Article

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Secretion of GPNMB from Neural Stem Cells Induced by ET-1 Contributes to Angiogenesis after Spinal Cord Injury

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Background: The feeble plasticity of spinal cord microvascular endothelial cells (SCMECs) after trauma is one of the major causes of spinal cord injury (SCI). Neural stem cells (NSCs) play an important role in nerve repair. Glycoprotein nonmetastatic B (GPNMB) has neuroprotective effects and can be stimulated by endothelin 1 (ET-1), and its expression is upregulated in SCI. Here, we aim to investigate whether elevated ET-1 levels stimulate NSCs to secrete GPNMB, thereby further promoting angiogenesis.
Methods: Mouse SCMECs and NSCs were isolated, cultured, and identified by flow cytometry and immunofluorescence staining. NSCs were treated with ET-1, while SCMECs were cocultured with NSCs, followed by treatment with ET-1. NCS and SCMEC viability were evaluated using cell counting kit 8 (CCK-8) assay, while cell proliferation, migration, invasion, and angiogenesis were examined using 5′-Ethynyl-2′-Deoxyuridine (EdU) staining, wound healing assay, Transwell assay, and tube formation assay. GPNMB expression in NCSs and SCMECs was quantified by western blot assay, quantitative Real-Time polymerase chain reaction (qRT-PCR), or enzyme-linked immunosorbent assay (ELISA).
Results: Mouse SCMECs and NSCs were successfully isolated and cultured. ET-1 promoted NSC viability and proliferation and upregulated GPNMB expression. NSCs and ET-1-treated NSCs promoted the viability, migration, invasion, angiogenesis, and GPNMB expression in SCMECs compared with control group cells, while GPNMB antibody reversed the above effects of ET-1 on the SCMECs.
Conclusion: ET-1 promotes SCMEC migration and invasion, along with angiogenesis, by enhancing NSC-mediated GPNMB secretion, so ET-1 may be a novel therapeutic target for SCI.

Keywords: spinal cord injury; spinal cord microvascular endothelial cells; neural stem cells; glycoprotein NMB; endothelin 1

Introduction

Spinal cord injury (SCI) is defined as damage to nerves, including the spinal cord and nerve roots, in the spinal canal caused by various pathogenic factors such as trauma, inflammation, and tumors, resulting in spinal cord nerve dysfunction [1,2]. The lack of effective treatment for SCI leads to heavy burdens on families and societies. Therefore, it is important to identify novel approaches for SCI prevention and treatment.

Spinal cord microvascular endothelial cells (SCMECs) injury occurs immediately after SCI [3,4]. Interruption of angiogenesis, reduction of blood supply, and disruption of nutrition supply lead to poor spinal cord plasticity, which is the initiating factor of secondary injury in SCI [5,6]. Therefore, the promotion of microvascular regeneration in SCI may provide the elements needed for nerve regeneration to facilitate the recovery of nerve function.

Neural stem cells (NSCs), which are responsible for nerve tissue repair, are the most promising cells because of their ability to proliferate, renew, differentiate, and undergo passage [3]. In 2010, Salazar *et al*. [7] discovered that NSC transplantation promotes locomotor recovery in an SCI model mouse, and in 2018, Rosenzweig *et al*. [8] further demonstrated that NSC transplantation enhances spinal cord plasticity in primates with SCI. In a recent study, NSC-derived small extracellular vesicles were reported to inhibit apoptosis and neuroinflammation by activating autophagy in SCI [9]. However, the mechanism by which NSC can alleviate SCI-mediated damage remains unclear.

Glycoprotein nonmetastatic B (GPNMB), an endogenous glycoprotein, enhances bone formation by promoting angiogenesis [10]. Aside from the effect on bone formation, it also has an anti-inflammatory role, suggesting that it can alleviate damage and repair tissues [11]. The neuroprotective effects of GPNMB were first reported by Ono *et al*. [12] in 2016, and it can mediate the neuroinflammatory response in patients with Alzheimer's disease [13]. While we have examined GPNMB expression levels in spinal cord tissues in a model of NSC transplantation in SCI mice, the role of GPNMB in SCI remains to be defined.

