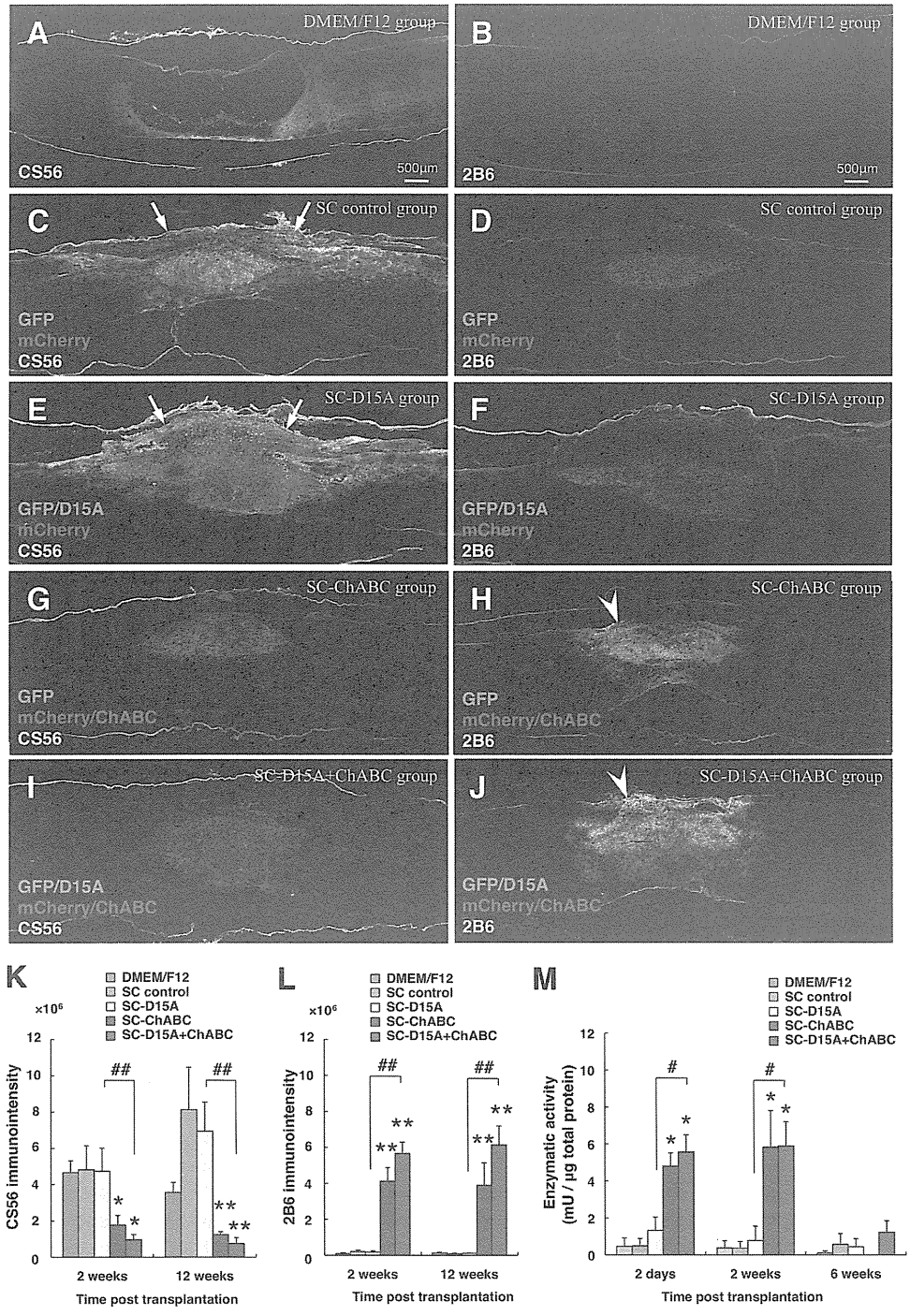


**Figure 5.** ChABC reduced CSPG immunoreactivity in injured spinal cords at 12 weeks after transplantation. In staining for CS56 at 12 weeks after transplantation (***A***, ***C***, ***E***, ***G***, ***I***), the groups without ChABC showed immunoreactivity both inside and around the transplant (***C***, ***E***, arrows). In contrast, the groups with ChABC did not show heightened immunoreactivity (***G***, ***I***), confirmed by quantitation of immunodensity (***K***). 2B6 staining at 12 weeks revealed higher immunoreactivity both inside and around the graft in the groups with ChABC (arrowheads in ***H*** and ***J*** vs ***B***, ***D***, ***F***), as confirmed by immunodensity measurements (***L***). The enzymatic activity as assessed by the DMB assay is graphed in ***M***. Data are presented as mean ± SEM; $n = 6$ per group in ***K*** and ***L***; $n = 3$ per group in ***M***. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with SC control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with SC-D15A or SC-ChABC group.

volume than did the SC-D15A ($p < 0.05$) and SC-ChABC (0.8 ± 0.2 $mm^3$, $p < 0.01$) groups.

In comparing the total number of GFP- and mCherry-labeled SCs in two sections from the center of the graft (Fig. 3*G*), the SC-D15A (18,665 ± 1928, $p < 0.01$) and SC-D15A + ChABC groups (17,265 ± 1455, $p < 0.05$) showed significantly higher numbers of SCs within the lesion site compared with the SC control group (9900 ± 1001). The SC-D15A + ChABC group also showed significantly higher numbers than the SC-ChABC group (8536 ± 2196, $p < 0.05$). For GFP-labeled SCs, the SC-D15A + ChABC group (7485 ± 2813) showed a higher number than the SC control group (4395 ± 754, $p < 0.05$); mCherry-labeled SCs were higher in the SC-D15A group (12015 ± 858, $p < 0.01$) than in SC controls (5505 ± 876). There were no statistically significant differences between the numbers of GFP-labeled and mCherry-labeled SCs in each group. Transplanted SC num-

