996 [32], following its isolation, cloning, and characterization from rat retinal cells; a study which also detailed the interaction between GDNF, GFRα-1, and the cRET receptor. Interestingly, the following week a Nature publication [63] revealed concurrent work with similar findings on a cloned and characterized GFRα-1, as well as the GDNF, GFRα-1, and cRET multi-subunit receptor complex.

Localization of GDNF and its receptors

Expression patterns of GDNF, GFRα-1, and cRET indicate that the three are not mutually exclusive for GDNF's trophic actions, as GFRα-1 is expressed in regions lacking cRET, and cRET has expression in regions lacking GFRα-1 expression, well-characterized by [67]. In 1996, Trupp et al. [66] identified GDNF's activation of the cRET proto-oncogene, resulting in neuronal survival, while Jing et al. [32] identified GFRα-1 as mediating the interaction between GDNF and cRET. In 2001, Nicole et al. [46] demonstrated the expression of GDNF mRNA and protein, as well as GFRα-1 and cRET on both neurons and astrocytes. Heparan sulphate, a key glycosaminoglycan, was identified

as crucial for the phosphorylation of the c-Ret co-receptor, thus, also necessary for GDNF signaling through its GFRα-1 receptor [6].

Satake et al. [53] showed a dramatic upregulation of GDNF mRNA expression within 3 hours post SCI that was maintained for approximately 2-4 weeks following injury. Additionally, changes in GDNF's expression pattern following CNS injury are nicely illustrated by Trupp et al. [65, 67] and Donnelly and Popovich [18]. GDNF targets in the CNS and PNS, as well as the administration of GDNF gene therapy for motoneuron protection were highlighted in a review by Bohn [9].

GDNF promotes cell survival and growth

One of the earliest studies to report GDNF induced reduction of astrogliosis was a study by Trok et al. [64], in which spinal cord explants were allotransplanted into Sprague-Dawley anterior eye chambers. GDNF was shown to promote graft survival and growth, in addition to the reduced GFAP immunoreactivity. Klöcker et al. [34] identified a new subpopulation of neurons responsive to GDNF in a study showing significantly reduce cell death of axotomized retinal ganglion cells in response to GDNF treatment. The upregulation of GDNF in the distal portion of peripheral injured nerves was assessed and quantified, along with the localization of its cRET receptor, as reported by Bär et al. [5]. Similarly, Höke et al. [24] showed upregulation of GFRα1 receptor on the distal segment of the sciatic nerve following injury; this upregulation and the upregulation of GDNF by Schwann cells was maintained for approximately six months following injury. The GFRα1 receptor was localized to peripheral Schwann Cells in a study by Hase et al. [21], showing another target of GDNF for the repair of injured nervous system. Arce et al. [4] reported a 75% inhibition of neuron survival after exposure to Schwann cell cultured media containing a blocking antibody against GDNF; thus, demonstrating the importance of GDNF for the Schwann cell-mediated neuroprotection. Paratcha et al. [49] highlighted the recruitment of cRET to neuronal cell membrane lipid rafts, in response to soluble GFRα1. Rind et al. [52] showed anterograde transport of GDNF in dorsal root ganglia (DRG) and motor neurons, both with undetectable levels of GDNF mRNA in their current state. The radiolabeled GDNF in this study was provided to the DRGs and motor neurons and by Schwann cells and oligodendrocytes, respectively. In 2004, a novel *in vivo* study was published showing for the first time the endogenous release of GDNF from astrocytes, which was neuroprotective to neighboring neuronal populations, in utero during development [76].

Molecular signaling of GDNF promotion of cell survival

In addition to its neuroprotective effects [48, 7, 61], GDNF has also been shown to: 1) attenuate astrocyte cell death via reduced activation of caspase-3 [74] as well as through caspase-3/Akt independent mechanisms [13]; 2) minimize activation of microglia and production of nitric oxide [73, 23]; and 3) promote the survival [39] and proliferation [25, 75] of Schwann cells. GDNF activates rat primary cortical microglial cells through GFRα-1 and cRET receptors, with downstream signaling through the MAPK pathway, as illustrated in a study by Honda et al. [26]. This study demonstrates microglia as another putative therapeutic target for GDNF in CNS injury and disease. However, a pro-inflammatory response, resulting in increased levels of IL-1β likely led to the GDNF neuroprotection observed in a lipopolysaccharide (LPS)-induced nigral degeneration model of Parkinson's disease [30].

