

# GFR $\alpha$ 1 promotes sensory axon regeneration after spinal cord injury

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## INTRODUCTION:

Spinal cord injury (SCI) causes disruption of axonal connections, resulting in devastating paralysis. In spite of recent advancement of medicine, the effective therapy to regenerate axons after SCI remains to be developed. Recently, we have identified the fact that Schwann cells promoted axon regeneration of peripheral nerve by expressing glial cell line-derived neurotrophic factor (GDNF) receptor  $\alpha$ 1 (GFR $\alpha$ 1). GFR $\alpha$ 1 is known to function as a receptor for GDNF as a cell surface molecule or a soluble molecule released from cell surface. At the same time, GFR $\alpha$ 1 stimulates injured axons through binding to the complex of neural cell adhesion molecule (NCAM) and integrin  $\alpha$ 7 $\beta$ 1, resulting in the promotion of axon regeneration after peripheral nerve injury. Further, we confirmed that GFR $\alpha$ 1 stimulated neurite outgrowth of cultured dorsal root ganglion (DRG) neurons. Since DRG neurons project their axons to not only peripheral organs but also brain stem through dorsal column in spinal cord, we hypothesize that GFR $\alpha$ 1 could promote regeneration of axons derived from DRG neurons after spinal cord injury. The purpose of the current study is to determine whether GFR $\alpha$ 1 promotes regeneration of dorsal column sensory axons after spinal cord injury.

## METHODS:

To examine the expression of the receptor complex to GFR $\alpha$ 1 in dorsal column sensory axons, adult Lewis rats received dorsal column injury (DCI) at C4 with wire-knife, followed by the injection of cholera toxin subunit B (CTB) into sciatic nerves 4 days later. One week after injury, subjects were perfused for immunolabeling of NCAM, Integrin  $\alpha$ 7, and  $\beta$ 1. To provide GFR $\alpha$ 1 and cell substrate for axonal growth into lesion sites, syngeneic bone marrow stromal cells (BMSCs) expressing GFR $\alpha$ 1 and GFP (GFR $\alpha$ 1-BMSCs) were prepared by primary cultured BMSCs with lentivirus mediated gene transduction and cell sorting against GFP. Their expression of GFR $\alpha$ 1 was examined by Western blotting. As a control, BMSCs expressing GFP alone was prepared. After making C4 DCIs in adult Lewis rats, 100,000 cells of BMSCs were transplanted into lesion sites, followed by the CTB injections. Four weeks after injury, subjects were perfused. Sagittal sections of spinal cords were immunolabeled with CTB, S100, and GFAP. Growth of CTB labeled axons into the cell graft, the infiltration of Schwann cell into the cell graft, glial scar formation, and the size of cell grafts were quantified.

## RESULTS:

Immunoreactivities of NCAM, integrin  $\alpha$ 7, and  $\beta$ 1 were not detected in CTB labeled intact sensory axons, whereas injured sensory axons exhibited immunoreactivities of all of them (Fig.1), indicating a possibility that injured dorsal column sensory axons have a sensitivity to GFR $\alpha$ 1. Cultured BMSCs exhibited GFP, and only GFR $\alpha$ 1-BMSCs showed staining for GFR $\alpha$ 1. The protein expression of GFR $\alpha$ 1 was detected in the cell lysates and the conditioned media of GFR $\alpha$ 1-BMSCs but not control BMSCs (Fig.2), indicating the secretion of GFR $\alpha$ 1 from GFR $\alpha$ 1-BMSCs. CTB labeled sensory axons robustly regenerated into the grafts of GFR $\alpha$ 1-BMSCs with significantly more regenerating axons in the cell graft compared to the control (Fig.3). Moreover, some of these axons extended beyond the injury site. The size of the cell grafts, the number of infiltrated Schwann cells, and the GFAP immunoreactivity around lesion sites did not significantly differ between these two groups.

## DISCUSSION:

The current study demonstrated that the provision of GFR $\alpha$ 1 in lesion sites by the graft of GFR $\alpha$ 1-BMSCs promote regeneration of dorsal column sensory axons after SCI. Importantly, sensory axons express the receptor complex against GFR $\alpha$ 1 after SCI, and GFR $\alpha$ 1 did not show any effects in enhancing the proliferation of BMSCs, promoting Schwann cell infiltration, or reducing glial scarring. These findings suggest that the axon promoting effect of GFR $\alpha$ 1 is due to the direct stimulation on injured axons but not the modulation of lesion environment, that GFR $\alpha$ 1 could serve as a novel axon regeneration factor for central nervous system, and that GFR $\alpha$ 1 has a therapeutic potential for SCI. Currently, the investigation of regenerative effects on other spinal tract axons such as corticospinal tract is ongoing.

## CLINICAL RELEVANCE:

GFR $\alpha$ 1 has a therapeutic potential for SCI by promoting axon regeneration.