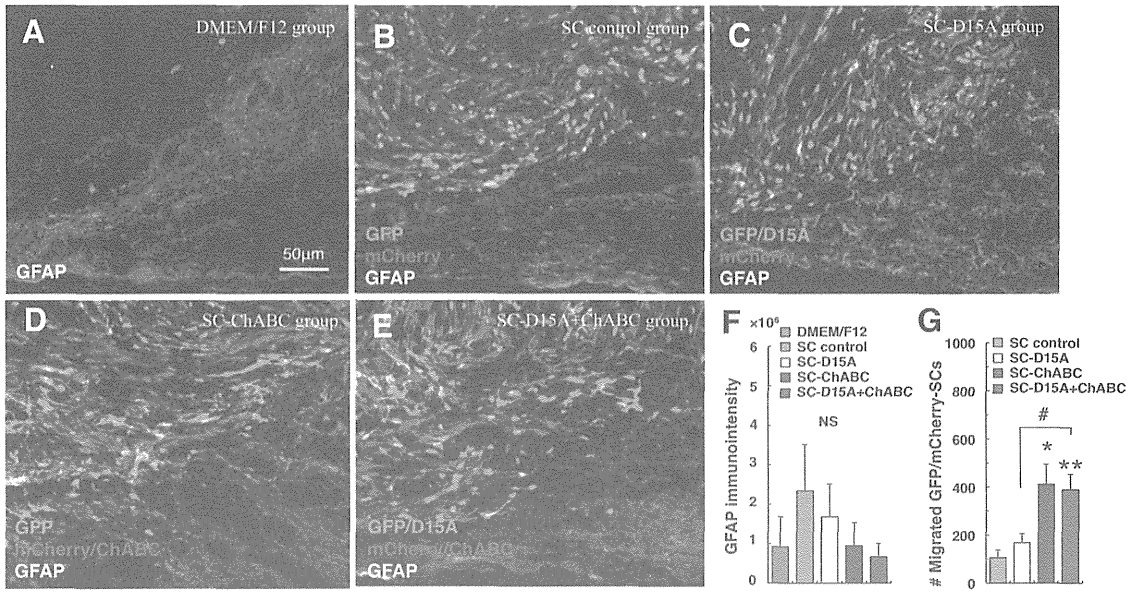


**Figure 6.** ChABC leads to irregular graft-host borders. At higher magnification of the caudal edge of the graft, more SCs entered the host spinal cord and more astrocyte processes appeared to extend into the graft in groups with ChABC (*D*, *E*) compared with other groups (*B*, *C*). In quantifying GFAP immunointensity, there was no significant difference between the groups including the DMEM/F12 group (*A*, *F*). The number of SCs that migrated into the host spinal cord was significantly higher in the groups with ChABC (*G*). Data are presented as mean ± SEM; $n = 5$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with SC control group. $^{\#}p < 0.05$ compared with SC-D15A or SC-ChABC group.

ber measurements were obtained in a blinded fashion from two serial sagittal sections around the midline of the largest graft per rat.

The NT-3 ELISA of the transplanted spinal cord showed the levels of NT-3 to be significantly higher in the SC-D15A (1043 ± 72 pg/mg total protein, $p < 0.05$) and SC-D15A + ChABC groups (1199 ± 70 pg/mg total protein, $p < 0.05$) compared with SC control grafts (182 ± 29 pg/mg total protein) at 2 d after transplantation (Fig. 3*H*), diminishing by 2 weeks. At this time, the levels in the SC-D15A (116 ± 16 pg/mg total protein, $p < 0.05$) and SC-D15A + ChABC (123 ± 16 pg/mg total protein, $p < 0.05$) groups remained significantly higher than in the SC control group (67 ± 5 pg/mg total protein).

### ChABC is active inside and outside the graft

After immunostaining for CS56 in spinal cord sections at 2 weeks after transplantation, the SC control and SC-D15A groups and the DMEM/F12 group showed immunoreactivity around the graft/lesion sites (Fig. 4*A*,*C*,*E*). In contrast, the SC-ChABC and SC-D15A + ChABC groups did not exhibit this immunoreactivity (Fig. 4*G*,*I*). At 12 weeks, some immunoreactivity was seen around the lesion (Fig. 5*A*); the SC control and SC-D15A groups showed CS56 immunoreactivity both inside and outside the graft, especially in the dorsal spinal cord (Fig. 5*C*,*E*). In contrast, the SC-ChABC and SC-D15A + ChABC groups did not show this immunoreactivity (Fig. 5*G*,*I*). The quantification of CS56 immunointensity confirmed these observations at 2 ($p < 0.05$) and 12 weeks ($p < 0.01$; Fig. 5*K*).

Upon immunostaining for 2B6 at 2 and 12 weeks, the SC-ChABC and SC-D15A + ChABC groups showed 2B6 immunoreactivity both inside and around the graft (Figs. 4*H*,*J*, 5*H*,*J*), whereas the other groups did not (Figs. 4*A*,*B*,*D*,*F*, 5*A*,*B*,*D*,*F*). The quantification of 2B6 immunointensity confirmed the significant increase in the SC-ChABC and SC-D15A + ChABC groups compared with the SC control group at 2 ($p < 0.01$) and 12 weeks ($p < 0.01$; Fig. 5*L*).

The DMB assay of the transplanted spinal cord revealed that the enzymatic activity of ChABC was significantly higher in the SC-ChABC (4.8 ± 0.7 mU/μg total protein, $p < 0.05$) and SC-D15A + ChABC (5.6 ± 0.9 mU/μg total protein, $p < 0.05$) groups than in the SC control group (0.5 ± 0.4 mU/μg total protein) at 2 d after transplantation (Fig. 5*M*). Such increased enzymatic activity was observed until 2 weeks but disappeared by 6 weeks.

### ChABC leads to irregular graft-host borders and promotes SC migration

In inmmunostaining for GFAP after transplantation, there was no striking difference in immunoreactivity inside and around the graft among the treatment groups (Figs. 3*A–E*, 6*A–E*). The quantification of GFAP immunointensity revealed no statistical differences among the groups (Fig. 6*F*). At the caudal edge of the graft, SCs were less confined to the graft and more astrocyte processes were observed to extend into the graft in groups with ChABC (Fig. 6*D*,*E*) than in those without (Fig. 6*B*,*C*). The numbers of transplanted SCs that migrated into the surrounding host spinal cord tissue or adjacent were significantly higher in the groups with ChABC compared with the other groups (Fig. 6*G*).

### D15A increases SC-myelinated axons in the transplant

Representative images after toluidine blue staining show more dense and larger SC transplants in the SC-D15A and SC-D15A + ChABC groups (Fig. 7*C*,*F*,*H*,*J*) than in the other groups (Fig. 7*B*,*E*,*G*,*I*), which was at least in part due to there being more SC-myelinated axons in the transplant. The numbers of SC-myelinated axons were significantly higher in the SC-D15A and SC-D15A + ChABC groups than in the SC control group ($p < 0.05$ and $p < 0.01$, respectively; Fig. 7*K*). In addition, the SC-D15A + ChABC transplants contained a significantly higher number than the SC-ChABC groups ($p < 0.05$).

