Molecules and Cells

Research Article

Dipeptidyl peptidase 4 as an injury-responsive protein in the mouse sciatic nerve

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ABSTRACT

Dipeptidyl peptidase 4 (DPP4) is a membrane-bound protease known for its roles in immunity and metabolism; however, its function in the nervous system remains largely unexplored. We found that DPP4 is predominantly expressed in the Schwann cells of the sciatic nerve, and its systemic depletion in postnatal mice resulted in a decline in motor function. Importantly, the inhibition of its proteolytic activity did not affect axon regeneration, indicating that DPP4's protease activity may not be directly involved in axon regeneration. Instead, we observed a reduction in DPP4 protein levels in the sciatic nerve after injury and increased in serum postinjury, suggesting that DPP4 may be shed into circulation, potentially mediating systemic responses following injury. These findings highlight DPP4's importance in sensory function and its potential role in systemic responses after peripheral nerve injury.
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Keywords: Axon regeneration, Dipeptidyl peptidase 4, GLP-1, Schwann cells, Sciatic nerve

INTRODUCTION

Dipeptidyl peptidase 4 (DPP4), also known as CD26, is a membrane-bound serine protease involved in cleaving bioactive peptides such as incretins, cytokines, and neuropeptides (Mentlein, 1999). DPP4 is widely expressed in various tissues and is best known for its role in glucose metabolism, particularly through the degradation of GLP-1, a hormone critical for insulin secretion and glucose homeostasis (Cho and Lim, 2024; Gao et al., 2024; Rohrborn et al., 2015; Shao et al., 2020). Beyond its metabolic functions, DPP4 regulates immune responses by acting as a costimulatory molecule for T cell activation and proliferation, while also modulating B cells, macrophages, and natural killer cells (Klemann et al., 2016; Ohnuma et al., 2015; Shao et al., 2020).

Despite its well-established roles in immunity, metabolism, and viral infections, the function of DPP4 in the nervous system remains largely unexplored. Given its expression in various tissues, its regulatory roles in immune and metabolic pathways, and its broad substrate specificity, DPP4 participates in the cleavage of various bioactive peptides, including incretins such as GLP-1, cytokines, and neuropeptides. This substrate diversity highlights the potential for DPP4 to modulate neuronal signaling and influence responses to nerve injury (Wei et al., 2024). To investigate these unexplored neural functions, we used an axon regeneration model in sciatic nerve injury to explore DPP4's potential role in peripheral nerve function and regeneration.

MATERIALS AND METHODS

Antibodies, Immunohistochemistry, and Western Blot

The following antibodies were used in the present study: anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (Santa Cruz Biotechnology, sc-32233, clone 6C5, 0.2 mg/ml, 1:200), anti-βIII tubulin (TUJ1) antibody (Abcam, ab41489, 0.3 mg/ml, 1:300), anti-superior cervical ganglion 10 (SCG10) antibody (Novus Biologicals, NBP1-49461, 1.0 mg/ml, 1:1,000), anti-myelin basic protein (MBP) antibody (Santa Cruz Biotechnology, sc-271524 AF594, 0.2 mg/ml, 1:50), anti-neural cell adhesion molecule 1 (NCAM1) antibody (Bio-Techne, AF2408, 0.2 mg/ml, 1:1,000), and anti-DPP4 antibody (Abcam, ab187048, 0.155 mg/ml, 1:1,000).

Sciatic nerve tissues were dissected either longitudinally or transversely, fixed in 4% paraformaldehyde for 1 hour at room temperature immediately following dissection, and then placed in 30% sucrose for cryoprotection. Samples were embedded in OCT medium (Tissue-Tek), sectioned at 10 μm thickness, and immunostained as outlined previously (Cho et al., 2015). Briefly, tissues were blocked in a solution containing 5% normal goat serum and 0.1% Triton X-100 in PBS for 1 hour, then incubated overnight at 4°C with primary antibodies diluted in the same blocking solution. Following 4 washes with PBS containing 0.1% Triton X-100, the tissues were incubated for 1 hour at room temperature with secondary antibodies, washed again 4 times in PBS containing 0.1% Triton X-100, and mounted with VectaShield (Vector Laboratories, H1000 or H1200). Imaging was performed as z-stacks using a Zeiss LSM800 microscope, followed by z-projection.