Soler et al. [58] characterized the downstream signaling of GDNF in motoneurons, which includes activation of both the PI3K and ERK-MAPK pathways. Further investigation revealed that the neuroprotective effects of GDNF signaled through the PI3K pathway [58]. In 2001, Nicole et al. [46] described a novel mechanism of cortical neuroprotection from excitotoxicity-induced necrotic cell death after GDNF application; however, in this study GDNF failed to rescue cortical neurons from apoptotic cell death. Moreover, this study illustrated the indispensable nature of the MAPK (MEK) pathway, and GDNF's reduction of NMDA-triggered calcium influx, resulting in the attenuation of necrotic cell death. However, glutamatergic excitotoxicity induced by non-NMDA

agonists (AMPA and kainate) was unable to be attenuated by GDNF administration [46]. Additionally, this study highlighted GDNF's neuroprotective effects were likely through diminished NMDA receptor activity and not the result of free radical scavenging. Cheng et al. [12] investigated the downstream neuroprotection signaling of GDNF and determined that GDNF activated the MAPK signaling pathway and resulted in increased levels of Bcl-2. Liu et al. [39] described a similar upregulation of Bcl-2 and downregulation of Bax, which provided neuroprotection *in vitro* and Schwann cell survival *in vivo*, in rats treated with Schwann cells overexpressing GDNF, as compared to SCI rats.

Studies employing GDNF for repair of SCI

After avulsion injury, axotomized motoneuron cell death was reduced by 50% and somatic atrophy was reduced, after treatment with GDNF [36]. In another study of avulsion injury, GDNF administered via AAV-viral vector significantly attenuated spinal cord ventral horn motor neuron death [70]. In one of the earliest studies of GDNF administration after SCI, Ramer et al. [51] reported the ability of GDNF to rescue spinal cord motoneurons. In a contusive SCI model, GDNF showed significant improvement in motor function (Basso, Beattie, Bresnahan, BBB locomotor rating scale), increased cell survival and number of spared neuronal fibers compared to PBS-controls [12].

Iannotti et al. [29] reported significantly increased spared white matter and significantly attenuated lesion volume in response to GDNF administration via an osmotic minipump, following contusive SCI. Quite noteworthy, Mills et al. [44] described the GDNF enhancement of axonal regeneration occurs within a narrow therapeutic dosage range. In a compressive clip model of SCI, Kao et al. [33] demonstrated significantly improved motor functional recovery (inclined plane), significantly reduced infarct zone, a dramatic increase in the number of VEGF-positive and GDNF-positive cells (undetectable in sham and SCI-only groups), and significantly reduced TUNEL staining.

Studies using GDNF in combinational therapies for SCI repair

Iannotti et al. [28] showed robust remyelination, axonal regeneration, and reduced cavitation, as well as modest yet significantly reduced astrogliosis and immune infiltration, in response to GDNF releasing matrigel guidance channels transplanted following hemisection SCI. Additionally, there was synergistic promotion of axonal regeneration and myelination in response to guidance channels containing both Schwann cells (SCs) and GDNF [28]. Despite significant axonal regrowth into the SCI lesion site, accompanied by the recruitment of myelinating Schwann cells, Blesch and Tuszynski [8] highlighted the difficulty of promoting axonal regrowth through and beyond the lesion site, following secretion of GDNF from genetically modified, transplanted fibroblasts. In a novel study of chronic spinal cord injury, using a peripheral nerve graft, GDNF treatment enhanced axonal regeneration by 7-fold compared to controls [17]. In a study with Schwann cell seeded-guidance channels [75] observed significantly enhanced axonal regeneration, myelination, and number of blood vessels within the regenerated tissue. GDNF was also shown to increase the diameter of the regenerated axons in this study [75].

The observed inhibitory astrogliosis was positively modulated and an intermingling of host and graft tissue was observed at the hemisection lesion interface, in a combinational study of GDNF and Schwann cells (SCs) in semi-permeable guidance channels [15]. Noteworthy, is a study by Zhao et al. [77] in which GDNF reduced axotomy-induced astrogliosis of the facial nerve. In a more recent study, a growth-promoting bridge was formed by transplantation of Schwann cell-seeded guidance channels, with Schwann cells overexpressing GDNF [16]. This GDNF overexpression modulated the astrocytic glial scar, created a more permissive environment for propriospinal axonal regrowth through and beyond the distal end of the lesion, conducted electrical signals through the lesion gap, and improved functional recovery [16]. This study highlights the importance of combinational treatment approaches for traumatic spinal cord injury.