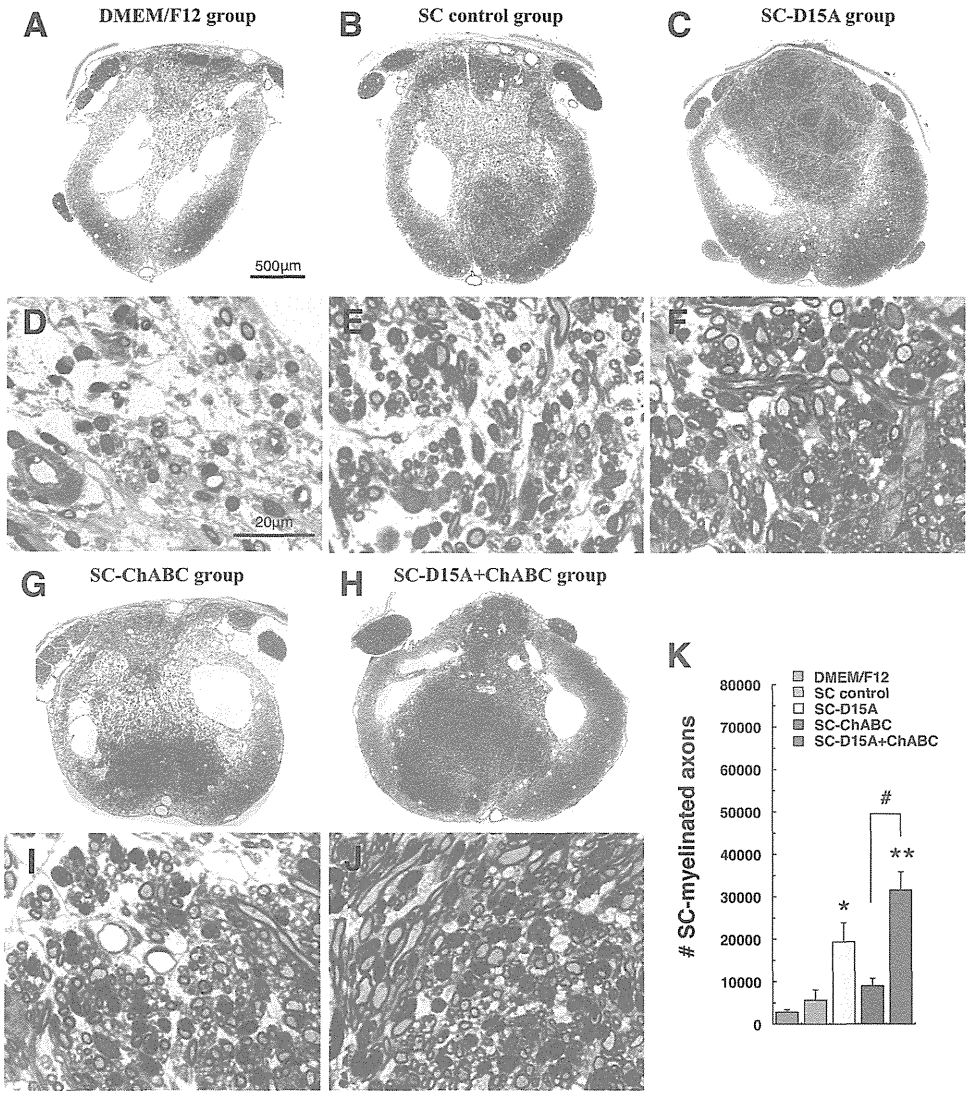


**Figure 7.** SC myelination in the transplant is promoted by D15A. In toluidine-blue-stained, semithin sections of the transplanted spinal cord, groups with D15A showed larger and denser SC transplants (***C***, ***H***) compared with the other groups (***B***, ***G***); the grafts are apparent compared to the DMEM/F12 injected spinal cord (***A***). At higher magnifications, the groups with D15A showed an increased number of SC-myelinated axons in the transplant (***F***, ***J***) compared with the other groups (***E***, ***I***), as confirmed by quantitation (***K***). SC myelin may appear next to the SC nucleus; enabling distinguishing SC from oligodendrocyte myelin that was spared around the cord periphery. Data are presented as mean ± SEM; $n = 5$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the SC control group. $^{\#}p < 0.05$ compared with the SC-ChABC group.

### There is a higher number of propriospinal axons in the transplant and host tissue with ChABC

The presence of propriospinal axons inside and around the transplant was evaluated using anterograde axonal tracing. In the SC control group (Fig. 8*A–E*), BDA-labeled axons in the rostral spinal cord were observed only occasionally to grow into the graft (Fig. 8*A*,*B*,*C*), into host tissue at the epicenter (Fig. 8*D*), or into the caudal spinal cord (Fig. 8*E*). However, the SC-D15A + ChABC group (Fig. 8*F–J*) exhibited many BDA-labeled axons in the transplant (Fig. 8*G*,*H*). This combination group showed more BDA-labeled axons within surrounding, spared host tissue at the epicenter (Fig. 8*I*) and in the caudal spinal cord (Fig. 8*J*) compared with the SC control group.

In counting the BDA-labeled propriospinal axons in the transplant (Fig. 8*K*), the numbers of axons were significantly higher in the SC-D15A, SC-ChABC, and SC-D15A + ChABC groups than in the SC control group ($p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively). The SC-D15A + ChABC group showed a significantly higher number of BDA-labeled axons in the surrounding host tissue at the epicenter than did the SC-D15A group ($p < 0.01$) and the SC control group ($p < 0.05$). At 500 μm caudal to the graft (Fig. 8*L*), the numbers of axons were significantly higher in the SC-ChABC and SC-D15A + ChABC groups than in the SC control group ($p < 0.05$ and $p < 0.01$, respectively)

### There are more CST axons in the caudal spinal cord with D15A and ChABC

In the SC control and SC-D15A groups, BDA-labeled CST axons in the rostral spinal cord were not present close to the graft (Fig. 9*A*,*B*,*J*) and were rarely observed in the surrounding host tissue at the epicenter and the caudal spinal cord (Fig. 9*C*,*D*,*I*). In contrast, CST axons were closer to the graft in the SC-ChABC and SC-D15A + ChABC groups, although not in the graft (Fig. 9*E*,*F*,*I*). In the SC-D15A + ChABC group, BDA-labeled fibers were observed in surrounding host tissue at the epicenter and in the caudal spinal cord (Fig. 9*G*,*H*). The BDA-labeled fibers reached at least 500 μm beyond the caudal edge of the transplant. These tracing results were confirmed by axon counts (Fig. 9*I*,*J*).

### Combination of D15A and ChABC leads to more labeled projection neurons in the brainstem after retrograde tracing

Labeling of supraspinal projection neurons in the brainstem was evaluated by placing the retrograde tracer (fast blue) caudal to the transplant, the numbers of fast-blue-labeled neuronal somata in the brainstem thereby reflecting the number of axons reaching the caudal spinal cord to take up the tracer. Figure 10 shows representative pictures of fast blue-labeled neurons in the raphe nuclei after tracing. In the SC-D15A + ChABC group (Fig. 10*E*), there were more labeled neurons compared with the SC control group (Fig. 10*B*). The SC-D15A + ChABC group showed a significantly higher number in the raphe nuclei compared with the SC control, SC-D15A, and SC-ChABC groups ($p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively, Fig. 10*G*). In the reticular formation, the number of fast blue-labeled neurons in the SC-D15A + ChABC group was significantly higher than in the other groups ($p < 0.01$) and the number of neurons in the SC-D15A group was significantly higher than in the SC control group ($p < 0.05$).

