Supplementary Information – Technical Results

Experimental validation of transgenic models to prevent astrocyte scar formation

Astrocyte scar formation is complete by two weeks after adult murine SCI and is critically dependent on astrocyte proliferation and STAT3 signaling [15-18]. To prevent astrocyte scar formation, we used two well-characterized transgenic loss-of-function mouse models that either (i) apoptotically kills proliferating scar-forming astrocytes via astrocyte-specific expression of herpes-virus thymidine kinase (TK) plus ganciclovir (GCV) given for 7 days after SCI [15,16] or (ii) disrupts scar formation by cell-type-specific conditional knock-out (CKO) of the *Stat3* gene from astrocytes using a well characterized Cre-loxP system [17,18]. We refer to these models respectively as TK+GCV and STAT3-CKO, and compared these with wild-type (WT) control mice.

To generate CNS lesions, we used a severe crush SCI at T10 (Fig. 1; Extended Data Fig. 1a) in young adult (two to four month old) female mice. Two days after SCI, all mice were evaluated in open field and mice exhibiting any hindlimb movements were not studied further. At three and seven days after SCI, all mice studied exhibited no or few hindlimb movements, indicating comparable severity of SCI among all treatment groups (Extended Data Fig. 1b). At eight-weeks after SCI, mice exhibited minor, but functionally meaningless hindlimb movements with no significant differences among treatment groups (Extended Data Fig. 1b).

To determine the efficacy of our transgenic models for preventing astrocyte scar formation, we quantified the area occupied by GFAP-stained cells within zones extending 500μm on either side of SCI lesion centers, which in WT mice contain the majority of newly proliferated scar-forming astrocytes [18]. WT mice formed dense astrocyte scars by two weeks that persisted for at least eight weeks after SCI. In striking contrast, TK+GCV, and STAT3-CKO mice failed to form scars by two weeks and reliably exhibited an approximately 1000μm wide region around the SCI lesion center that was essentially devoid of astrocyte scar from two to eight weeks after SCI (Fig. 1b-d) and consisted of non-neural cells and extracellular matrix of lesion core [13,14,16-18].

Visualization and quantification of axonal tracts

Axon regeneration was quantified in three major axonal systems commonly studied after SCI and regarded as functionally critical, the (i) descending (motor) corticospinal tract (CST), (ii) ascending sensory (gracile cuneate) tract (AST) and (iii) descending serotonergic (5HT) tract. Descending CST axons were visualized by injecting biotinylated dextran amine (BDA) into motor cortex (Fig. 1c). Ascending AST axons were visualized by injecting cholera toxin B (CTB) into the sciatic nerve (Fig. 1e). Descending 5HT axons were visualized by using immunohistochemistry (Fig. 1f). For quantification shown in various figures, intercepts of CST, AST or 5HT axons with lines drawn at various distances from lesion centers were counted in and expressed as a percent of the number of axons intercepting a line at 3mm proximal to the SCI (Fig. 1a).

Experimental validation of DTR-DTX model of ablating mature astrocyte scars

Chronic astrocyte scars no longer proliferate and are insensitive to TK+GCV. To delete chronic astrocyte scars, we used genetically targeted diphtheria toxin receptor (DTR), which is not expressed by mice, thereby allowing selective ablation of targeted cells with ultralow doses of diphtheria toxin (DTX) [21]. To target DTR to mature astrocyte scars, we injected the viral vector, AAV2/52/5-GfaABC1D-Cre, into SCI lesions at two weeks after SCI in two months old loxP-DTR mice. To ablate chronic astrocyte scars, we then administered DTX three weeks after AAV injection, which was five weeks after SCI (Extended Data Fig. 1d). Axonal tract tracing was conducted and mice perfused after a further five weeks (or 10 weeks total after SCI) (Fig. 2). Distribution and specificity of Cre targeting was verified using AAV2/5-GfaABC1D-Cre injected into loxP-tdTomato (tdT) reporter mice after SCI (Fig. 2b; Extended Data Fig. 1c). Hindlimb locomotor activity showed no significant differences among groups prior to DTX injection and significant reduction after DTX ablation of chronic astrocyte scars (Extended Data Fig. 1d). GFAP immunohistochemistry verified efficient deletion of mature astrocyte scars in loxP-DTR mice that received AAV plus DTX (Fig. 2c). AAV2/5-GfaABC1D-Cre injections were initially titrated to restrict astrocyte ablation primarily to chronic astrocyte scar borders (Fig. 2b,c; Extended Data Fig. 1c). To increase the area of astrocyte ablation in order to reach axons that had died back away from the lesion and its primary border of scar-forming astrocytes, we injected AAV2/5-GfaABC1D-Cre over larger areas to delete both chronic astrocyte scars and adjacent astrocytes. This approach caused tissue degeneration and large

lesions (Extended Data Fig. 1e) with essentially no detectable CST, AST or 5HT axons in the resulting large lesion cores.