Endothelin 1 (ET-1) triggers melanogenesis via the microphthalmia-associated transcription factor (MITF)-regulated GPNMB pathway to upregulate GPNMB expression levels [14]. ET-1, a 21-amino acid peptide initially isolated from porcine aortic endothelial cells as a vasoconstricting agent, promotes NSC proliferation and is upregulated in SCI [15,16]. Therefore, we aim to investigate whether elevated ET-1 levels stimulate NSCs to secrete GPNMB, thereby further promoting angiogenesis.

Corresponding, in this research, after primary mouse SCMECs and NSCs were cultured, the roles of ET-1 in GPNMB secretion of NSCs and the role of ET-1-treated NSCs in SCMECs were investigated by performing cell function assays to detect the cell viability, proliferation, migration, invasion and angiogenesis. Our research is committed to exploring novel biomarkers and therapeutic targets for SCI treatment.

Materials and Methods

Animals

Six C57 mice (6–8 weeks old, 18–22 g body weight) were purchased from Cavens (Changzhou, China) and used in this research.

Isolation, Culture, and Identification of SCMECs

Mouse SCMECs were isolated from the spinal cord tissues of five male C57 mice after intraperitoneal administration of 1% pentobarbital sodium (P010, 150 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) and euthanized via cervical dislocation [17]. After the SCMECs were isolated, they were purified using CD31 microbeads (130-097-418, Miltenyi Biotec, Cologne, Germany). In brief, SCMECs were cultured for 7 days, followed by digestion with accutase (25200072, Gibco, Grand Island, NY, USA), incubation with CD31 microbeads, and passage through a mass spectrometry/laser spectroscopy sorting column to collect the cells. Next, SCMECs were cultured in Endothelial Cell Growth Medium-2 medium (CC-3156, Lonca, Basel, Switzerland) for 3 days and then stained with CD144 antibody (ab91064, Abcam, London, UK), CD146-fluorescein isothiocyanate (FITC) antibody (ab75769, Abcam), or CD31 antibody (ab7388, Abcam). This step was followed by incubation with rabbit Immunoglobulin G (IgG)-cFITC (ab6717, Abcam) or rat IgG-FITC (ab6840, Abcam). SCMECs were analyzed by flow cytometry (Attune NxT model; Thermo Fisher Scientific, Waltham, MA, USA).

Isolation, Culture, and Identification of NSCs

NSCs were isolated from the cortex tissue of a fetus from a 2-week pregnant C57 mouse [3]. NSCs were cultured in an NSC complete medium (CM-M139, Procell, Wuhan, China) for 7 days, followed by immunofluorescence staining using a nestin antibody (CL594-19483, Proteintech, Wuhan, China). NSCs routinely underwent mycoplasma contamination tests and were mycoplasma-free.

Different media were used to assess the differentiation potentials of NSCs. To assess differentiation into astrocytes and oligodendrocytes, NSCs were cultured in Dulbecco's Modified Eagle Medium-F12/GlutaMAX (10565018, Gibco) supplemented with B27 (A1895601, Gibco) and insulin-like growth factor 1 (PA5-27207, Invitrogen, Carlsbad, CA, USA), respectively. Next, NSCs were incubated with Alexa Fluor 488 anti-glial fibrillary acidic protein (GFAP) antibody (ab194324, Abcam) or Alexa Fluor 488 anti-oligodendrocyte transcription factor 2 (Olig2) antibody (ab225099, Abcam), followed by staining by 4′,6-diamidino-2-phenylindole (DAPI) (C0065, Solarbio, Beijing, China). GFAP- and Olig2-positive cells were observed under a fluorescence microscope (DM2500 model; Leica, Weztlar, Germany) at $\times$200 magnification.