### There is a higher number of 5-HT axons in SC grafts and the caudal spinal cord with combined D15A and ChABC

In representative images of 5-HT fiber immunostaining (Fig. 11*A–F*), these axons were rarely observed in the transplant, surrounding host tissue at the epicenter, or caudal spinal cord in the SC control group (Fig. 11*A–C*). In contrast, the SC-D15A + ChABC group showed more 5-HT-labeled fibers in all of these locations (Fig. 11*D–F*). Counts of 5-HT-labeled axons revealed that their total number in the graft was significantly higher in the SC-ChABC and SC-D15A + ChABC groups than in the SC control group ($p < 0.05$; Fig. 11*G*). The numbers of 5-HT axons in the SC-D15A + ChABC group were significantly higher in the caudal spinal cord ($p < 0.01$; Fig. 11*H*).

### Combination of D15A and ChABC improves locomotor function and reduces allodynia in the hindlimbs

BBB scores and subscores were measured for 13 weeks (Fig. 12*A*,*B*). After contusion injury, all animals exhibited gross locomotor impairment, showing only joint movement for the first few days and no weight-supported stepping for the first week (BBB score <10) and partial improvement over the 13 weeks after injury. After 9 weeks, most animals displayed frequent plantar stepping without forelimb/hindlimb coordination, which appeared to plateau. Animals in the SC-D15A + ChABC group showed additional improvement at 13 weeks; 3 of 7 animals in this group exhibited consistent stepping with occasional forelimb/hindlimb coordination (BBB score ≥12),

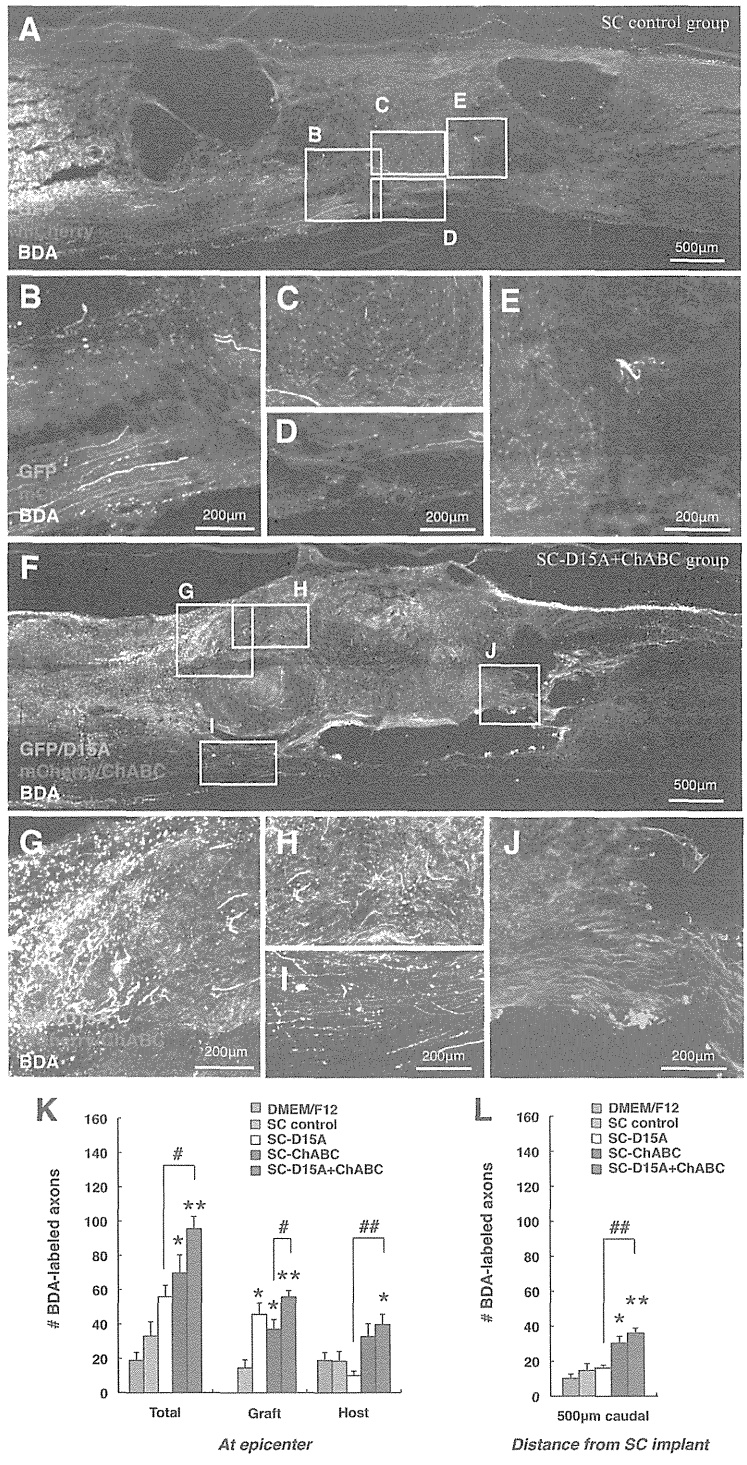


**Figure 8.** ChABC leads to more propriospinal axons in the graft and host tissue. In the SC control group, an occasional BDA-labeled propriospinal axon was observed in the graft (*A–D*), but most did not appear to enter the graft (*B*). BDA-labeled fibers were rarely observed in host tissue at the epicenter (*D*, *K*) and in the caudal spinal cord (*E*, *L*). In the SC-D15A + ChABC group, many fibers grew into the graft (*F–H*). Labeled fibers also were observed in host tissue at the epicenter (*I*, *K*) and in the caudal spinal cord (*J*, *L*). The SC-D15A groups showed a significant increase in propriospinal axons only in the graft (*K*). A significant increase in axons in the SC-ChABC and SC-D15A + ChABC groups also was seen in the caudal spinal cord (*L*). Data are presented as mean ± SEM; $n =$ 5 per group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with SC control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the SC-D15A or SC-ChABC group.