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In another combinational treatment approach, GDNF was embedded into an alginate hydrogel for slow release and employed in a hemisection SCI model [3]. In this study, GDNF promoted increased functional recovery, increased numbers of intralesional and perilesional neurites, reduced astrogliosis, and increased intralesional vasculature, as compared to controls. Using PLGA (polylactide-co-glycolic acid) microspheres for slow release, Zhang et al. [76] administered GDNF, Chondroitinase ABC, and a Nogo A antibody following a transection SCI. Lu et al. [40] showed remarkably robust axonal regeneration up to 12mm in length, in a severe SCI transection model (2mm of cord removed), with a combinational treatment approach including transplantation of neural stem cells in fibrin matrices containing a trophic factor cocktail (GDNF, BDNF (brain-derived neurotrophic factor), PDGF-AA (platelet-derived growth factor), NT3 (neurotrophin-3), IGF-1(insulin-like growth factor 1), EGF (epidermal growth factor), aFGF (acidic fibroblast growth factor), bFGF (basic fibroblast growth factor), HGF (hepatocyte growth factor), and calpain inhibitor/MDL28170). Moreover, this tissue graft resulted in: 1) significantly enhanced motor recovery, 2) significantly improved electrical signals across the lesion gap, 3) survival and differentiation of the neural stem cells, 4) an intermingling of host axons into tissue grafts, 5) increased myelination, and 6) functional synapse formation likely leading to the observed significant improvement in locomotion [40].

Chen et al. [11] used a combinational approach consisting of hydrogel scaffolds containing Schwann cells overexpressing GDNF, transplanted into the transected rat spinal cord, and observed increased axonal growth and axon myelination (by host Schwann cells). Shahrezaie et al. [56] observed significant functional recovery (BBB) and axon number, with a combined treatment of bone marrow mesenchymal stem cells (BMSCs) with lentivirus for GDNF expression, more so than SCI alone, BMSCs alone, or BMSCs with an empty lentiviral vector. Another novel combinational treatment approach was utilized by Zhao et al. [78], with a temperature-sensitive heparin-poloxamer hydrogel with high GDNF-binding affinity, orthotopically injected following thoracic compression SCI in rats. Rats receiving hydrogel with GDNF showed dramatically increased functional recovery (BBB and inclined plane) compared with hydrogel treatment or SCI alone. Furthermore, this treatment showed reduced astrogliosis, increased axon regeneration, and both autophagy-dependent and autophagy-independent neuroprotection. In a 2016 study [45], human umbilical cord blood mononuclear cells (hUCB-MCs) were combined with an adenoviral vector containing GDNF, following rat thoracic contusion SCI. Adenoviral vectors carrying GDNF as well as hUCB-MCs with adenoviral GDNF showed significantly more tissue sparing than either of the control groups lacking GDNF. The combined hUCB-MCs with GDNF (adenoviral vector) showed a significant increase in myelination compared to hUCB-MCs or adenoviral GDNF alone. Significant functional recovery (BBB) was observed for the adenoviral-GDNF group compared to the adenoviral control; in addition, hUCB-MCs adenoviral-GDNF showed similar improvements to the adenoviral-GDNF group. The GDNF-containing treatment groups also showed distinct changes in various glial cells (astrocytes, oligodendrocytes, and Schwann cells) throughout the injured area.

Jiao et al. [31] employed a silk fibroin/alginate GDNF scaffold seeded with human umbilical cord mesenchymal stem cells (hUCMSCs) for a thoracic contusion injury in a rat model. The silk fibroin scaffold combined with alginate had a prolonged release of GDNF compared to either scaffold alone. Moreover, the combination scaffold including GDNF seeded with hUCMSCs, resulted in significant functional improvement (BBB), neuroprotection, increased expression of neuronal markers, and significantly reduced inflammatory cytokine expression, compared to the combination scaffold with GDNF alone, combination scaffold without GDNF, and SCI alone. A similar combinational study utilized placental-derived mesenchymal stem cells (PMSCs) plus GDNF compared to bone marrow-derived mesenchymal stem cells (BMSCs) plus GDNF accompanied by copolymer scaffolds [41]. Interestingly, PMSCs expressing GDNF did not significantly differ in their SCI repair capability from BMSCs expressing GDNF. However, untransfected PMSCs and BMSCs showed significantly less tissue repair than transfected PMSCs and BMSCs expressing GDNF.

Collectively, these studies demonstrate the high potential of GDNF, particularly in combinational treatment approaches, for use for repair of the injured spinal cord.

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237 **Conflicts of Interest**
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