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For biochemical analysis of the sciatic nerve, equal lengths (3 mm) from the proximal and distal sections were homogenized in a RIPA lysis buffer consisting of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 1 mM PMSF, supplemented with a protease and phosphatase inhibitor cocktail (Roche). Equal amounts of protein from each sample were subjected to SDS-PAGE followed by Western blot analysis.

In Vivo Axon Regeneration and Surgery

All procedures involving mice were conducted in accordance with the guidelines approved by the Institutional Animal Care & Use Committee at Daegu Gyeongbuk Institute of Science and Technology. Mice had unrestricted access to food (PicoLab 5053, LabDiet) and water, and were maintained in a controlled environment with a 12-hour light/dark cycle (lights on from 6 am-6 pm). For all experiments, mice of the same age and sex were used at the specified experimental time points. For sitagliptin treatments, 50 μl of 10 mM sitagliptin or DMSO vehicles were applied to sciatic nerves using Surgifoam (Johnson and Johnson). For the in vivo axon regeneration assay, mouse sciatic nerves were crushed using forceps. The nerves were dissected for 2- or 3-days postinjury and fixed for immunohistochemistry analysis. Axon regeneration was assessed by measuring SCG10 fluorescence intensity using ImageJ software, as previously reported (Shin et al., 2014).

Serum Preparation and ELISA

To investigate whether DPP4 proteins potentially shed from the injured sciatic nerve were introduced into vessels as a soluble form and circulating in the blood, we performed an ELISA using serum samples. Blood was collected from the tail or heart of anesthetized mice 2 or 24 hours after injury, incubated at 37°C for 1 hour and centrifuged at 3,000 rpm at 4°C for 10 minutes. The supernatants were gently pipetted to avoid blood cell contamination and used for ELISA with a mouse DPP4 ELISA kit (Abcam, ab264630). Serum samples were diluted 1:100 or 1:200, and concentrations were calculated based on the standard curve for each experiment.

Embryonic DRG Neuron Culture

To prepare embryonic DRG neuron cultures, DRG tissue was dissected from E12.5 mice and dissociated in 0.05% trypsin-EDTA. The cells were plated on poly-D-lysine/laminin-coated dishes in Neurobasal Medium (Gibco), supplemented with 2% B-27 (Gibco), 1% Glutamax, 1 μM 5-fluoro-2′-deoxyuridine (Sigma), 1 μM uridine (Sigma), 1% penicillin-streptomycin, and 50 ng/ml 2.5S nerve growth factor (Envigo, BT-5017). To prevent the death of non-neuronal cells in embryonic DRG tissues, an fluorodeoxyuridine (FDU)-free medium was prepared with the addition of 5% FBS. In the +FDU condition, the same culture medium was used but supplemented with FDU to eliminate dividing non-neuronal cells.

In Vivo AAV Delivery and Behavioral Test

To knockdown *Dpp4* in vivo, 8 μl for low-dose injection or 12 μl for high-dose injection of AAV (serotype 9) encoding GFP- (1×10^{13} GC/ml) or shRNA to *Dpp4* (1×10^{13} GC/ml) was injected into neonatal CD-1 mice (postnatal day 1) via facial vein injection using a Hamilton syringe (Hamilton, 1710 syringe with a 33G/0.75-inch small hub removable needle). All behavioral tests were conducted in a soundproof room with controlled temperature, humidity, and light. The mice were acclimated for 1 hour in the test room before training and testing. Open field test: the open field test was conducted for analyzing spontaneous explorative activity. Each mouse was initially placed in a center of the open field box (Jeungdo Bio & Plant). Mice were allowed to freely explore the box for 15 minutes. Distance moved (cm) and velocity (cm/s) were recorded by Ethovision xt11.5 (Noldus). Rotarod test: the rotarod test was conducted to assess passive proprioceptive sensory-motor integration and motor learning (Ugo Basile). For training, mice were placed on the rotarod, with the speed increasing from 4 to 40 rpm over 300 seconds, and trained a total of 5 times across 2 days. One day after training, mice were tested with the speed accelerating from 5 to 50 rpm over 300 seconds, with 3 trials conducted. Treadmill running endurance test: to assess exercise capacity, mice underwent a treadmill running endurance test (Panlab/Harvard Apparatus). On the first day, they were acclimated to running at speeds ranging from 12 to 17 cm/s, and on the second day, the speed was increased to between 17 and 20 cm/s. For the endurance test, the mice were placed on a treadmill set to a 10° incline. The speed gradually increased from 17 to 61 cm/s over a duration of 48 minutes.