To assess differentiation into neurons, NSCs were cultured in Neurobasal Medium (21103049, Gibco). Next, NSCs were incubated with β-III tubulin antibody (ab78078, Abcam), followed by Alexa Fluor 488 goat anti-mouse IgG (ab150113, Abcam) and DAPI. β-III tubulin-positive cells were observed under a fluorescence microscope (DM2500 model; Leica, Weztlar, Germany) at $\times$200 magnification.

ET-1 Treatment of NSCs

NSCs were treated with ET-1 as previously described [18]. In brief, 10, 100, 200, and 300 nM ET-1 (117399-94-7, MERYER, Shanghai, China) was added to NSC Complete Medium, and NSCs were cultured for 1, 6, 12, and 24 h. NSCs were then harvested for later use.

5′-Ethynyl-2′-Deoxyuridine (EdU) Staining

NSC proliferation after ET-1 treatment was evaluated using the EdU-488 Cell Proliferation Detection Kit (C0071S, Beyotime, Shanghai, China). In brief, ET-1 treated NSCs were incubated with 1$\times$ EdU working buffer for 2 h at 37 °C, followed by fixation with 4% paraformaldehyde (P0099, Beyotime) for 15 min. After washing three times, NSCs were incubated with immunostaining wash solution (P01106, Beyotime) for 15 min and washed three times. Next, NSCs were incubated with reaction solution for 30 min in darkness and washed three times. NSCs were stained with DAPI for 20 min, and NSCs were observed under a fluorescence microscope (DM2500 model; Leica, Weztlar, Germany) at $\times$200 magnification.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Assay

The *GPNMB* mRNA level in NSCs after ET-1 treatment was quantified by qRT-PCR. In brief, total RNA was isolated using an RNA extraction kit (KGR203, KeyGEN, Nanjing, China), and the concentration of total RNA was measured using a NanoDrop UV spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Next, cDNA was synthesized using a cDNA first-strand synthesis kit (D7168S, Beyotime). Finally, cDNA was mixed with the real-time PCR Master Mix (KGA1339-1, KeyGEN) and *GPNMB* primers for qRT-PCR in a QuantStudio 6 System (Thermo Fisher Scientific). Primers were used as follows: *GPNMB* forward primer (5′-TGCCAAGCGATTTCGTGATGT-3′), *GPNMB* reverse primer (5′-GCCACGTAATTGGTTGTGCTC-3′), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer (5′-AGGTCGGTGTGAACGGATTTG-3′), *GAPDH* reverse primer (5′-GGGGTCGTTGATGGCAACA-3′).

Enzyme-Linked Immunosorbent Assay (ELISA)

The concentration of GPNMB in culture media from ET-1-treated NSCs was measured using the mouse GPNMB DuoSet ELISA Kit (DY2330, R&D Systems, Minneapolis, MN, USA). In brief, after treatment with ET-1 for 1, 6, 12, and 24 h, the culture medium was centrifuged at 12,000 ×*g*, the supernatant was collected, and 100 µL of the supernatant was added into each well of a 96-well plate. Next, 100 µL of the primary antibody was added, and the plate was incubated for 2 h. After washing with wash buffer, 100 µL of the secondary antibody was added, and the plate was incubated for 20 min, followed by washing with wash buffer. Next, 100 µL of the substrate solution was added, the plate was incubated for 20 min, and 50 µL of the stop solution was added. The optical density (OD) of each well was read using a microplate reader (Varioskan LUX, Thermo Fisher Scientific, Waltham, MA, USA) set to 450 nm, and the concentration of GPNMB in the culture media was calculated, according to the manufacturer's instructions.