Immunohistochemistry of CS56 (total CSPG) in WT, TK+GCV and STAT3-CKO mice, uninjured and after SCI

As expected in uninjured mice, total CSPG staining observed with the widely used CS56 antibody [9,22] was significantly greater in grey versus white matter (Figs. 3b,c; Extended Data Fig. 2a,b). In SCI lesions of WT mice, non-neural lesion core tissue exhibited significantly greater CSPG area, but not GFAP area, compared with uninjured grey or white matter, and contained few astrocytes and many CSPG-positive, GFAP-negative cells. The adjacent astrocyte scar exhibited significantly greater GFAP area in both grey and white matter compared with uninjured, whereas CSPG area was significantly greater only in white matter (Figs. 3b,c; Extended Data Fig. 2a,b). In SCI lesions of TK+GCV and STAT3-CKO mice, GFAP area was significantly reduced in both grey and white matter compared with WT, whereas CSPG area was not significantly reduced either in lesion core tissue or in regions of ablated astrocyte scar, which were filled with CSPG-positive, GFAP-negative, cells (Figs. 3b,c; Extended Data Fig. 2). These findings show that non-astrocyte cells produce substantive CSPGs and that preventing astrocyte scar formation fails to reduce total CSPG production in SCI lesions.

Experimental validation of astrocyte-specific ribosomal RNA precipitation

Ribosome-associated RNA (ramRNA) was precipitated specifically from astrocytes via a Rpl22-hemagglutinin (HA) tag [26] targeted to either WT or STAT3-CKO astrocytes by crossing mice of mGFAP-Cre line 73.12 alone or with floxP-STAT3 [17,18] with loxP-STOP-loxP-*Rpl22*-HA (RiboTag) mice [26]. Immunohistochemistry confirmed HA targeting specificity to astrocytes and not to neurons or oligodendrocytes at the single cell level (Fig. 4b; Extended Data Fig. 3a). Comparison of ribosomal RNA (ramRNA) precipitated from astrocytes to flow-through RNA demonstrated pronounced enrichment for known astrocyte genes [27] and pronounced de-enrichment for known genes from other cells (Extended Data Fig. 3b,c). Gene expression correlation analyses of biologically independent replicates

verified the specificity and reproducibility of our genomic profiling of astrocytes and non-astrocyte cells under different experimental conditions (Extended Data Fig. 3d).

Significantly altered gene expression by astrocytes and non-astrocyte cells in SCI lesions of WT and STAT3-CKO mice

At two weeks after SCI, WT and STAT3-CKO astrocytes around SCI lesions exhibited significantly altered expression of 6236 and 3102 genes, respectively, relative to uninjured (Extended Data Fig. 4a,b). Non-astrocyte cells in WT SCI lesions significantly regulated 6139 genes relative to uninjured, and exhibited substantively different changes when scar formation was disrupted by astrocyte STAT3-CKO (Extended Data Fig. 4a,b).

Delivery of NT3 and BDNF to SCI lesions via synthetic hydrogel depots

NT3 and BDNF were delivered via synthetic hydrogel depots [38-40] injected into lesion centers two days after SCI (Fig. 5d). These hydrogel depots do not modify astrocyte scar formation [39] and provide prolonged neurotrophin delivery [40]. NT3 and BDNF did not alter the appearance or density of GFAP-positive astrocyte scars (Extended Data Fig. 7a).

Laminin-dependence of AST axon regeneration

We looked for axon-matrix interactions that might support regrowing AST axons. Laminin is a preferred substrate for sensory axons *in vitro* where sensory axon growth can be severely attenuated using anti-CD29 function-blocking antibodies that disrupt laminin-integrin binding [37]. AST axons in intact gracile-cuneate tracts were coarsely beaded and were not in direct contact with laminin surfaces (Extended Data Fig. 9a,d). In striking contrast, AST axons stimulated to regrow in SCI lesions were much thinner and uniformly tracked along laminin surfaces, turning and even reversing direction along these surfaces (Figs. 5f; Extended Data Fig. 9c,e,f), as expected of regenerating axons [41]. Hydrogel delivery of anti-CD29 function-blocking antibodies [37] together with NT3 and BDNF significantly reduced AST axon regrowth in lesion cores by 73% (Figs. 5g-h; Extended Data Fig. 9g-i). Thus, laminin interactions were critical for AST axon regrowth in SCI

lesions, further demonstrating that these were regenerating axons rather than uninjured spared axons, which were not associated with laminin.