RESULTS

Dpp4 Knockdown Leads to Behavioral Deficits

Previous studies on *Dpp4* loss-of-function models have primarily focused on *Dpp4*'s metabolic and immune roles, with less attention given to its role in the nervous system. We systematically knocked down *Dpp4* using AAV-shRNA delivery in neonatal mice and assessed their behavioral performance at 5 weeks. In the tail suspension test, mice injected with *Dpp4* AAV-shRNA exhibited significant hindlimb clasping, indicating motor impairment (Fig. 1a and b). In addition, in the open field test, rotarod test, and treadmill running endurance test, the *Dpp4* knockdown mice displayed significantly reduced motor coordination and performance compared to controls (Supplementary Fig. 1a-c).

While previous research on *Dpp4* knockout models emphasized its metabolic and immune phenotypes, our analysis of neonatal systemic *Dpp4* depletion demonstrates that *Dpp4* deficiency results in significant behavioral deficits, highlighting its important role in maintaining proper coordination.

Dpp4 Is Expressed in Both Myelinating and Nonmyelinating Schwann Cells

Given the observed sensory and motor dysfunctions in systemic *Dpp4* knockdown mice, we next investigated the expression of DPP4 in the peripheral nervous system. We performed immunohistochemistry on cross-sections of the mouse sciatic nerve and found that DPP4 is predominantly located near areas where MBP, a marker of myelinating Schwann cells, is expressed, forming "ring-like" structures encircling axons marked by TUJ1 without directly overlapping with them (Fig. 1c and e). This pattern suggests that DPP4 is primarily expressed in myelinating Schwann cells.

