

Functional analysis of fibroblasts involved in fibrotic scar formation in spinal cord injury

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INTRODUCTION: Spinal cord injury is a disease that causes significant motor function impairment. Scar formation at the site of injury impedes axonal regeneration and inhibits recovery from paralysis. Scars in spinal cord injury include Glial scar, which is composed of astrocytes and fibrotic scar, which is composed of fibroblasts. Meflin, which was reported by our laboratory, is a specific marker of fibroblasts involved in the suppression of cancer and fibrotic diseases. In this study, we performed lineage analysis of Meflin-positive cells in fibrous scars of spinal cord injury to clarify the pathology of fibrous scar formation.

METHODS: Spinal cord injury was created in 10- to 12-week-old female mice by performing a laminectomy of the T10 thoracic vertebra and applying a 60 g clip to create a crush model. Meflin-CreERT2/Rosa26-tdTomato mice were used to evaluate spinal cord injury using in situ hybridization (ISH), immunohistochemical staining, and HE staining. Additionally, to evaluate Meflin accumulation in three dimensions, we used the Clear Unobstructed Brain/Body Imaging Cocktails and Computational Analysis (CUBIC) method to render the tissue transparent for observation. In addition to comparing Meflin knockout and Meflin wild-type mice, C57BL/6 mice were used to create control groups (0.1 ml/10 g) treated with 0.5% carboxymethylcellulose, Am80 acute phase (0–14 days post-injury), and subacute phase groups (14–28 days post-injury) (3 mg/kg) were administered orally. Post-injury evaluations included hematoxylin and eosin (HE) staining, in situ hybridization (ISH), immunohistochemical staining, and motor function assessment using the Basso Mouse Scale (BMS).

RESULTS SECTION: In three-dimensional structural analysis using ISH and the CUBIC method, Meflin was found to accumulate in the fibrotic scar along the new blood vessels from the seventh day after spinal cord injury. No difference in BMS was observed between MeflinWT and KO mice, but a difference in fibrosis at the site of spinal cord injury was observed. In the Am80 acute administration group, necrosis was severe in the epicenter. In the Am80 subacute administration group, improvement in BMS was observed, and less fibrosis was observed at the center of the injury.

DISCUSSION: Administration of Am80 immediately after spinal cord injury may exacerbate the inflammatory response during the acute phase, suggesting the existence of a therapeutic window for treatment intervention. Given the improvements observed in motor function and tissue, Meflin may be a potential target for treatment of spinal cord injury.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Meflin is a specific marker of fibroblasts. Meflin may be a treatment target of the fibrotic scar of the spinal cord injury.