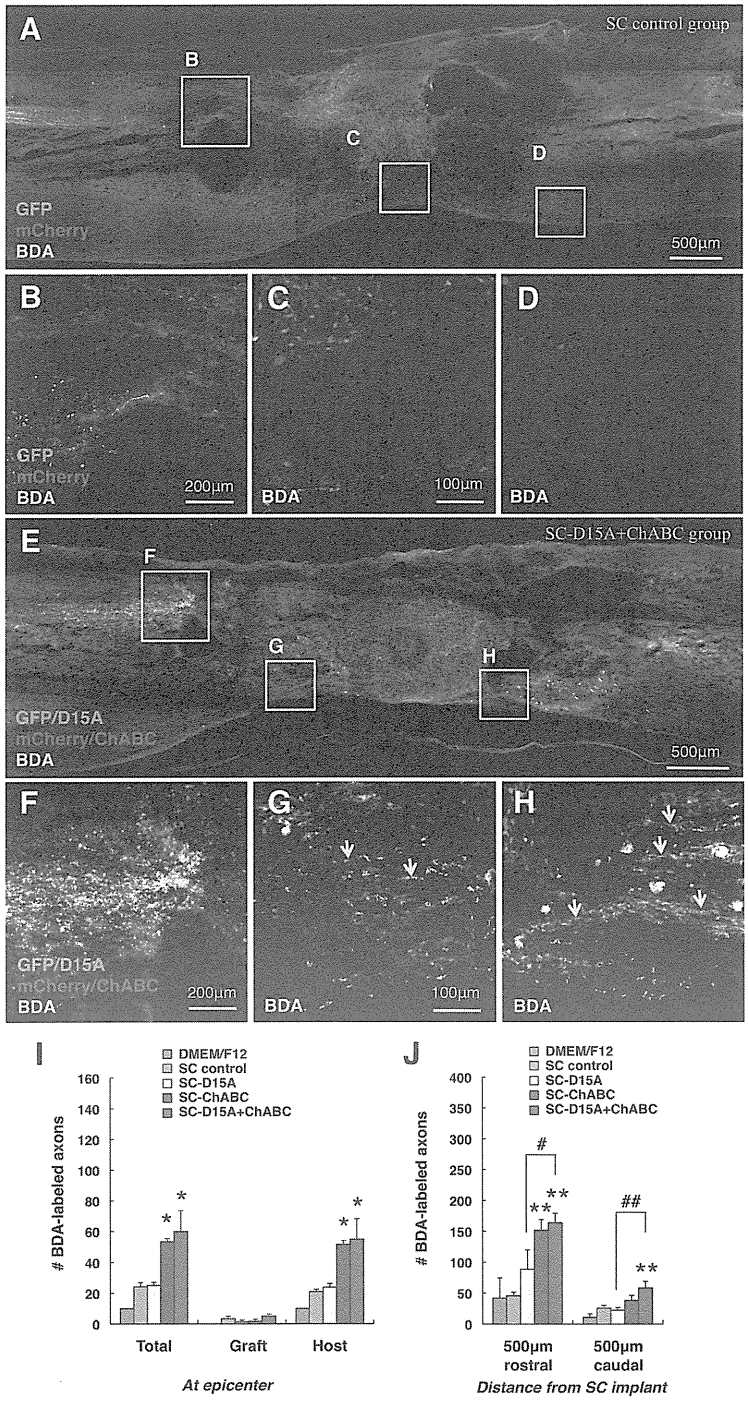


**Figure 9.** Combined D15A and ChABC leads to more CST axons in the caudal spinal cord. In the SC control group, BDA-labeled CST axons were not present close to the SC graft (***A***, ***B***) and were rarely observed in host tissue (***C***, ***D***). In the SC-D15A + ChABC group, labeled CST axons were present closer to the transplant rostrally (***E***, ***F***) and were observed in host tissue at the epicenter (***G***, arrows) and in the caudal spinal cord (***H***, arrows). Axonal counts at the epicenter were significantly increased in the SC-ChABC and SC-D15A + ChABC groups compared with the SC control group (***I***). The number of CST axons in the caudal spinal cord was significantly increased in only the SC-D15A + ChABC group (***J***). Data are presented as mean ± SEM; $n = 6$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the SC control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with SC-D15A group.

components such as paw position, toe clearance, trunk control, and tail position, the animals in the SC-D15A + ChABC group showed a significantly higher score than the SC control group at 5, 8, 11, 12, and 13 weeks ($p < 0.05$; Fig. 12*B*).

CatWalk analysis allows the determination of the regularity index, a fractional measure for interlimb coordination (Lankhorst et al., 2001). It relates the proportion of regular step patterns to the number of steps taken. In completely coordinated rats, it is therefore 100%. At 5 weeks after SCI, the regularity index decreased to 70–85% in all groups (Fig. 12*C*). From 9 weeks, the SC-D15A + ChABC group showed a significantly higher percentage (better coordination) compared with all other groups ($p < 0.05$; Fig. 12*C*). The regular step patterns were categorized as described previously (Cheng et al., 1997). The most commonly observed regular step pattern in rats is the Ab pattern (order of steps: left forelimb → right hindlimb → right forelimb → left hindlimb; Hamers et al., 2006). In our animals, the major regular step pattern before injury was the Ab pattern (>95%; Fig. 12*D*). The percentage of Ab pattern had decreased at 5 weeks after SCI in all groups and then gradually increased until 13 weeks. From 9 weeks, the SC-D15A + ChABC group showed a significantly higher percentage of Ab pattern (improved coordination) than the SC control group ($p < 0.05$). Stride length and base of support were analyzed as static locomotor parameters for the hindlimbs. From 5 weeks, the SC-D15A + ChABC group showed significantly longer stride length compared with the SC control group ($p < 0.05$; Fig. 12*E*). There was no significant difference in the base of support among the groups (Fig. 12*F*).

Mechanical and thermal allodynia using the von Frey and Hargreaves methods, respectively, were examined. Withdrawal thresholds to mechanical stimuli were decreased in all groups after SCI and then gradually increased until 13 weeks (Fig. 12*G*). Animals in the SC-D15A + ChABC group demonstrated significantly higher withdrawal thresholds at 5, 9, and 13 weeks, returning to values similar to baseline, than those in the SC control and SC-D15A groups ($p < 0.01$). The withdrawal thresholds in the SC-ChABC group were also significantly higher and closer to baseline values compared with those in the SC control group at 5 and 9 weeks ($p < 0.01$). The withdrawal latencies to a heat stimulus were decreased in all groups, but animals in the SC-D15A + ChABC group exhibited significantly higher withdrawal latencies than those in all other groups at 5, 9, and 13

whereas no animals in the other groups reached this functional level except one animal in the SC-D15A group. At 13 weeks, the SC-D15A + ChABC group showed a higher BBB score than the SC control group ($p < 0.05$; Fig. 12*A*), although more time points would be needed to verify a persistent, gross locomotor improvement. In the BBB subscore, to further examine specific locomotor

weeks ($p < 0.01$; Fig. 12*H*), with values being similar to those obtained at baseline.

