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Epidermal neural crest stem cell-derived glia enhance neurotrophic elements in an ex vivo model of spinal cord injury

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Abstract

Growing evidence that cell-based therapies can improve recovery outcome in spinal cord injury (SCI) models substantiates their application for treatment of human with SCI. To address the effectiveness of these stem cells, potential candidates should be evaluated in proper SCI platform that allows direct real-time monitoring. In this study, the role of epidermal neural crest stem cells (EPI-NCSCs) was elucidated in an ex vivo model of SCI, and valproic acid (VPA) was administered to ameliorate the inhospitable context of injury for grafted EPI-NCSCs. Here the contusion was induced in organotypic spinal cord slice culture at day seven in vitro using a weight drop device and one hour post injury the GFP- expressing EPI-NCSCs were grafted followed by VPA administration. The evaluation of treated slices seven days after injury revealed that grafted stem cells survived on the injured slices and expressed GFAP, whereas they did not express any detectable levels of the neural progenitor marker doublecortin (DCX), which was expressed prior to transplantation. Immunoblotting data demonstrated that the expression of GFAP, BDNF, neurotrophin-3 (NT3), and Bcl2 increased significantly in stem cell treated slices. This study illustrated that the fate of transplanted stem cells has been directed to the glial lineage in the ex vivo context of injury and EPI-NCSCs may ameliorate the SCI condition through releasing neurotrophic factors directly and/or via inducing resident spinal cord cells.

KEYWORDS
epidermal neural crest stem cell, glia, spinal cord injury, valproic acid

1 | INTRODUCTION

The restricted regenerative capacity of adult spinal cord has directed the focus of preclinical trauma investigation on finding new tools that reduce secondary degeneration and promoting regeneration. Stem cells could potentially address both of these requirements as they can contribute in replacement of injured neurons and glia, secretion of trophic factors and enhancement of axon elongation that ultimately benefit SCI condition.[1] So far numerous types of stem cells

Abbreviations: CNS, central nervous system; DCX, doublecortin; DIV, day in vitro; DPI, day post injury; ECL, enhanced chemo-luminescence; EPI-NCSC, epidermal neural crest stem cell; GFP, green fluorescent protein; HDAC, histone deacetylase; IHC, immunohistochemistry; MEM, minimum essential medium; NT3, neurotrophin-3; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; RT, room temperature; SEM, scanning electron microscopy; SCI, spinal cord injury; VPA, valproic acid; WB, Western blotting.

from neuronal and non-neuronal tissues have been examined in animal models and humans.[2–4] Among various cell types have been investigated for SCI regeneration, a considerable research was dedicated to address effectiveness of central nervous system (CNS) derived stem cells and those that are ontologically related as they are predetermined to differentiate into neural lineages.[5,6]

EPI-NCSCs are one of the adult multipotent stem cells that have received much attention from an increasing number of studies as a promising therapeutic option for SCI. These stem cells are among derivatives of the transient embryonic neural crest stem cells and reside in the bulge of the hair follicle. These cells are accessible in the bulge of hairy skin and can be isolated through a minimally invasive procedure.[7–9] EPI-NCSCs have been evaluated in various animal models of SCI, as they are ontologically related to spinal cord stem cells and express both neuronal, and glial markers.[10–13]

Moreover, due to limited restorative capacity of cell-based therapy and complexity of SCI condition, there is an agreement among researchers that combination therapies will lead to cumulative SCI improvement.[14] In this regard, therapeutical strategies that combine cell transplantation with drugs that activate growth promoting programs and/or attenuate the growth inhibitory pathways have been explored considerably.[15,16] Valproic acid (VPA; 2-propylpentanoic acid)-an established drug in treatment of epilepsy and bipolar mood disorders- recently has been appreciated as a potential pharmacological approach that promotes SCI recovery.[17–21] It has been widely reported that VPA imposes its positive impacts via inhibiting histone deacetylase (HDAC) and also activating ERK pathway that produce effects similar to neurotrophic factors in the CNS.[22,23] Therefore, VPA was employed to ameliorate the inhospitable context of injury to help grafted epidermal neural crest stem cells (EPI-NCSCs) exhibit their potential.

The consequences and efficacy of EPI-NCSCs transplantation have only been assessed in animal models of SCI; however, further elaboration and mechanistic studies require a more accessible platform. As organotypic slice cultures can simulate spinal cord injury ex vivo, they have become important tools in SCI research recently.[24–26] Using an ex vivo- derived SCI model, besides overcoming the in vivo challenges, allows direct real-time detection of effects following employed therapeutical strategies in various cellular, and molecular levels.[27] Although modeling contusion in slice culture does not replicate all the features related to traumatic injury in human, this system closely mimics the in vivo complex microenvironment. Collectively, this study was designed to find out the role of transplanted EPI-NCSCS in ex vivo SCI model and define their acquired fate seven days post transplantation in the presence of VPA. Taking advantage of the newly introduced ex vivo model of SCI, we reported here that grafting epidermal neural crest stem cells enhanced neurotrophic and neuroprotective factors in the context of injury, meanwhile they no longer expressed neuronal marker.

