Heparan sulfate proteoglycan overcomes the inhibition of nerve regeneration at the glial scar: A new treatment approach for spinal cord injury

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INTRODUCTION: Mature neurons cannot outgrow the glial scars that develop after spinal cord injury. One of the mechanisms involved entails the accumulation of chondroitin sulfate proteoglycans (CSPGs) in the glial scar. These act on the tyrosine phosphatase receptor (PTPσ) on the neuron surface, causing the axon's growth cone to assume a spherical "dystrophic endball" and arresting axonal elongation. In contrast, embryonic neurons vigorously extend axons toward their targets during development and maintain axon regenerative activity, even after injury. Although several intrinsic and extrinsic mechanisms have been reported to mediate these differences, a detailed understanding remains elusive. Chondroitin sulfate (CS) acts on PTPσ and inhibits axonal regeneration in neurons, whereas heparan sulfate (HS) competitively interacts with this receptor and promotes axonal elongation. We hypothesized that embryonic neurons have a specific mechanism in their heparan sulfate proteoglycan (HSPG) profiles that differs from those of adult neurons and that this mechanism competes with the effect of CSPG on PTPσ, enabling axonal outgrowth in a CSPG environment. This study aimed to elucidate the differences in endogenous mechanisms of nerve regeneration between embryonic and adult nerves using an in vitro model of glial scars and to contribute to a new approach for treating spinal cord injury.

METHODS: We used dorsal root ganglion (DRG) neurons from adult (8W) and embryonic (E15) mice to observe the behavior of neuronal axons in a concentration gradient of aggrecan (CSPG) in primary culture experiments using an in vitro model that mimics glial scarring. Quantitative PCR was used to investigate the expression profiles of HSPGs expressed by adult and embryonic cells-expressing. HSPGs were overexpressed in adult neurons using a lentivirus, and nerve regeneration experiments were conducted using a glial scar model.
All animal experiments were approved by the Committee of Animal Care of our institution's Ethics Committee in compliance with the national legislation. The datasets were analyzed using the Mann–Whitney U test for comparing two groups; the Kruskal-Wallis test was applied in cases of three or more groups. Statistical significance was set at $p < 0.05$.

RESULTS: The axons of adult DRG neurons stopped elongating at the edge of the spot where the concentration of CSPG increased and never crossed the gradient. In contrast, some axons of embryonic DRG neurons easily overcame the CSPG gradient (1.8% of adult and 25.1% of embryonic axons crossed the CSPG gradient; $p < 0.001$; Figure 1). The quantitative PCR analysis for the cell-surface HSPG family (including the Syndecan and Glypican families) revealed that Glypican-2 (Gpc-2) expression in the embryonic DRGs was approximately 6-fold higher than that in the adult DRGs. Furthermore, the axonal tips of adult DRG neurons in a CSPG environment did not exhibit dystrophic endball-like structures after overexpression of GPC-2 (the ratio of growth cone formation was 0.0 for the control and 35.3% for GPC-2 over-expression neurons; $p < 0.05$; Figure 2).

DISCUSSION: Our findings revealed the specific expression of GPC-2, a member of the HSPG family, in the axonal tips of embryonic neurons, enabling it to antagonize CS–PTPσ by competing with the receptor. Overexpression of GPC-2 in adult neurons successfully rescued the dystrophic endball, leading to the restoration of a healthy growth cone on the CSPG gradient. These results unequivocally establish the pivotal role of Glypican-2 in defining the axonal response to CS and identify it as a promising therapeutic target for axonal injury. It is important to acknowledge that further in vivo experiments using actual drugs to modify the HSPG-PTPσ axis in animal models are needed to validate its efficacy for clinical application. Regenerative therapy holds immense promise as an active treatment for spinal cord injury and is progressively being realized. However, the formulation of these therapies presents challenges and issues regarding limited treatment facilities and medical economics that persistently hinder their widespread use as standard treatments. This study elucidated the mechanism of nerve regeneration from a molecular biological perspective, focusing specifically on glycans, and introduced a novel approach for developing a useful therapeutic agent for spinal cord injury.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): No effective treatment for nerve regeneration after spinal cord injury has yet been established. In this study, we propose a new approach to nerve regeneration from a molecular biological perspective.

IMAGES:

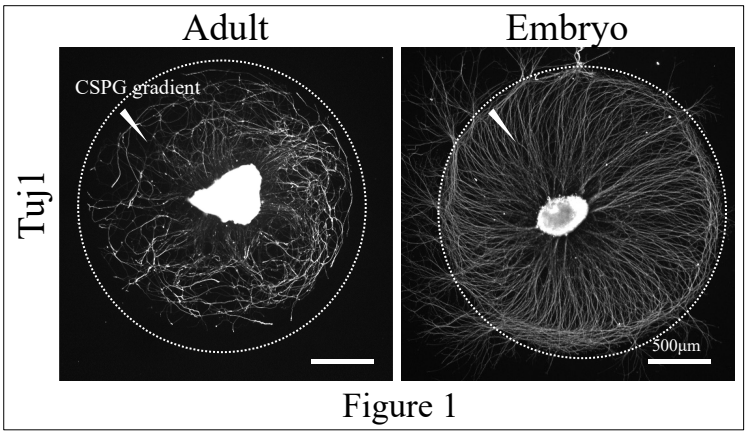

Figure 1

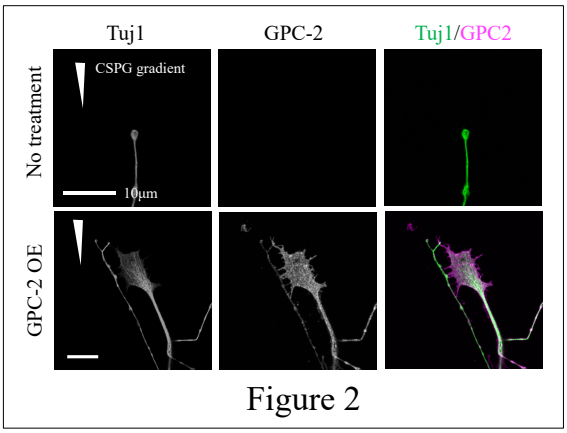

Figure 2

Figure 1. Gross view of the explant culture of adult and embryonic DRGs on the CSPG gradient model. The dotted line indicates the outer rim of the gradient. Some axons of embryonic DRG neurons overcame the CSPG gradient.
Figure 2. Immunocytochemistry for Tuj1 and GPC-2 in No treatment or GPC-2 overexpressing adult DRG neurons. Axons of GPC-2–overexpressing neurons keep growth cone formation even on the CSPG gradient.