**GFRα1 promotes sensory axon regeneration after spinal cord injury**

Hidemasa Terao1, Ken Kadoya1, Tomoaki Suzuki1, Takeshi Endo1, Norimasa Iwasaki1
1 Department of Orthopaedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

Disclosures: H. Terao (N), K. Kadoya (N), T. Suzuki (N), T. Endo (N), N. Iwasaki (N)

**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 α1 (GFRα1). GFRα1 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, GFRα1 stimulates injured axons through binding to the complex of neural cell adhesion molecule (NCAM) and integrin α7/β1, resulting in the promotion of axon regeneration after peripheral nerve injury. Further, we confirmed that GFRα1 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 GFRα1 could promote regeneration of axons derived from DRG neurons after spinal cord injury. The purpose of the current study is to determine whether GFRα1 promotes regeneration of dorsal column sensory axons after spinal cord injury.

**METHODS:**
To examine the expression of the receptor complex to GFRα1 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. Sagittal sections of spinal cords were immunolabeled with CTB, S100, and GFAP. Growth of CTB labeled axons into the cell grafts, infiltration of Schwann cell into the cell graft, glial scar formation, and the size of cell grafts were quantified.

**RESULTS:**
Immunoreactivities of NCAM, integrin α7, and β1 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 GFRα1. Cultured BMSCs were not stained for GFRα1, and only GFRα1-BMSCs showed staining for GFRα1. The protein expression of GFRα1 was detected in the cell lysate and the conditioned media of GFRα1-BMSCs but not control BMSCs (Fig.2), indicating the secretion of GFRα1 from GFRα1-BMSCs. CTB labeled sensory axons robustly regenerated into the grafts of GFRα1-BMSCs with significantly more regenerating axons in the cell graft compared to the control (Fig.3).

**DISCUSSION:**
The current study demonstrated that the provision of GFRα1 in lesion sites by the graft of GFRα1-BMSCs promote regeneration of dorsal column sensory axons after SCI. Importantly, sensory axons express the receptor complex against GFRα1 after SCI, and GFRα1 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 GFRα1 is due to the direct stimulation on injured axons but not the modulation of lesion environment, that GFRα1 could serve as a novel axon regeneration factor for central nervous system, and that GFRα1 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:**
GFRα1 has a therapeutic potential for SCI by promoting axon regeneration.