Treatment of SCMECs

SCMECs were divided into four groups: the control group, the NSC group, the ET-1 group, and the ET-1+GPNMB antibody (Ab) group. SCMECs in the control group were cultured normally for 48 h. SCMECs in the NSC group were cocultured with NSCs for 48 h. SCMECs in the ET-1 group were cocultured with NSCs for 48 h, where NSCs were treated with 100 nM ET-1 for 6 h before co-culture. SCMECs in the ET-1+GPNMB Ab group were cocultured with NSCs for 48 h, where NSCs were treated with 100 nm ET-1 for 6 h before co-culture. Next, the GPNMB antibody (ab188222, Abcam) was added to the culture medium. For coculture of SCMECs with NSCs, SCMECs were cultured in 12-well plates, and NSCs were cultured in Transwell inserts (141078; pore size, 0.4 µm; Thermo Fisher Scientific, Waltham, MA, USA), which were housed in 12-well plates. SCMECs were then harvested for later use.

Western Blot Assay

In brief, total protein from NSCs and SCMECs was extracted using a protein extraction kit (BB-31013, Bestbio, Beijing, China), and the concentration of total protein was determined using a protein quantitation kit (BB-3401, Bestbio) and a microplate reader (Varioskan LUX, Thermo Fisher Scientific, Waltham, MA, USA). Proteins were denatured by combining with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer (P0015A, Beyotime) and heating at 100 °C for 5 min, followed by cooling to 4 °C. Next, proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (P0012A, Beyotime) and transferred to polyvinyl difluoride membranes (FFP28, Beyotime), which were blocked with blocking buffer (P0252, Beyotime) for 2 h. Membranes were incubated with GPNMB antibody (ab188222, 1:5000, Abcam) or GAPDH antibody (ab8245, 1:8000, Abcam) for 16 h at 4 °C, followed by goat anti-rabbit IgG (ab97051, 1:10,000, Abcam) or goat anti-mouse IgG (ab205719, 1:10,000, Abcam) for 2 h. Immunoreactive proteins were visualized with an enhanced chemiluminescence (ECL) detection reagent (BB-3501, Bestbio) and captured using a ChemiDoc MP Chemiluminescence Detector (Bio-Rad, Hercules, CA, USA).

Cell Counting Kit 8 (CCK-8) Assay

NSC and SCMEC viability after treatment with ET-1 was evaluated using the CCK-8 assay. In brief, treated NSCs and SCMECs (1×10^3 cell/well) were seeded into a 96-well plate and cultured for 24, 48, 72, and 96 h. Next, 10 µL of CCK-8 regent (BB-4202, Bestbio) was added to each well, and the plate was incubated for 3 h. Finally, the OD value of each well was determined using a microplate reader (Varioskan LUX, Thermo Fisher Scientific, Waltham, MA, USA) set at 450 nm.

Wound Healing Assays

The cell migration ability of SCMECs after treatment with ET-1 was evaluated using a wound-healing assay. In brief, treated SCMECs were seeded in a 6-well plate and cultured until the cell confluence reached approximately 100%. A vertical line was created in each well using a sterile pipette tip. Next, the medium was replaced with serum-free medium, and SCMECs were cultured for an additional 48 h. The vertical wound image at 0 and 48 h was observed and captured under a DMi8 S optical microscope (Leica, Weztlar, Germany) at ×100 magnification.

Transwell Assay

The cell invasion ability of SCMECs after treatment with ET-1 was evaluated using the Transwell assay. Transwell units (3464; 8-µm pore size) and Matrigel (354230) were purchased from Corning (Corning, NY, USA). Next, treated-SCMECs (1.0×10^5/well) in 200 µL of serum-