2 | MATERIALS AND METHODS

In the present study, all animal experiments were undertaken in accordance with the Ethical Committee for the use and care of laboratory animals of Neuroscience Research Center, Shahid Beheshti University of Medical Sciences in compliance with the standards of the European Communities Council directive (86/609/EEC), and efforts were made to minimize animal suffering and reduce the number of animals used.

2.1 | Organotypic spinal cord slice culture

Organotypic spinal cord slice culture was prepared from adult male Wistar rats (weighing 200-250 g) according to regular interface method.[28] Animals were deeply anesthetized with CO_2 and back skin was removed and vertebral column was dissected. Once exposed, the spinal cord was flushed out with ice-cold phosphate buffered saline (PBS) using a 60 mL syringe. Next, the lumbar enlargement region of the cord was excised and transferred to another petri dish containing 40°C low melting point agarose (4% V/W in HBSS) and placed on ice to induce gelation. Then the embedded spinal cord was cut by vibratome at 400 μm. Finally, the undamaged slices with undisturbed morphology were transferred onto inserts with 0.4 micrometer pore size (Millipore, Burlington, MA) in six-well plate with 1 mL of culture medium consisted of 50% minimum essential medium (MEM) with L-glutamine, 25% heat-inactivated horse serum, 25% HBSS, 6.4 mg/mL D-glucose, and 1% penicillin/streptomycin. Slices were incubated in a 5% CO_2 humidified incubator at 37°C and the medium was changed thrice a week.

2.2 | In vitro preparation of epidermal neural crest stem cell

The EPI-NCSCs were isolated from bulge of whisker hair follicles of adult rats as described earlier.[7,29] Briefly, follicles of whisker pad were dissected and bulge region was rolled out by cutting the follicle capsule longitudinally. Isolated bulges were explanted in collagen-coated plates and fed with alpha-modified MEM supplemented with 10% fetal bovine serum, 5% day-11 chick embryo extract, and 1% penicillin/streptomycin and were cultured at 37°C with 5% CO_2. Following the migration of EPI-NCSCs, stem cells were trypsinized and replated. Afterward, stem cells were transduced with lentiviral particles (pGreenPuro, SBI, Palo Alto, CA) encoding green fluorescent protein (GFP) produced in LentiX-293T cells (Clontech, Mountain View, CA).

Using puromycin resistance gene as a selection marker, non-transduced cells were omitted by puromycin containing (2 μg/mL, Sigma, St. Louis, MO) medium.

2.3 | Immunofluorescent staining

Indirect immunofluorescent staining was performed on the population of migrated EPI-NCSCS on day 10 in vitro before and after transduction. Briefly, cells were washed twice with PBS for 2 min and fixed with 4% paraformaldehyde at room temperature (RT) for 12 min, followed by three washes of TPBS (0.05% Tween-20 in PBS). Subsequently, cells were permeabilized with 0.2% Triton X-100 for 10 min, and non-specific sites were blocked with 1% BSA in TPBS at RT for 1 h. Primary antibodies used were: rabbit anti-Double-cortin (DCX) (Abcam, Boston, MA, Cat No: ab77450, 1:200), rabbit anti- β-III tubulin (Abcam, Cat No: ab18207, 1:200) and rabbit anti- GFAP (Abcam, Cat No. ab7260, 1:1000). Secondary antibody used was goat anti-rabbit IgG FITC conjugated (Sigma, Cat No. F1262, 1:100), and the cells' nuclei were counterstained with DAPI (Sigma).

2.4 | SCI model and combination therapy

After 7 days of organotypic culture, SCI was induced at day 7 in vitro (DIV: 7) using a weight drop device adopted from Krassioukov et al.[30] Briefly, an impactor (Figure 1A) with a 3 mm diameter head and 0.5 g total weight was dropped from a height of 3 cm on the entire slice surface and subsequently slices were returned to incubator. Experimental groups were assigned as: (i) Non-injured; (ii) injured slices (SCI); (iii) injured slices treated with VPA (SCI+VPA); (iv) or stem cells (SCI+Cell); and (v) injured slices that received combinatory therapy (SCI+VPA+Cell).