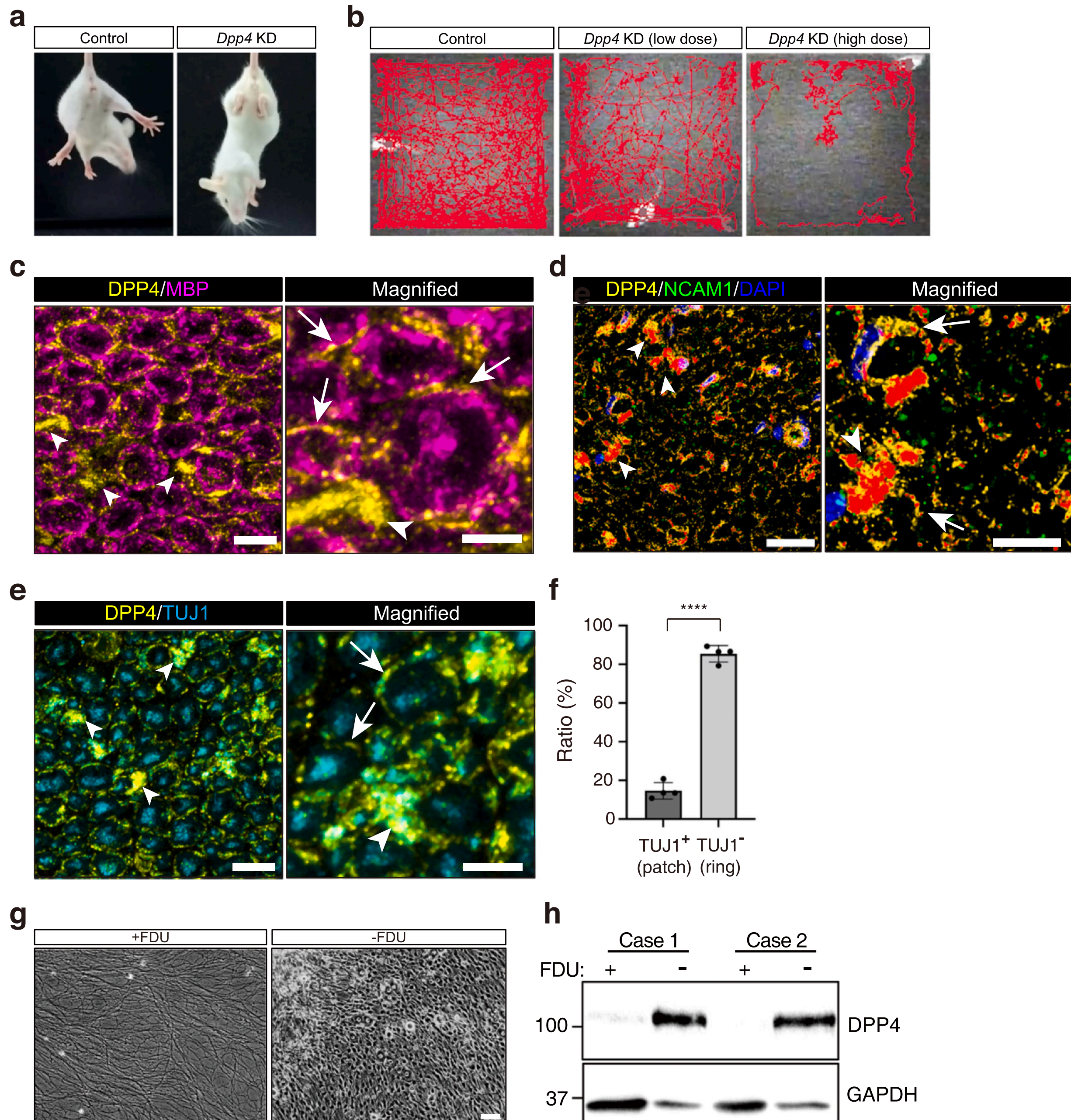

Fig. 1. Systemic *Dpp4* depletion results in significant behavioral abnormalities in mice and DPP4 is predominantly expressed in nonaxonal cells of the mouse sciatic nerve. (a) Tail suspension assay demonstrating that *Dpp4* knockdown mice show hindlimb clasping, indicating impaired neurological function. (b) Open field test results with tracking of mouse movement (red lines). (c-e) Immunofluorescence staining of cross-sections from adult mouse sciatic nerve, showing DPP4 localization in comparison to markers for myelinating Schwann cells (MBP, myelin basic protein, c), non-myelinating Schwann cells (NCAM1, neural cell adhesion molecule, d), and axons (TUJ1, β3-tubulin, e). DPP4 shows ring-like pattern (arrows) and patch-like pattern (arrowheads). Scale bar, 10 µm (left) or 5 µm (right, magnified). (f) Quantification of ratio of non-TUJ1-overlapping "ring"-like DPP4 staining and TUJ1-overlapping "patch"-like DPP4 staining ($n = 4$ of unit areas from the individual mouse sections, $^{****}P < .0001$). (g) Embryonic DRG tissues from E12.5 mice were dissected, dissociated, and cultured. The +FDU group represents a pure neuronal culture where fluorodeoxyuridine was added to eliminate dividing non-neuronal cells, while the −FDU group allowed non-neuronal cells to survive alongside neurons (scale bar, 100 µm). (h) Western blot analysis of total lysates collected on day 6 from the cell cultures shown in (g). Cases 1 and 2 represent independent biological replicates.

In certain regions, however, DPP4 staining appeared in "patch-like" patterns, distinct from the circular shapes associated with myelinating Schwann cells. To investigate whether these patch-like structures represent nonmyelinating Schwann cells, we performed additional immunohistochemistry with NCAM1, a marker for nonmyelinating Schwann cells. The results showed that DPP4 colocalized with NCAM1 in these regions (Fig. 1d), indicating that these patch-like signals likely represent DPP4 expression in nonmyelinating Schwann cells (Supplementary Fig. 2a-c, arrowheads).

To quantify these observations, we analyzed the proportion of DPP4 signals forming ring-like versus patch-like patterns. The results showed that the majority of DPP4 signals were associated with ring-like patterns around TUJ1, indicative of myelinating Schwann cells, while a smaller proportion exhibited patch-like patterns along TUJ1-positive axons, consistent with nonmyelinating Schwann cells (Fig. 1f). These findings suggest that DPP4 is expressed in both myelinating and nonmyelinating Schwann cells, potentially playing distinct roles in each cell type and contributing to different aspects of nerve function and regeneration.

