UBL3 Deficiency Impairs Post-Injury Functional Recovery with Prolonged Neuroinflammation and CD99L2 Upregulation

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INTRODUCTION:

Spinal cord injury (SCI) is a devastating disorder of the central nervous system that can be life-threatening and currently lacks effective treatment strategies. Functional recovery after SCI depends on successful nerve regeneration, a process influenced by complex molecular and cellular mechanisms. UBL3, a ubiquitin-like protein, has been found to interact with 35 novel proteins in the mouse brain, suggesting an expanded functional network within the central nervous system. Several of these interacting proteins are associated with spinal cord function and pathology, raising the possibility that UBL3 may contribute to SCI-related pathological processes.

This study investigates whether UBL3 participates in the response to SCI and clarifies its potential role in promoting functional recovery.

METHODS:

In this study, we used eight-week-old female wild-type (WT) mice and female *Ubl3* knockout (KO) (Ubl3−/−) mice, with a weight range of 18–22 g. The wild-type mice (C57BL/6JJmsSlc) were purchased from SLC Inc. (Hamamatsu, Japan). The *Ubl3*-KO mice were acquired from the previously established laboratory colony.

Cross-sections of the mice spinal cord (T9-T10) were immunofluorescently stained with UBL3 (ABclonal, A4028) and TUBB3 (ab78078) antibodies (n = 3). Subcellular fractions—including nuclear, cytosolic, and synaptosomal components—were isolated by cell fractionation, and UBL3 protein levels were quantified via Western blotting (n = 3; one-way ANOVA followed by Tukey's post hoc test; $p < 0.05$).

To evaluate changes in UBL3 expression following injury, tissue was harvested from the injury site 2 hours post-injury in both SCI and sham-operated mice. UBL3 levels were quantified by Western blotting (n = 3; unpaired t-test with Welch's correction; $p < 0.05$).

Functional studies were performed using WT and *Ubl3*-KO mice subjected to SCI simultaneously. Basso Mouse Scale (BMS) scores were recorded on days 1, 3, 7, 10, 14, 21, and 28 post-injury and averaged between two blinded observers (WT: n = 4, *Ubl3*-KO: n = 3; unpaired t-test with Welch's correction at each time point; $p < 0.05$). Three-dimensional MRI reconstructions of the spinal cord were obtained on days 0, 7, 14, and 28 post-injury (n = 3).

To assess inflammation, spinal cord tissue from the injury site was collected on day 7 post-injury from both WT and *Ubl3*-KO mice, and the microglial marker Iba1 (ab178846) was quantified by Western blotting (n = 3; unpaired t-test with Welch's correction; $p < 0.05$). On day 14 post-injury, LC-MS/MS-based proteomic analysis was performed on injured spinal cord tissues from WT and *Ubl3*-KO mice (n = 3). Differentially expressed proteins were identified by calculating the log□ fold change (FC) in expression levels and applying two-tailed unpaired t-tests. Proteins with FC ≥ 2 or FC ≤ 0.5 and $p < 0.05$ were considered significantly altered.

RESULTS: Our results showed that UBL3 is primarily localized in neurons within the gray matter of the spinal cord and is highly enriched in synaptosomes. Notably, UBL3 levels at the injury site decreased as early as 2 hours after spinal cord injury (SCI).

To further investigate its role, we established a SCI model in both Ubl3 knockout (KO) and wild-type (WT) mice. Behavioral assessments—including BMS scoring, 3D spinal cord MRI, and immunostaining of the injury site—consistently showed that Ubl3-KO mice exhibited impaired recovery compared to WT controls.

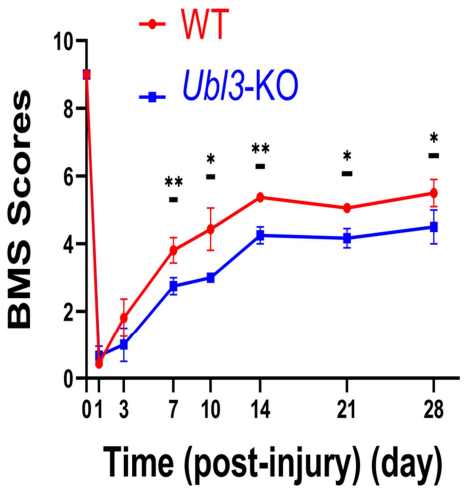
Given the observed recovery deficits, we considered that this impairment was associated with sustained inflammatory infiltration. This was supported by higher levels of Iba1 in the injury site of Ubl3-KO mice at 7 days post-injury, indicative of increased and prolonged microglial accumulation or activation.

Proteomic analysis of spinal cord tissues at 14 days post-injury identified seven significantly altered proteins in KO mice compared to wild-type controls: CD99L2, FAM177A, Dnaja3, TMEM256, FKBP8, Endophilin A2, and Glg1. These proteins are broadly associated with immune regulation, mitochondrial dynamics, neurodevelopmental signaling pathways, and cellular trafficking. Among them, CD99L2 was the most prominently upregulated protein in the KO group, suggesting a potential role in the observed inflammatory phenotype.

DISCUSSION AND CONCLUSION:

Our findings show that UBL3 is predominantly localized in spinal cord neurons, with enrichment in synaptosomes. This distribution pattern reflects its role as a membrane-anchored protein involved in vesicle transport. Furthermore, UBL3 protein levels were reduced following SCI, indicating a molecular response to injury. UBL3 deficiency impaired functional recovery after SCI and was associated with prolonged neuroinflammation. This is accompanied by elevated Iba1 levels and upregulation of CD99L2, an adhesion molecule involved in neuroinflammation.

These results establish UBL3 as a regulator of the inflammatory response following SCI and identify it as a potential therapeutic target.


WT
Ubl3-KO
BMS Scores
10
8
6
4
2
0
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*
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*
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0 1 3 7 10 14 21 28
Time (post-injury) (day)