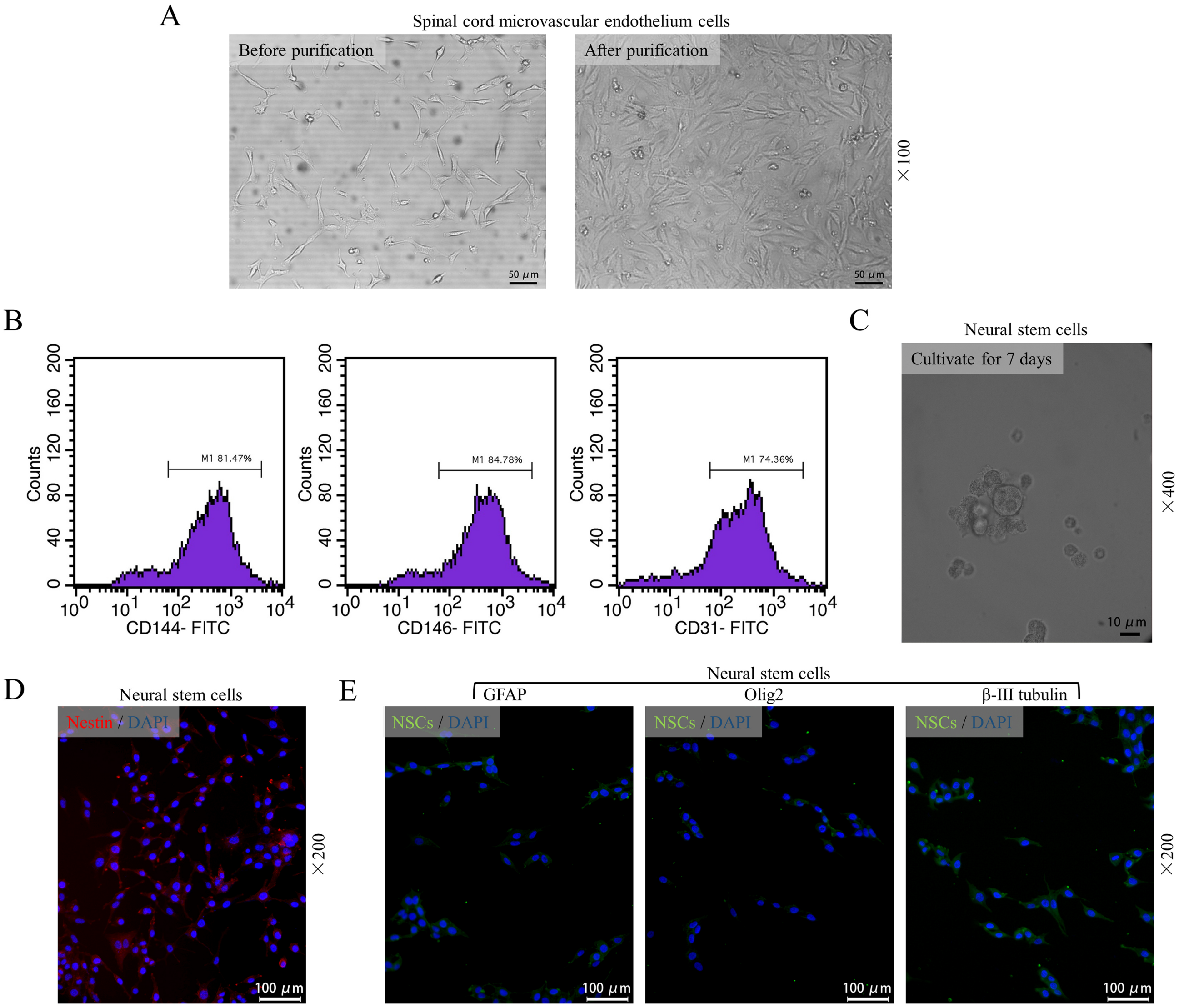

Fig. 1. Mouse SCMECs and NSCs were successfully isolated and cultured. (A) SCMEC morphology before purification and after purification (×100 magnification). (B) Purified SCMECs were analyzed using flow cytometry. (C) NSC neurospheres were observed after culture for 7 days (×400 magnification). (D) NSCs were identified using nestin immunofluorescence staining (×200 magnification). (E) Under different culture conditions, the differentiation potentials of NSCs were identified using immunofluorescence staining (×200 magnification), and the green channel fluorescence represented glial fibrillary acidic protein (GFAP), oligodendrocyte transcription factor 2 (Olig2) and β-III tubulin; DAPI, 4′,6-diamidino-2-phenylindole; SCMECs, spinal cord microvascular endothelial cells; NSCs, neural stem cells. n = 3.