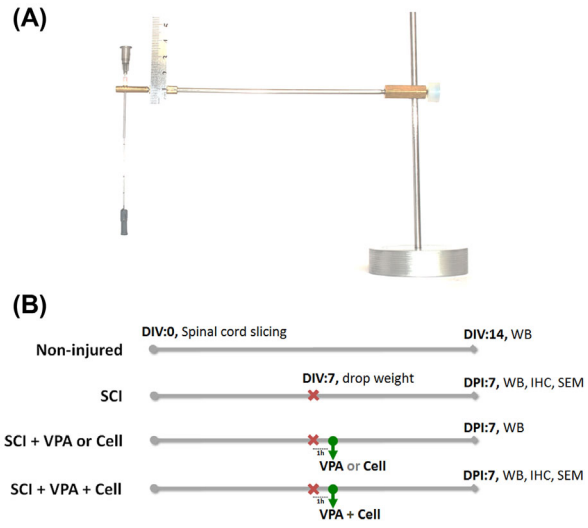

FIGURE 1 The injury device and the schematic protocol of experiment. The injury was induced by dropping the rod and attached grey pin head on top surface of spinal cord slices. The total weight of rod and its head was 0.5 gram that was dropped from a 3 cm height (A). The stem cell and VPA treatment of injured spinal cord slices and following evaluations in each experimental group (B). DIV: day in vitro, DPI: day post injury, WB: western blotting, IHC: immunohistochemistry, SEM: scanning electron microscopy

One hour after injury, GFP- expressing EPI-NCSCs were trypsinized and number of 3×10^4 cells in 3 μL medium were transplanted on top surface of injured slices using a fire-polished capillary tube attached to pipette tip to avoid touching slices. Valproic acid was provided from the Darou Pakhsh Pharma. Chem. Co., Tehran, Iran and 5 μM concentration was prepared in the complete medium and applied one hour following injury as its effectiveness in the aforementioned injury context was established formerly.[31] The procedure of injured slices treatment was carried out at acute phase and following evaluations were illustrated in Figure 1B.

2.5 | Western blot

For western blot analysis, in each experimental group, almost seven spinal cord slices from at least three different rats were collected and snap frozen in liquid nitrogen. Samples were homogenized in ice-cold modified RIPA lysis buffer (50 mM Tris-HCl pH 7.5, 1% Triton X-100, 0.5% sodium deoxycholate [Sigma], 0.2% SDS, 100 mM NaCl and 1 mM EDTA). Protein concentration was determined using bicinchoninic acid kit (BCA Protein Assay Reagent, Thermo Fisher Scientific, Rockford, IL). Protein extract (20 μg/lane) were separated on a 12% sodium dodecylsulfate-polyacrylamide mini-gel and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were then incubated with 2% skimmed milk blocking buffer for 1 h. Afterward, membranes were incubated in primary antibodies overnight at 4°C: rabbit anti-GFAP (Abcam, Cat No. ab7260, 1:10000), rabit anti- β-III tubulin (Abcam, Cat No: ab18207, 1:200), rabbit anti-Bcl-2 (Cell Signaling, Cat No. 2876, 1:1000), rabbit anti-BDNF (Thermo Fisher Scientific Cat No. OSB00017W, 1:500, ~28KDa), rabbit anti-neurotrophin 3 (Abcam, Cat No. ab65804, 1:500) and rabbit anti-β-actin (Cell Signaling, New York, NY, Cat No. 49687, 1:750). After incubation with secondary antibody conjugated to horseradish peroxidase (Cell Signaling, Cat No. 7074, 1:3000) protein bands were detected by an enhanced chemo-luminescence kit (ECL kit, GE Healthcare Life Science, Marlborough, MA) in accordance with the manufacturer's instructions and blots were exposed to X-ray film (Kodak, Rochester, NY) and intensities were quantified with Image J software.

2.6 | Slice processing and immunohistochemistry

The immunostaining of slices was carried out seven days after cell transplantation according to introduced protocol of

Gogolla and her colleagues.[32] Firstly, slices were fixed with 4% paraformaldehyde for 5 min and following washing with PBS, they were fixed with 20% methanol in PBS for another 5 min. After washing slices with PBS, they were permeabilized (0.5% Triton X-100 in PBS) for 2 h and then slices were transferred to 24-well plate and blocked in 10% BSA for overnight at 4°C. Then, slices were incubated with primary antibody overnight. On third day, slices were washed trice with PBS over 45 min time span. Afterward slices were incubated with goat anti-rabbit alexa Fluor 633 conjugated secondary antibody (Thermo Fisher Scientific Cat No. A-21070, 1:100) for 2 h at RT and then washed with PBS and observed with confocal microscope. Confocal microscope (Nikon, Tokyo, Japan) was set to observe upper 20 μm layer of slices.

2.7 | Electron microscopy

For scanning electron microscopy (SEM), the treated and non-treated spinal cord slices were fixed in 2.5% glutaraldehyde for 24 h followed by two consecutive washes in PBS for 20 min and 2 h post fixation in 1% tetroxidosmium. Samples were dehydrated in ascending concentrations of ethanol and then air-dried for 24 h. They were mounted on stubs, coated with gold-palladium and examined by Hitachi SU3500 SEM (Hitachi High-Tech, Tokyo, Japan) at 15 kV.