## Discussion

We demonstrate here for the first time that the combination of genetically modified, transplanted SCs secreting D15A and ChABC promotes axonal regeneration and improves function after SCI, the combination being more efficacious than either treatment alone. D15A-secreting SCs led to larger implants with more SC-myelinated axons than did control SC grafts, confirming earlier results (Golden et al., 2007). The ChABC-transduced SCs secreted biologically active enzyme after transplantation and significantly decreased CSPGs both within the graft and in the surrounding spinal cord. With SC-ChABC transplantation, the border of the implant was more irregular due to SCs emerging from the transplant. These SCs secreting ChABC led to more propriospinal and 5-HT-positive axons in the graft, more 5HT-positive and CST axons in spared host tissue around the lesion epicenter, and more propriospinal axons in tissue caudal to the graft compared with control SCs. Compared with SC-ChABC transplants, SC-ChABC + SC-D15A transplantation led to significantly more propriospinal and 5-HT-positive axons in the graft and more 5-HT-positive axons in tissue caudal to the graft. The numbers of propriospinal axons in spared host tissue at the epicenter and CST axons caudal to the lesion/graft were significantly higher only in the combination group. Furthermore, retrograde tracing revealed the presence of more labeled neurons in the raphe nuclei and reticular formation with axons projecting caudal to the lesion/graft than after SC-ChABC or SC-D15A transplantation. Finally, the combination strategy led to an improvement in locomotor function and lessened mechanical and thermal allodynia on the hindpaws compared with control groups. In sum, the highest numbers of axons were observed within the graft and in spared tissue around the graft, including caudal to the graft with the combination of SCs, D15A, and ChABC; this was the only approach that improved locomotion and sensation. These results suggest that the transplantation of genetically modified SCs to secrete neurotrophin and ChABC has significant therapeutic potential for treating SCI.

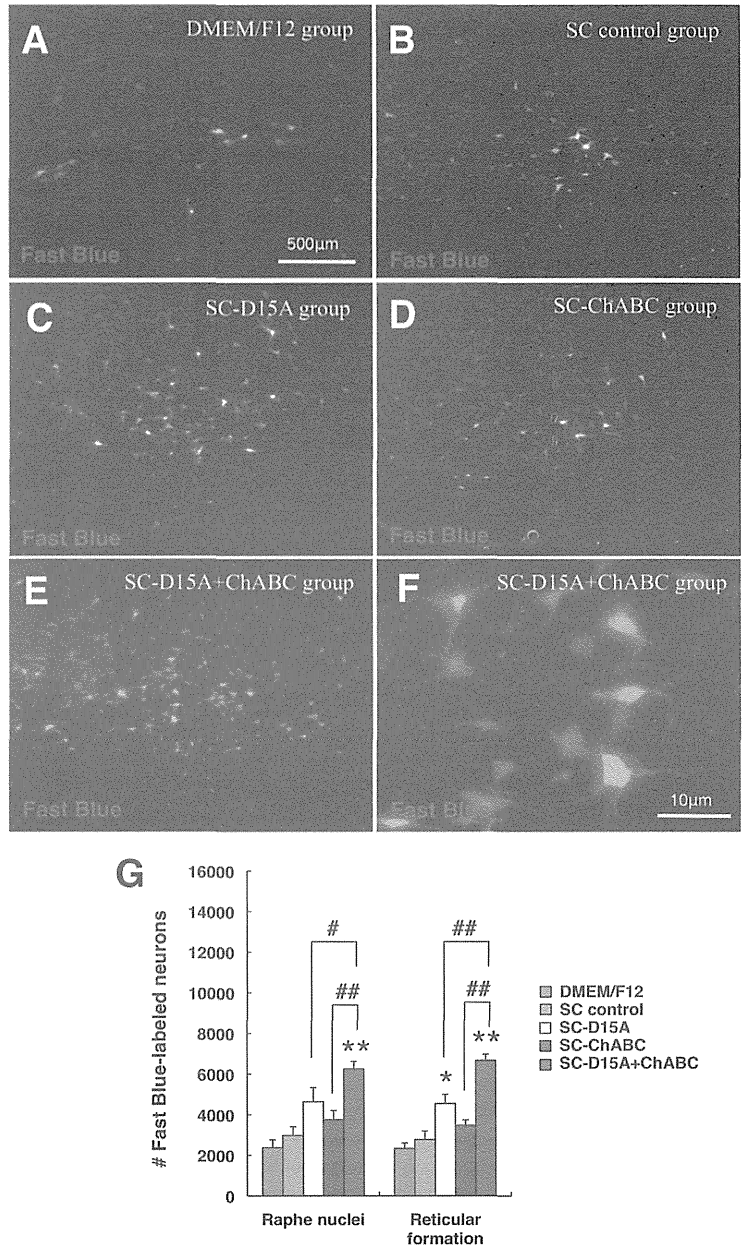


**Figure 10.** The combination of D15A and ChABC leads to more fast blue-labeled neurons in the brainstem after retrograde tracing. ***A–F***, Representative pictures of fast blue-labeled neurons in the raphe nuclei show increased numbers in the SC-D15A group (***C***) compared with the SC control group (***B***); the SC-D15A + ChABC group shows a further significant increase (***E***; at higher magnification, ***F***). ***G***, In the reticular formation, the SC-D15A and SC-D15A + ChABC groups showed a significantly higher number of labeled neurons than in the SC control group. In addition, the number of labeled neurons in the SC-D15A + ChABC group was significantly higher than that in the SC-D15A and SC-ChABC groups. Data are presented as mean ± SEM; $n = 7$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with SC control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the SC-D15A or SC-ChABC group.

The permissivity of the interfaces between SC grafts and the contiguous spinal cord tissue is key to the success of this transplantation strategy. Both rostral and caudal interfaces need to allow ingrowth and exit of regenerating axons to cross the area of injury and enter the cord. Often, an interface appears as a sharp boundary containing GFAP antibody staining and CSPGs that are inhibitory to axonal growth. ChABC is used to diminish CSPGs to improve such growth (Bradbury and Carter, 2011). Here, the reduction of CSPGs around the SC graft with ChABC most likely enabled more axons to cross the interfaces with SCs, leaving the graft and astrocytes extending into the graft to form a more irregular host-graft interface. Such irregular borders are more permissive for axons to cross from astrocyte to SC territory (Afshari et al., 2010; Williams and Bunge, 2012). Williams et al. (2013) found a correlation between the numbers of elongated astrocyte processes and brainstem axons entering the SC graft and an improvement in hindlimb movement scores.