free medium were added into the upper Transwell chamber, which was precoated with Matrigel, and 650 µL of complete medium was added into the lower Transwell chamber. SCMECs were cultured for 48 h, and those that invaded the lower Transwell chamber were fixed with 4% paraformaldehyde for 20 min, followed by staining with crystal violet staining buffer (BB-44577, Bestbio) for 20 min. After staining, SCMECs were washed with sterile water three times, and cells were viewed and imaged under an optical microscope at ×200 magnification.

Tube Formation Assays

The angiogenic ability of SCMECs after treatment with ET-1 was evaluated using the tube formation assay. In brief, each well of a 48-well plate was pre-coated with EC-Matix gel (ECM625, Millipore, Burlington, MA, USA) at 37 °C for 1 h, and 2.0 × 10^5 treated-SCMECs were added into each well. After the SCMECs were cultured for 4 h, the tube-like structures formed by SCMECs were observed under an optical microscope at ×100 magnification.

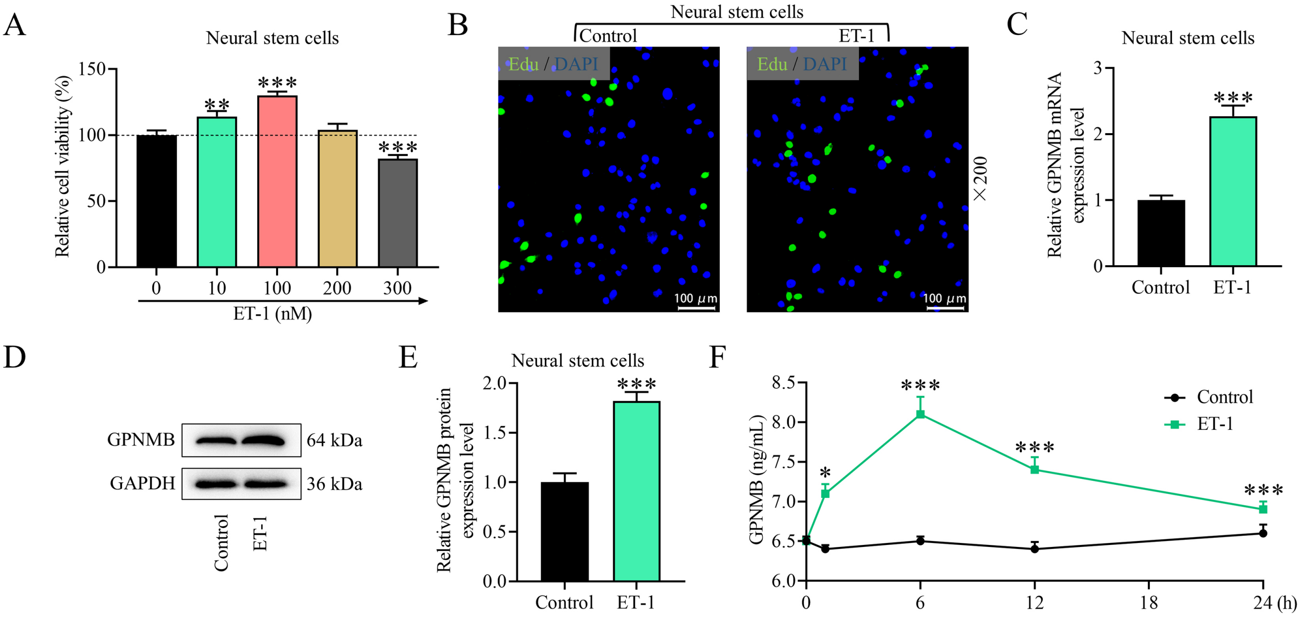

Fig. 2. ET-1 promoted NSC viability, proliferation, and GPNMB expression. (A–E) After the NSCs were treated with different concentrations of ET-1 for 24 h, NSC viability was evaluated using the cell counting kit 8 (CCK-8) assay (A), NSC proliferation was evaluated using EdU staining (×200 magnification; B), and GPNMB expression in NSCs was quantified using quantitative Real-Time polymerase chain reaction (qRT-PCR) (C) and western blot (D,E). (F) After the NSCs were treated with 100 nm of ET-1 for 1, 6, 12, and 24 h, the concentration of GPNMB in the medium was evaluated using enzyme-linked immunosorbent assay (ELISA). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Control. NSCs, neural stem cells; GPNMB, glycoprotein nonmetastatic B; EdU, 5′-Ethynyl-2′-Deoxyuridine; ET-1, endothelin 1. n = 3.

Statistical Analyses

The experiments were repeated three times in the study. Independent *t-test* was used for comparisons between two groups, and one-way analysis of variance was used for comparisons among multiple groups. All statistical analyses were carried out using GraphPad 8.0 software (GraphPad Software, San Diego, CA, USA). Measurement data were described as mean ± SD, and $p < 0.05$ was considered a statistically significant difference.