2.8 | Statistical analysis

Statistical analysis was performed on GraphPad Prism (Version 6.02, 1992-2013 GraphPad Software, Inc., San Diego, CA) using one-way ANOVA and Tukey post hoc test. $P < 0.05$ was considered significant.

3 | RESULTS

3.1 | EPI-NCSCs were characterized before transplantation

In this study EPI-NCSCs were isolated from bulge area of whisker pad hair follicles. Within 2-3 days after bulge explantation, cells with stellate morphology migrated with increasing number over time (Figure 2A). To confirm the migrated cells as an epidermal neural crest stem cells, the expression of DCX, a neuronal precursor marker, β-III tubulin, an immature neurons marker and GFAP, traditional astrocyte marker were evaluated. Immunostaining demonstrated the expression of all three assessed markers before (Figures 2B and 2D) and after transduction (data not shown) and this observation was in agreement with findings of previous studies.[12,29]

3.2 | Transplanted EPI-NCSCs can survive on injured spinal cord slice

In the next step, a highly pure population of GFP expressing stem cells was generated following transduction (Figure 2E and E') to track and distinguish transplanted stem cells from resident spinal cord cells in a noninvasive fashion. Seven days following transplantation of GFP expressing EPI-NCSC on the top surface of injured spinal cord slices, grafted stem cells were identified by their green fluorescence. Herein, confocal images illustrated survival of implanted cells and their morphology within both white and grey matter (Figure 3B and 3B1), However, no green cells were detected on top surface of injured slices that have not been grafted (Figure. 3A and 3A1) . Also slices were assessed with SEM to reinforce the presence of transplanted stem cells after seven days. Here, obtained images depicted transplanted stem cells attached properly to the surface of SCI slices Assessing top surface of injured spinal cord slices with SEM illustrated the presence of grafted cells (indicated by arrows) whereas they were not detected on non-treated injured slices (Figure 4).

3.3 | High expression level of neurotrophic and neuroprotective factors in injured slices following cell therapy

We next determined the expression level of two neurotrophic factors, BDNF, and NT3 and obtained data were analyzed with one-way ANOVA. According to previous reports, NT3 plus BDNF are two stimulatory cues that play substantial role in axonal regrowth in SCI lesion.[33–35] BDNF immunoblotting revealed that injured slices treated with VPA (SCI+VPA), stem cells (SCI+Cell) or combination of both (SCI+VPA +Cell) expressed significantly higher level of BDNF (Figure 5A). NT3 was another neurotrophic factor that has been examined in the context of injury.[36,37] NT3 level was significantly higher in experimental group transplanted with stem cells compared with either SCI or VPA treated groups (Figure 5B). The next factor examined by immunoblotting was Bcl-2, a neuroprotective agent which its overexpression prevents cells from undergoing apoptosis.[38] Our study demonstrated elevation of Bcl-2 in cell grafted experimental group. Nonetheless, while based on growing number of evidence treatment of SCI with VPA yields in significant increase of Bcl-2,[18,39] in our study VPA treatment alone showed no greater expression level compared with untreated slices (Figure 5C).

3.4 | Enhanced level of glial marker in injured slices after cell transplantation

In the subsequent set of experiments, immunoblotting was performed to define the expression level of β-III tubulin and GFAP which acquired data were analyzed with one-way ANOVA. Immunoblotting indicated no significant change in β-III tubulin expression between various experimental groups (Figure 6A). However, GFAP expression was significantly higher in stem cell treated group and the one that treated with