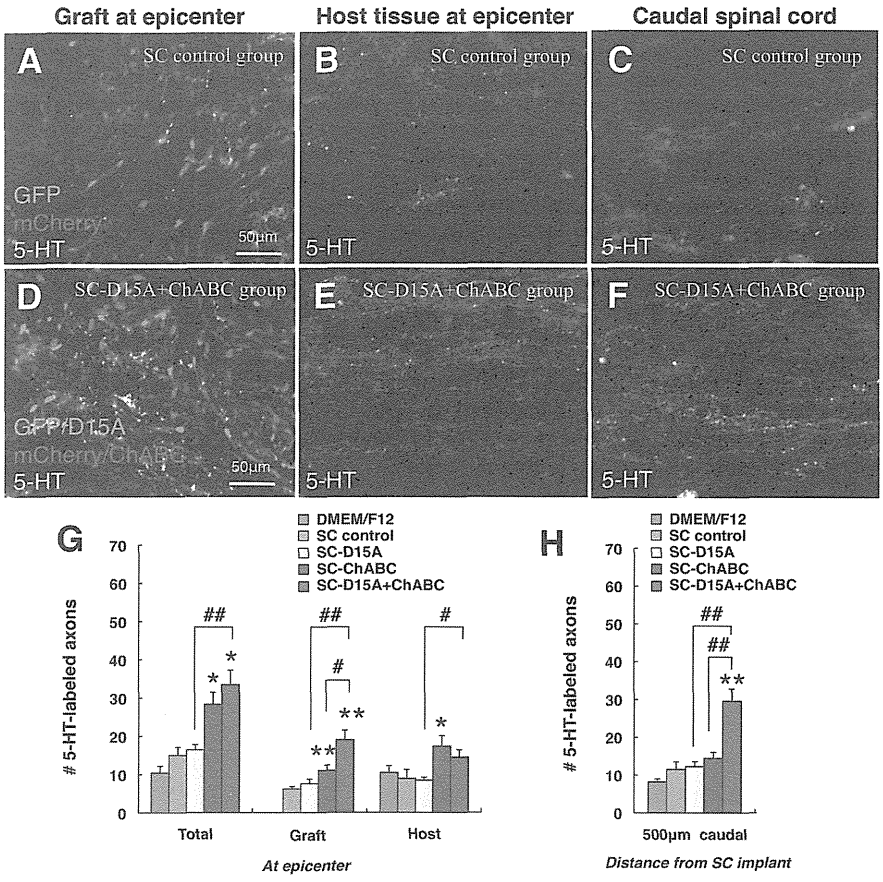


**Figure 11.** Combined D15A and ChABC leads to more 5-HT-labeled axons in grafts and the caudal spinal cord. ***A–H***, 5-HT-labeled axons were rarely observed in the SC graft (***A***), host tissue at the epicenter (***B***), or caudal spinal cord (***C***) in the SC control group. In contrast, the SC-D15A + ChABC group showed more 5-HT-labeled fibers in the graft (***D***) and the caudal spinal cord (***F***), as confirmed by counting (***G***, ***H***). 5-HT axons also were more numerous in the transplant and in the SC-ChABC group compared with the SC control group. Data are presented as mean ± SEM; $n = 7$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with SC control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the SC-D15A or SC-ChABC group.

It has long been questioned whether, if SCs left a graft to migrate into the neighboring spinal cord, then exit of regenerated axons across the "off-ramp" would be improved. Recent studies have shown this to be true. Papastefanaki et al. (2007) improved SC migration by adding polysialic acid (PSA) to the neural cell adhesion molecule NCAM on the transplanted SC surface by introducing, by viral vector, the enzyme sialytransferase X. With the ensuing migration of SCs from the transplant after compression injury, functional recovery was improved, along with more axonal growth and enhanced myelination. A more recent study by Ghosh et al. (2012), in which PSA-SCs again were highly migratory, extended this investigation by using a clinically relevant contusion injury. PSA-overexpressing SCs supported regeneration of both CST and serotonergic fibers across the transplant and beyond its caudal edge,and improved locomotor outcome. In our results, SCs were less confined to the transplant in groups with ChABC. This finding suggested that ChABC enabled SCs to emerge from the transplant site, thereby improving the extension of descending axons into the host tissue.

Modifying CSPGs in the scar with ChABC treatment could increase the availability of endogenous neurotrophic or other protective factors in the injured spinal cord to promote neuroprotection (Crespo et al., 2007). In our study, CST axons were observed closer to the rostral aspect of the transplant with ChABC, suggestive of lessened die back (or increased sprouting) of the axons. It is likely that the CST axons observed caudal to the graft had been spared because only rarely have CST fibers extended into a SC milieu (Cheng et al., 1996). Because the more clinically relevant contusion injury instead of transection was chosen for our study, it was not possible to distinguish spared/sprouted from regenerated axons. It is likely that axons well within the graft had regenerated in the "new tissue," one of the criteria defined by Steward et al. (2003) to distinguish between truly regenerated and spared/sprouted axons. In our results, axons in the tissue around the SC graft were likely to have been spared, suggesting that the ChABC treatment was also, at least in part, neuroprotective. It also has been reported that ChABC present at the injury site can rescue axotomized supraspinal projection neurons from atrophy, as do neurotrophic factors at the injury site (Carter et al., 2008). In our results, the combination of ChABC and D15A induced significantly more fast blue-labeled brainstem neurons than only D15A. These findings could suggest that a neuroprotective effect of ChABC led to the higher number of brainstem neurons, although the presence of ChABC could have caused increased permissivity of the interfaces for regenerating axons.

Recent studies have shown that CSPGs interact with the transmembrane receptor protein tyrosine phosphatase $\sigma$ in axons and inhibit their growth in the injured spinal cord (Shen et al., 2009; Fry et al., 2010), an interaction abolished by ChABC treatment (Shen et al., 2009). ChABC is known to induce CST sprouting (Barritt et al., 2006; Houle et al., 2006). A previous study showed that ChABC administration enhanced regeneration of transected propriospinal axons after SCI and, importantly, allowed them to grow across the caudal SC/spinal cord interface (Chau et al., 2004). In this case, modification of the scar led to further axonal regrowth in the spinal cord beyond the SC bridge. Our results suggested that ChABC promoted regrowth of 5-HT-positive and propriospinal axons in the SC graft, although, again, improved interface permissivity could have been a factor. In sum, the overall outcomes that we have documented here may have resulted from a combination of axonal regeneration, sprouting, and neuroprotection (including reduced die back), all of which have been implicated in repair after provision of ChABC (Bradbury and Carter, 2011). Analysis to compare neuroprotection of the spared white matter among the experimental groups was found not to be feasible because the graft/white matter interfaces were not distinct enough in the ChABC-treated animals.