To further verify these observations, we performed an experiment using embryonic DRG (dorsal root ganglion) neuron cultures, where DRG dissociates were cultured with or without FDU, a compound used to eliminate dividing non-neuronal cells (Fig. 1g). After 5 days, total cell lysates were analyzed by western blot. In the FDU-treated condition, where non-neuronal cells were removed, very little DPP4 was detected. However, in cultures without FDU, a significant amount of DPP4 was present, suggesting that DPP4 is primarily expressed in non-neuronal cells within embryonic DRG tissues (Fig. 1h).

DPP4 Levels Show Spatially Restricted Decrease in Proximal Part of Sciatic Nerve After Transection

Since DPP4 is expressed in Schwann cells of the sciatic nerve, we investigated how its levels change following sciatic nerve injury, which involves demyelination and remyelination. We performed a sciatic nerve transection and harvested protein lysates from the proximal and distal parts of the injured nerve at 24 hours postinjury (Fig. 2a). DPP4 showed a specific reduction in the regenerating proximal part (SP) 24 hours after injury, displaying a significant reduction to 0.25-fold compared to uninjured sciatic nerves (SU) (Fig. 2b and c). In contrast, the distal part, which undergoes degeneration, did not exhibit any significant decrease in DPP4 levels. This suggests that the reduction in DPP4 is a proximal-specific response to nerve injury.

To determine how early this response occurs, we examined various time points and found that the reduction of DPP4 in the proximal part is observed as early as 2 hours post-transection, indicating an immediate reaction to injury (Supplementary Fig. 3a). Interestingly, the staining patterns of DPP4 differed between MBP-positive and NCAM1-positive regions as shown in Figure 1. Following sciatic nerve injury, the ring-like DPP4 signals nearly disappeared, whereas the patch-like DPP4 signals remained visible even after the injury (Supplementary Fig. 3b). This suggests that DPP4 in myelinating cells and nonmyelinating cells may play distinct roles, with each potentially contributing differently to the regenerative process.

Dpp4 Is a Potential Mediator of Nerve Injury Responses With Distinct Functions

DPP4 is a membrane-bound protease known to have both protease-dependent and protease-independent functions. Given its potential role in nerve regeneration, we sought to determine whether DPP4′s protease activity contributes to axon regeneration. To assess this, we locally applied the DPP4 protease inhibitor, Sitagliptin, to the injured sciatic nerve to inhibit its protease activity (Fig. 2d). However, axon regeneration assays revealed that Sitagliptin treatment did not result in any significant enhancement or reduction in axon regeneration (Fig. 2e and f). This suggests that DPP4′s protease activity is not directly involved in the axon regeneration process following sciatic nerve injury. Instead, the observed decrease in DPP4 levels at the proximal injury site likely represents a Schwann cell-specific response to nerve damage, rather than a direct role in promoting axon regeneration.

DPP4 is known to undergo shedding, where it is released from the membrane under specific stimuli (Lee et al., 2020). Given the rapid decrease in DPP4 levels near the sciatic nerve injury site, we hypothesized that DPP4 may be shed from Schwann cells postinjury and then released into the bloodstream, acting as a signal that transforms a local injury event into a systemic response. To test this, we measured DPP4 levels in the serum at 2 and 24 hours following sciatic nerve injury (Fig. 2g). Our results showed a significant increase in serum DPP4 at 2 hours postinjury, while no increases were observed at 24 hours (Fig. 2h). Although it is not direct proof of injury-responsive DPP4 shedding, the correlation between the decrease in DPP4 at the injury site and the increase in serum DPP4 supports our hypothesis.

In conclusion, while DPP4′s protease activity does not appear to be directly involved in axon regeneration, the rapid reduction of DPP4 levels in Schwann cells near the injury site, coupled with its increase in the bloodstream, suggests that DPP4 may act as a shedding-mediated signal. This mechanism could contribute to a broader systemic response to peripheral nerve injury, indicating a dual role for DPP4 in both local tissue responses and systemic signaling after injury.