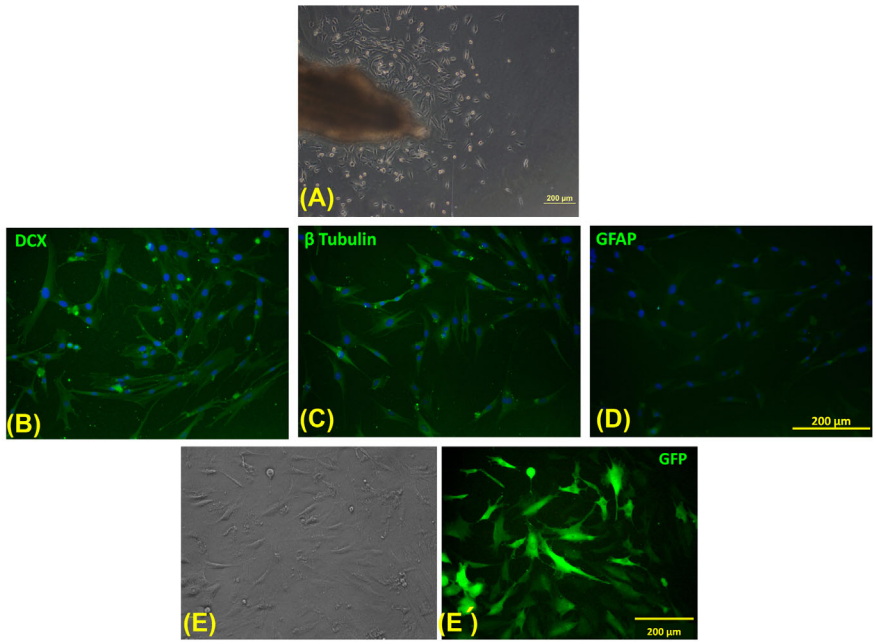

FIGURE 2 Characterization of migrated EPI-NCSCs in primary culture. Within 2 or 3 days, cells migrated from explanted hair bulge with increasing number over time (A). Immunofluorescent staining of migrated cells at day 10 in primary culture demonstrated expression of DCX (B), β- III tubulin (C), and GFAP (D). The transduction of EPI-NCSCs with lentiviral vector encoding GFP and following treatment with puromycin resulted in pure population of GFP expressing EPI-NCSCs (E, E')

combination of cell and VPA. VPA administration alone did not significantly increase the GFAP expression, whereas the GFAP was significantly higher in injured slices that simultaneously treated with VPA and stem cells compared to stem cells alone (Figure 6B).

3.5 | Survived EPI-NCSCs express GFAP following transplantation

The main objective after confirming the survival of GFP expressing EPI-NCSCs on the injured slices was to examine

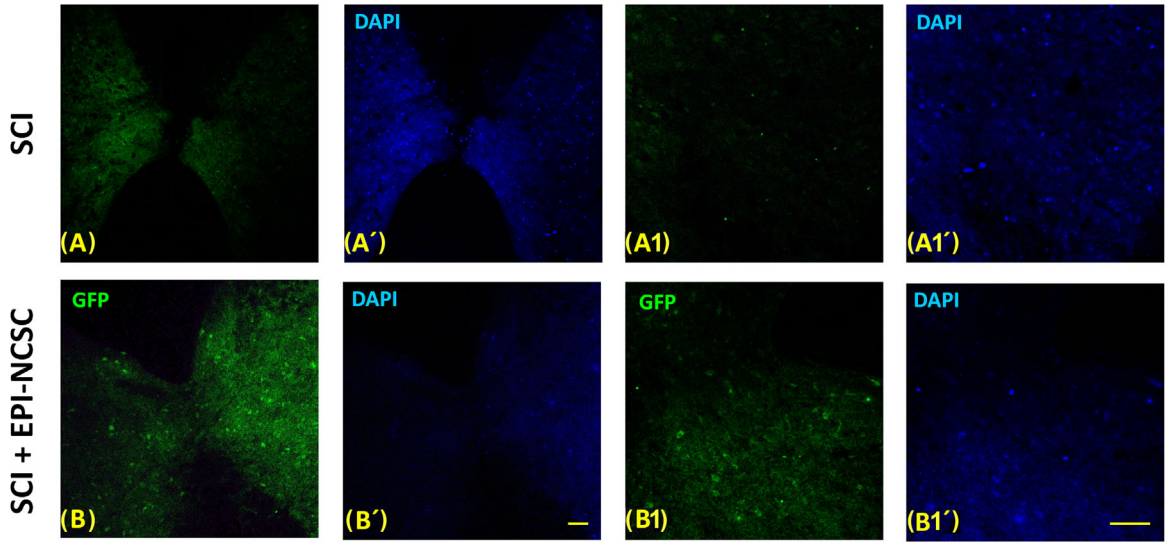

FIGURE 3 Survival of GFP- expressing stem cells on top surface of injured spinal cord slices. Confocal images of injured slices seven days after cell transplantation revealed the presence of GFP expressing cells on slices grafted with stem cells (B, B1). However no GFP- expressing cells were detected on the surface of injured slices which have not been grafted (A, A1). Cell nuclei were counterstained with DAPI. Scale bar: 100 µm

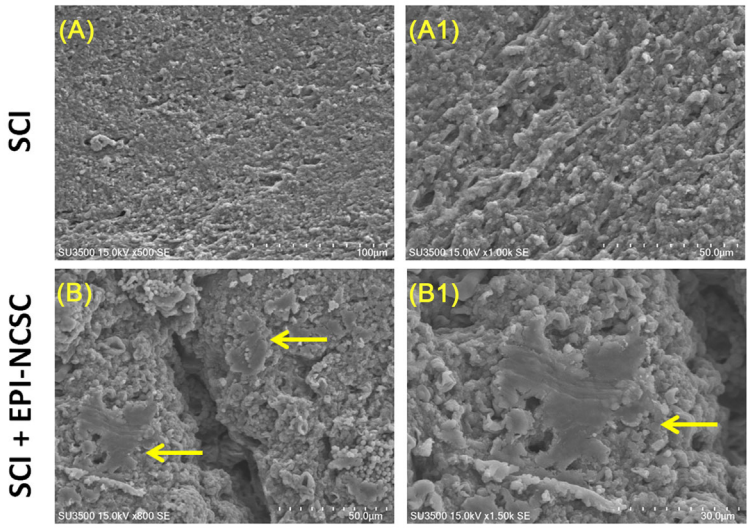

FIGURE 4 Confirming the presence of grafted stem cells by SEM. Assessing top surface of injured spinal cord slices with SEM illustrated the presence of grafted cells (indicated by arrows) (B, B1) whereas they were not detected on non-treated injured slices (A, A1)