Results

Mouse SCMECs and NSCs were Successfully Isolated and Cultured

Mouse primary SCMECs and NSCs were isolated and cultured. As shown in Fig. 1A, the SCMEC morphology before and after purification was exhibited. The purified SCMECs were analyzed using flow cytometry, as shown in Fig. 1B, and CD144-positive cells accounted for 81.47%, CD146-positive cells accounted for 84.78%, and CD31-positive cells accounted for 74.36%, indicating SCMECs were successfully isolated and cultured. Meanwhile, NSC neurospheres were formed after culture for 7 days (Fig. 1C). NSCs were identified using nestin immunofluorescence staining, as shown in Fig. 1D. Nestin-positive NSCs could differentiate into astrocytes, oligodendrocytes, and neurons under different culture conditions. Under these conditions, nestin-positive NSCs differentiated into GFAP-positive astrocytes, Olig2-positive oligodendrocytes, and β-III tubulin-positive neurons (Fig. 1E), indicating SCMECs and NSCs were successfully isolated from mice and cultured *in vitro*.

ET-1 Promoted NSC Viability, Proliferation, and GPNMB Expression

To examine the role of ET-1 in NSCs, different concentrations (10, 100, 200, and 300 nM) of ET-1 were used, as shown in Fig. 2A, and we observed that NSC viability increased by 10 nm and 100 nm ET-1 ($p < 0.01$) and decreased by 300 nm ET-1 ($p < 0.001$), and 100 nM ET-1 was selected for all subsequent experiments. NSC proliferation was evaluated after treatment with 100 nM ET-1 for 24 h using EdU staining and we observed that the number of EdU-positive cells increased compared to the control (Fig. 2B). In addition, upregulated GPNMB mRNA ($p < 0.001$; Fig. 2C) and protein ($p < 0.001$; Fig. 2D,E) levels were noted in NSCs after 100 nM ET-1 treatment for 24 h. Next, the effect of different treatment times of ET-1 on the expression of GPNMB in NSCs was investigated, as shown in Fig. 2F. The concentration of GPNMB in the NSC culture media after 100 nM ET-1 treatment for 6 h was the highest ($p < 0.001$); therefore, the NSCs were treated with 100 nM ET-1 for 6 h for all subsequent experiments.