DISCUSSION

Our study highlights the critical role of DPP4 in peripheral nerve function and its distinct response to nerve injury. While previous research on DPP4 has primarily focused on its metabolic and immune roles, our findings suggest an underexplored role for DPP4 in the nervous system, particularly in the context of nerve injury and regeneration. This underscores the need for further investigation into the neuronal functions of DPP4, especially in sensory neuron signaling and Schwann cell responses following peripheral nerve damage.

A key finding in this study is the rapid reduction of DPP4 protein levels near the sciatic nerve injury site, accompanied by a corresponding increase in serum DPP4 shortly after injury. Although this is not direct proof of DPP4 shedding, it raises the possibility that DPP4 is released from Schwann cells into the circulation following nerve damage. This shedding could represent a mechanism by which DPP4 mediates both local and systemic responses to injury. Locally, DPP4 may influence the environment around the injury, potentially affecting Schwann cell behavior or other aspects of the regeneration process. On a systemic level, DPP4 could act as a signal to initiate broader physiological responses, possibly serving as a key mediator in the connection between peripheral nerve injury and systemic inflammation (Han et al., 2020; Tang et al., 2023).

Given the potential for DPP4 to mediate both local and systemic responses, it is important to explore whether DPP4

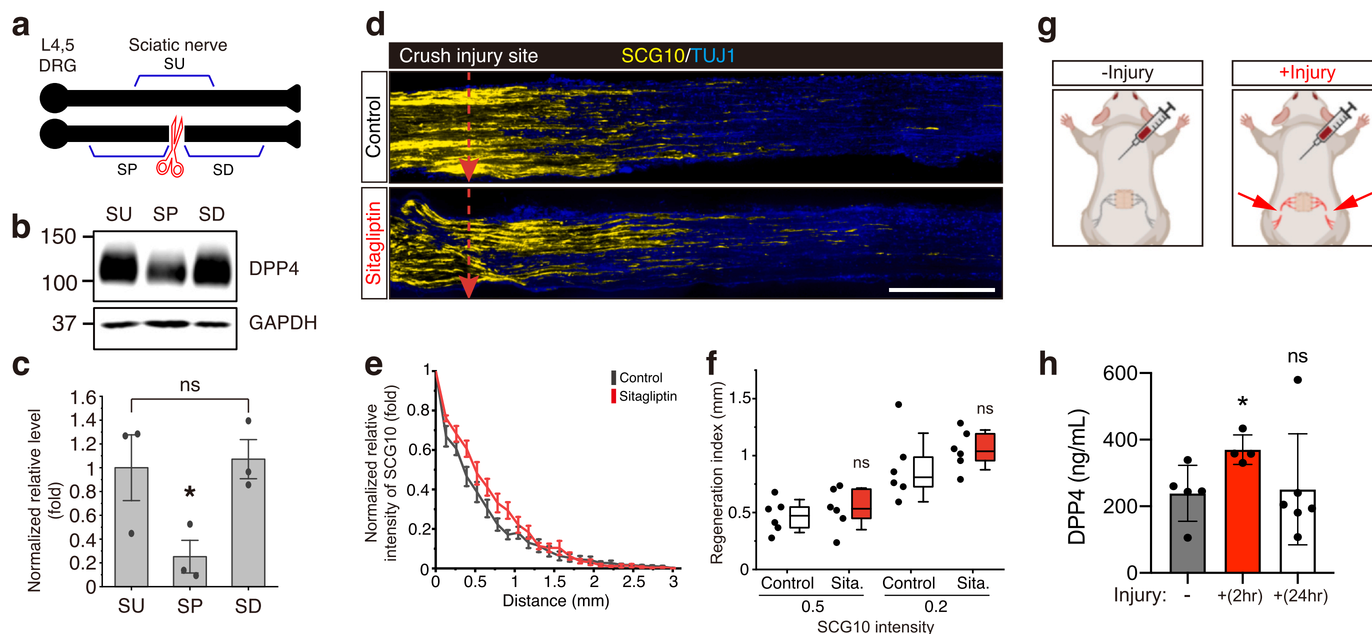

Fig. 2. DPP4 protein levels decrease in the proximal injured nerve, and serum DPP4 increases acutely after injury. (a) Schematic diagram of sciatic nerve injury model. L4,5 DRG, L4, and L5 dorsal root ganglions of a mouse. SU, an intact sciatic nerve with no injury. SP, a transected sciatic nerve part proximal to the DRGs. SD, a transected sciatic nerve part distal to the DRGs. (b) Mouse sciatic nerves were transected, and after 24 hours, total lysates were collected for western blot analysis. (c) Quantification of the western blot data shown in (b), normalized to GAPDH ($n = 3$ for each condition; mean ± SD, $^*P < .05$, ns: not significant). (d) Crush injury was applied to the sciatic nerve, and 48 hours later, longitudinal sections were obtained. Nerve sections were immunostained with SCG10 (yellow) to label regenerating axons and TUJ1 (blue) for general axon staining. The red dashed line with arrows marks the injury site. Scale bar, 500 µm. (e) Quantification of SCG10 fluorescence intensity in the distal part of the nerve, normalized to the crush site. Data are plotted as SCG10 intensity versus distance from the crush site ($n = 6$ for each condition; mean ± SD). (f) Box plot of the regeneration index, calculated as the distance where SCG10 intensity reaches 50% or 20% of the value at the crush site (Box: 25-75%; median ± SD; ns: not significant). (g) Schematic of experimental design. Mice were either uninjured (−Injury) or both sciatic nerves were transected (+Injury). At indicated time points postinjury, blood was collected from the heart and serum was isolated. (h) ELISA analysis of Dpp4 protein levels in serum collected at the indicated times following the experiment in (d) ($n = 5$, 4, and 6 for each condition; mean ± SD; $^*P < .05$, ns: not significant).