their phenotype. To do so, immunohistochemistry was carried out for DCX and GFAP which subsequently obtained data were analyzed with one-way ANOVA. Based on confocal images at day 7 after injury, GFP expressing EPI-NCSCs did not express any detectable level of DCX (Figure 7A); whereas high level of DCX expression was detected in EPI-NCSCs ahead of transplantation (Figure 2B). Moreover, we looked for expression of GFAP by grafted stem cells, which found the co-expression of GFP and GFAP (Figure 7B).

4 | DISCUSSION

Over the past decades, numerous strategies have been devised to cure spinal cord injury and examined in animal models and humans that some are now in, or moving towards clinical trials.[40] In this study, we provide further evidence showing that epidermal neural crest stem cells are potential candidate for SCI treatment as they enhance neurotrophic and neuroprotective factors in the context of injury. In spite of widely reported neuronal fate of mouse EPI-NCSCs in the SCI,[10–13] this study provided evidence that transplanted cells may impose their protection by differentiation to glial cells as they expressed GFAP and no longer expressed neuronal marker, DCX.

In the current investigation, the beneficial role of EPI-NCSCs was demonstrated in a SCI model induced in organotypic spinal cord slice culture. So far, spinal cord slices have been utilized to mimic the inhospitable post-injury microenvironment for the evaluation of therapeutic of stem cells such as mesenchymal stem cells and neural stem/

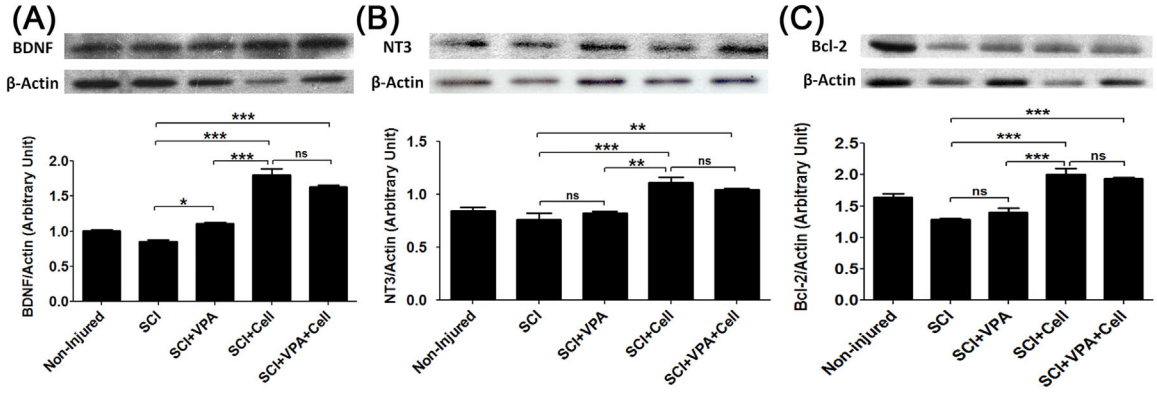

FIGURE 5 Expression of BDNF, NT3 and Bcl-2 in various experimental groups. The expression level of BDNF (A), NT3 (B), and Bcl-2 (C) was evaluated by immunoblotting in slices treated with VPA, cell or their combination seven days post treatment. The band intensity was normalized to β- actin. The data are expressed as mean ± SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (one-way ANOVA and Tukey post hoc test)

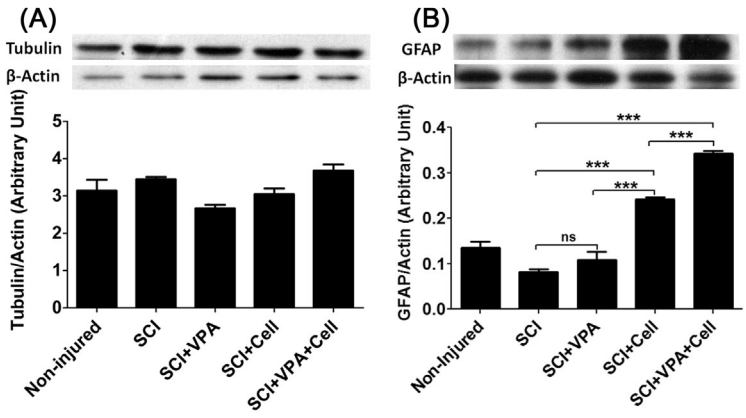

FIGURE 6 Expression of β- III tubulin and GFAP in various experimental groups. The expression level of β- III tubulin (A) and GFAP (B) was measured by western blot analysis. The band intensity was normalized to β- actin. The data are expressed as mean ± SEM. ***$P < 0.001$ (one-way ANOVA and Tukey post hoc test)

progenitor cells.[41,42] As these investigations used chemical agents to induce second phase of injury in slices excised from pre and postnatal animals, none of them fully imitate the clinical condition. Therefore, contusion model of injury was induced in adult rat spinal cord slices based on introduced model of Krassioukov et al.[30]