Although the combination strategy improved function, additional factors may have precluded greater improvement. ChABC treatment will not reduce other inhibitory ligands such as semaphorins, ephrins, NoGo, OMpg, and MAG. In addition, the higher concentration of neurotrophin in the implant than in the surrounding host tissue may prevent axons from leaving the

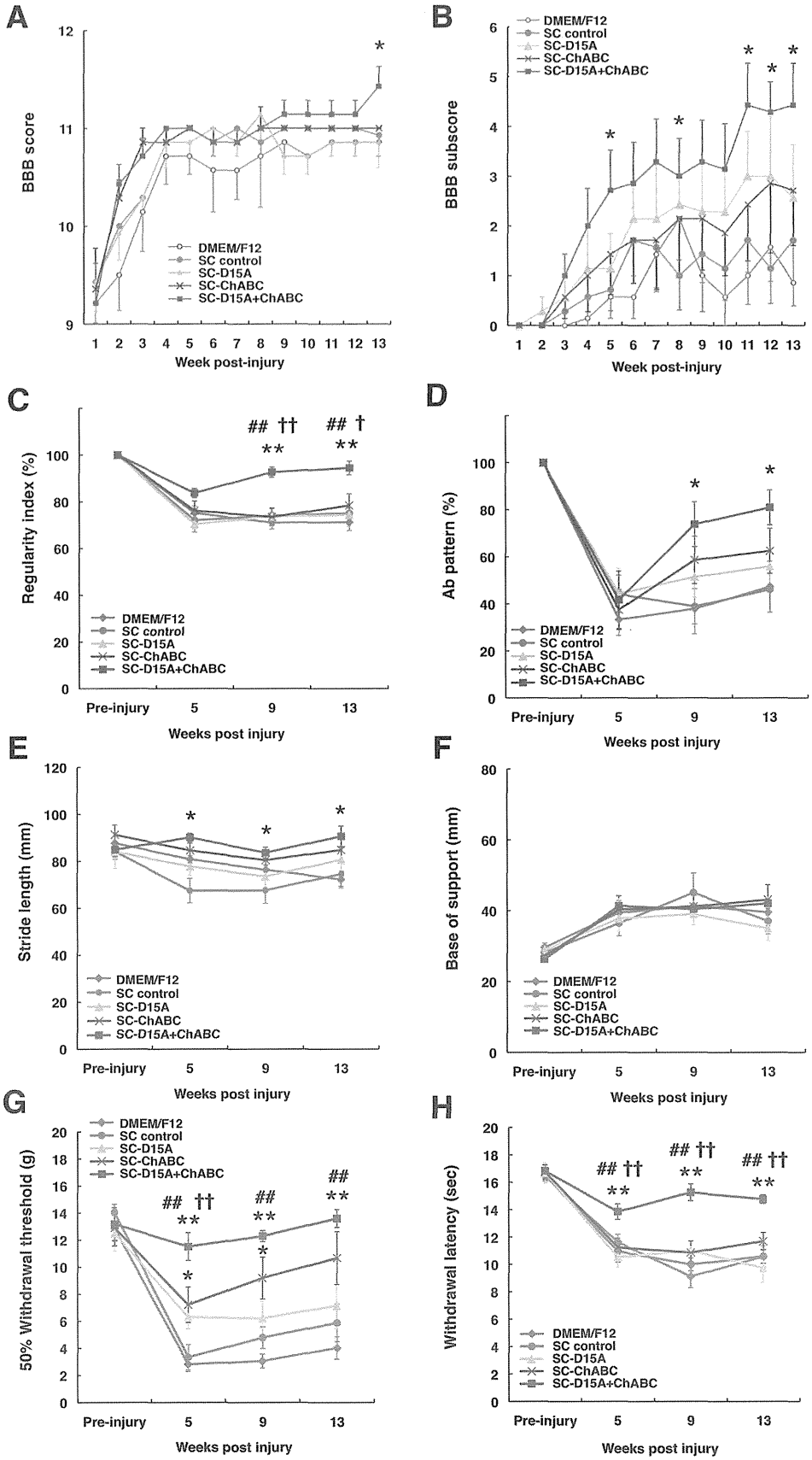


**Figure 12.** Combination of D15A and ChABC improves locomotor function and reduces allodynia in the hindlimbs. *A*, Locomotor function assessed by BBB scoring for 13 weeks after injury. The SC-D15A + ChABC group showed a small locomotor improvement compared with the SC control group at 13 weeks. *B*, In the subscore, the SC-D15A + ChABC group showed a significantly better score than the control group at 5, 8, 11, 12, and 13 weeks. *C–F*, Catwalk data for 13 weeks after injury. *G*, *H*, Graphs showing significant improvements in mechanical and thermal sensitivity in animals receiving the combination treatment. Data are presented as mean ± SEM; $n = 7$ per group. $^{**}p < 0.01$ compared with the SC control group. $^{\#\#}p < 0.01$ compared with SC-D15A group. $^{\dagger\dagger}p < 0.01$ compared with SC-ChABC group.

transplant. A study of ascending axons revealed that they left an NT-3-containing transplant only when NT-3 was present rostral to the transplant and only if the gradient of NT-3 was continuous with the transplant; any break in the NT-3 gradient from the transplant resulted in failure of axons to extend into the host tissue (Taylor et al., 2006). Therefore, a trail of neurotrophin was needed for regrowing axons to extend into the cord from the transplant. It should be noted that Taylor et al. (2006) achieved exit of axons from the transplant without the additional treatment of ChABC. We submit that neurotrophin gradients and modification of the graft/spinal cord interfaces will be among the essential components of a successful SC combination therapy.

The combination strategy significantly improved locomotor function and mechanical and thermal allodynia on the hindpaws. The combination treatment group demonstrated more obvious locomotor improvement in Catwalk parameters than in the BBB score. Previous studies have suggested that BBB scoring may be somewhat subjective, so it would be difficult to achieve a reliable assessment of interlimb coordination (Basso, 2004; Hamers et al., 2006), whereas the Catwalk may allow a more objective analysis of this coordination (Hamers et al., 2001; Deumens et al., 2007). The advantage of the Catwalk analysis in our studies was clarification of the significant locomotor improvement in the combination treatment group.

Previous reports showed that specific parameters in locomotor assessment were affected by mechanical allodynia in the limb (Vrinten and Hamers, 2003; Gabriel et al., 2007). The pain-induced gait change reflects, at least in part, the results in the BBB scoring and in the Catwalk. It was also suggested that paw withdrawal responses to mechanical stimulation were influenced by not only allodynia, but also by recovery of motor function in the hindlimb (Detloff et al., 2010). Therefore, our behavioral data would result from reciprocal influences of the locomotor and sensory conditions in the hindlimb.

### Conclusion

The goal of this experimentation was to improve function after contusion injury by increasing axons in the SC graft and modifying the caudal interface to enable exit of axons from the graft with a combination of transplanted SCs with the provision of neurotrophin and ChABC. This combination of ChABC and D15A led to the following: (1) increased numbers of SCs and SC-myelinated axons in the graft; (2) increased propriospinal axons in the graft and surrounding host tissue; (3) more CST axons closer to the transplant and around it, including caudally; (4) more axons from supraspinal projection neurons in the brainstem present caudal to the transplant; (5) increased serotonergic fibers in the transplant and caudal to it; (6) improvement in aspects of locomotion; and (7) lessened mechanical and thermal hindpaw allodynia. The combination was more efficacious than SCs plus D15A or ChABC. This study is the first to demonstrate a successful SC-based combinatorial strategy with ChABC and neurotrophin for the treatment of SCI. These findings point to the clinical application of genetically modified, autologous SC transplantation for spinal-cord-injured persons.

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