levels or activity are altered in the serum of patients with conditions such as peripheral neuropathy. Assessing DPP4 activity or concentration in these patients could offer valuable insights into whether DPP4 plays a role in the development of these conditions and whether it could serve as a biomarker for nerve injury or regeneration. Additionally, if DPP4 is shown to mediate systemic responses following peripheral nerve injury, this could suggest that repeated nerve injury might lead to chronic systemic inflammation, potentially contributing to inflammatory diseases in patients who experience recurrent peripheral nerve damage (Kiraly et al., 2018).

Further research is also needed to understand the precise mechanisms by which DPP4 exerts its effects after injury. While our data suggest that DPP4′s protease activity is not directly involved in axon regeneration, the role of DPP4 in systemic signaling remains unclear (Han et al., 2020; Tang et al., 2023). Shedding of DPP4 into the bloodstream could activate inflammatory pathways or immune responses, leading to a systemic response following what is primarily a local injury. A deeper investigation into DPP4′s functions in the blood is essential to fully understand its role in these processes. Moreover, studies exploring the protease-independent functions of DPP4, such as its interactions with other cell types or its role in modulating the immune system, are necessary to grasp the broader implications of DPP4 shedding in the context of nerve injury and regeneration (Wronkowitz et al., 2014; Zhong et al., 2015). These observations highlight the need to develop models capable of directly demonstrating whether the increased serum levels of DPP4 following sciatic nerve injury originate from Schwann cells or from immune cells activated in response to the injury, to better understand the interplay between systemic and local mechanisms in nerve damage and recovery.

In conclusion, our study identifies DPP4 as a potential key player in both local and systemic injury responses following peripheral nerve damage. Future studies should focus on understanding the neuronal functions of DPP4, its role in systemic signaling through shedding, and its possible contribution to systemic inflammatory conditions that arise from repeated nerve injuries. These findings underscore the necessity of developing Schwann cell-specific *Dpp4* knockdown or knockout models to further elucidate the tissue-specific roles of *Dpp4* in peripheral nerve injury and repair. Additionally, investigating DPP4′s role in the blood could provide important insights into how peripheral nerve injury affects the systemic inflammatory response, potentially leading to new therapeutic strategies aimed at modulating DPP4 activity in conditions such as peripheral neuropathy and related disorders.

AUTHOR CONTRIBUTIONS

Y.O. conceived and performed experiments. Y.O. and Y.C. wrote the manuscript. Y.C. secured funding.

DECLARATION OF COMPETING INTERESTS

The authors declare no competing interests.

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APPENDIX A. SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: doi:10.1016/j.mocell.2024.100159.

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