To our knowledge, characteristics of EPI-NCSs only were identified in numerous in vivo SCI models[10,12,43] and this is the first report that explored positive properties of epidermal neural crest stem cells in ex vivo model of injury. Based on scanning electron microscopy and confocal images of our previous study,[44] transplanted EPI-NCSCs can survive on top surface of injured slices which revealed organotypic culture is a proper platform to evaluate EPI-NCSCs alone or in combination with therapeutic enhancers. In order to achieve optimum level of regeneration, the cell treatment strategy was combined with VPA administration, a well-known neuroprotective agent. We defined earlier that adding 5 μM VPA, 1 h after injury in ex vivo spinal cord slice culture can diminish death of majority of cells and preserve neuronal integrity in the first three days post injury.[31] In this regard, 5 μM VPA was applied to attenuate inhospitable context of injury and also provide a better environment for subsequent cell therapy. Although the neuroprotective effect of VPA has been reported in numerous SCI models, in our study the employed single dose only increased BDNF and not seem to be pertinent to enhance NT3 and Bcl-2. It deserves to mention that lack of VPA effectiveness on NT3 and Bcl-2 in this study is likely due to long or short interval between VPA administration and evaluation of aforementioned parameters on day seven post injury or activation of other protective pathways. In contrast to VPA results, the BDNF, NT3, and Bcl-2 levels were significantly higher in stem cell grafted group. It was demonstrated previously that neurotrophic factors such as BDNF and NT3 play critical roles in axonal growth, survival of existing neurons, and neurogenesis.[45] The increase of BDNF in VPA treated group can be due to HDAC inhibitory function of VPA that can stimulate upregulation of various neurotrophins like BDNF in resident spinal cord neurons and glia.[46,47]

On the other hand, the elevated level of BDNF in cell treated group can be the result of its release by induced local spinal cord cells following transplantation or due to grafted cells secretion, as BDNF has been documented in the library of EPI-NCSCs' expressed growth factors.[10] In turn, the enhanced level of Bcl-2 in cell treated group probably is consequence of BDNF over expression which imposes its impact through Ras-MAP-kinase signaling pathway[48] whereas this impact was not detected in VPA administered group. Although treatment with VPA increased BDNF, the level of Bcl-2 was not changed that may be owing to insufficient availability of BDNF ligand to trigger downstream cascades. It is worth noting, higher level of BDNF and NT3 can be due to enhanced expression of these neurotrophins in the endogenous spinal cord cells following employed treatments or their release from transplanted cells. In both scenario, neurotrophins activate their own specific tyrosine kinase receptors that subsequently initiate their common downstream signaling cascade which promote CREB-regulated genes transcription that ultimately can resulted in neuroprotection and/ or neurogenesis.[47]

Moreover, findings of immunoblotting revealed neither VPA nor stem cells treatment changed the β- III tubulin expression between experimental groups. However, VPA is known for its ability to promote neurogenesis via ERK pathway in a dose and time-dependent manner.[49] No changes detection in β- III tubulin expression can be due to limited

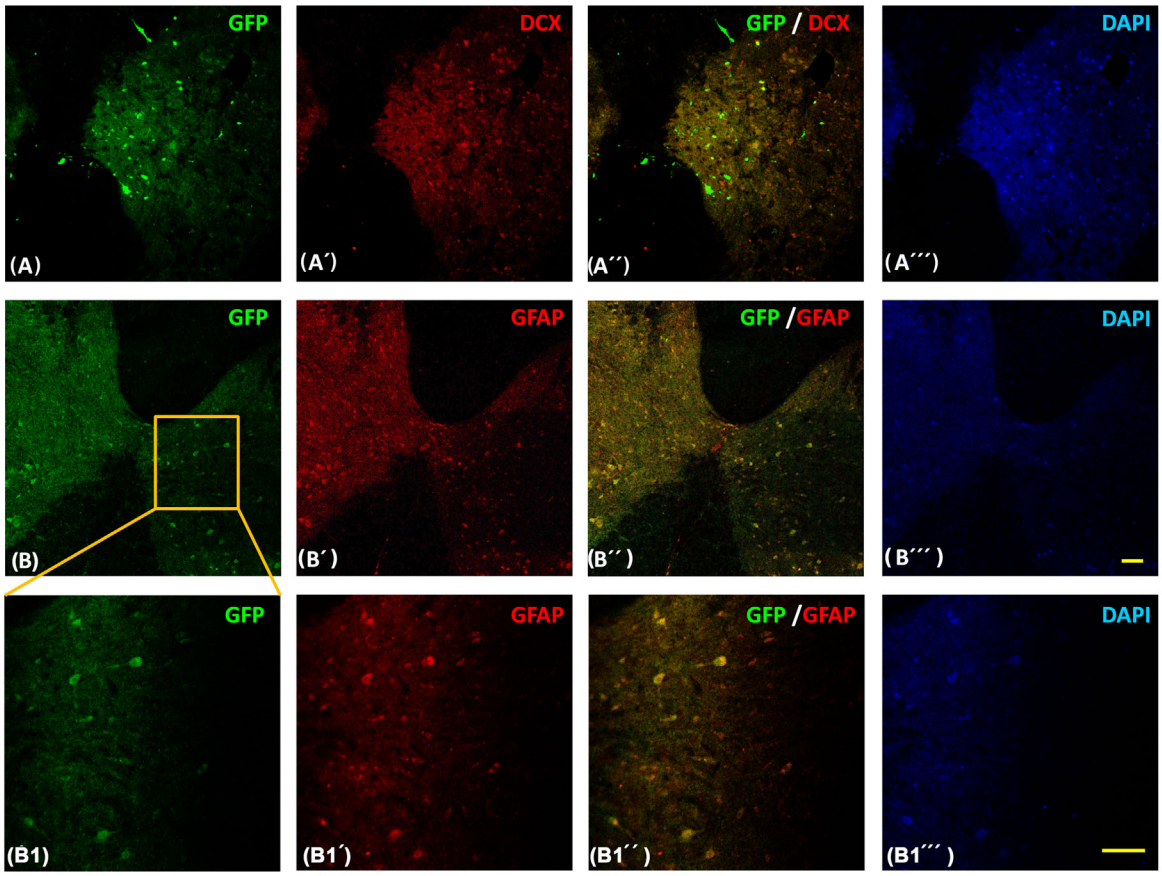

FIGURE 7 Expression of GFAP by transplanted stem cells in the injured spinal cord slices. The representative confocal images of slices after immunohistochemistry revealed very limited co-localization of GFP and DCX expression (A) while co-expression of GFP and GFAP was detected (B) in grafted stem cells 7 days post transplantation. Evaluation of specific region of slice (defined box) showed the expression of GFP by transplanted stem cells and its co-expression with GFAP at higher magnification (B1). Cell nuclei were counterstained with DAPI. Scale bar: 100 μm

sensitivity of evaluation method and genuinely high expression level of this protein.

In addition, the expression of intermediate filament protein GFAP elevated following cell and combinatory therapy. Here a synergistic effect was observed after simultaneous administration of VPA with stem cells. The increased level of GFAP following cell therapy can be result of grafted cells differentiation to glial lineage and/or activation of endogenous astroglia in response to released trophic factors from transplanted cells.

Subsequently, the immunohistochemistry of injured slices on day seven post transplantation defined the fate of grafted stem cells were directed into more glial lineage in the injured slices, as the co-expression of GFP and GFAP was observed in EPI-NCSCs, whereas they did not express any detectable level of DCX. This result is strikingly in contrast with findings of Sieber-Blum and her colleagues that reported no detectable level of GFAP by EPI-NCSCs in the site of lesion. They indicated that engrafted stem cells can differentiate into β- III tubulin-immunoreactive putative neuronal cells and/or cells that express the GABAergic marker GAD67,[12] while Amoh and his colleagues reported many of the GFP-expressing hair follicle stem cells differentiate into GFAP positive cells in severely injured mice spinal cord.[50–52]

Recently growing lines of evidence suggest, contrary to prevailing dogma that astrocytes significantly impede axonal regeneration, astrocytes perform detrimental role in promoting neuronal survival and axonal regeneration after spinal cord injury.[53–56] During last two decades increasing evidence from diverse animal models has indicated not only endogenous astrocytes, but also grafted-derived astrocytes like derived from glial-restricted precursors promote rather than inhibit axon regeneration in injury context.[57–60] Therefore, the improving effects observed in the introduced SCI platform can be due to differentiation of transplanted cells to glial lineage and/or consequence of their effects on endogenous cells.

5 | CONCLUSIONS

Collectively, it is possible that endogenous residents in synergy with transplanted cells create permissive environment that could be beneficial for injury in multiple ways including neuroprotection. Overall, this study suggests that EPI-NCSCs and VPA due to presenting various absorbing function in SCI can be considered as a promising candidates for SCI treatment but both approaches require some fine-tuning to work synergistically for future cure of SCI.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SP carried out all experiments, SP, and MSS have substantial contribution in data analysis and interpretation and manuscript writing. MSZ designed the impactor. AA and MN participated in design of study. LD designed the experiment and studied the final approval of manuscript. All authors read and approved.

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