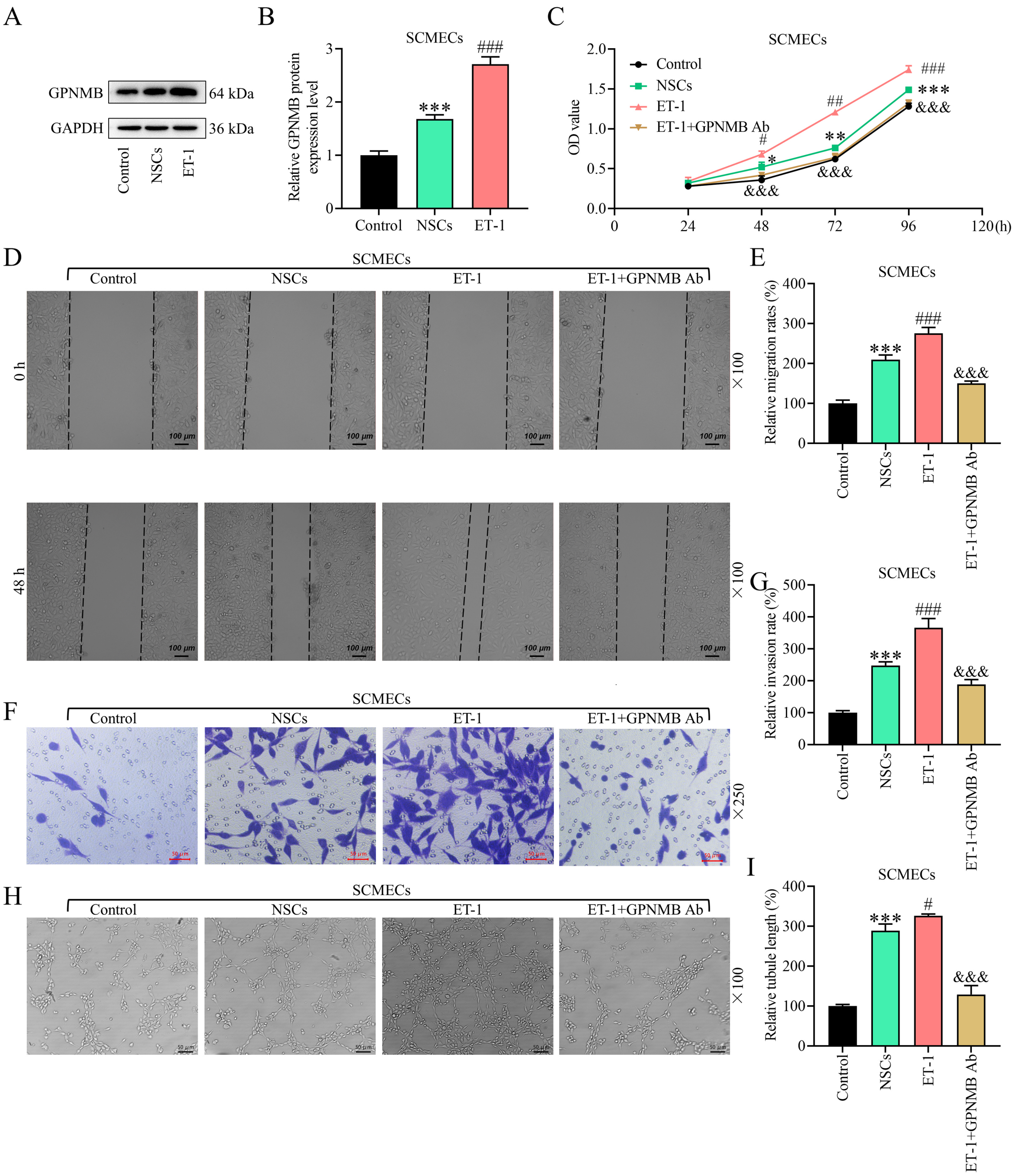

Fig. 3. High concentration of ET-1 promoted SCMEC viability, migration, invasion, and angiogenesis by upregulating GPNMB. After the SCMECs were cocultured with NSCs, which were treated/not treated with ET-1, the expression of GPNMB in SCMECs was detected using western blot (A,B), and the viability, migration, invasion, and angiogenesis of SCMECs were evaluated using the CCK-8 assay (C), wound healing assay (D,E), Transwell assay (F,G), and tube formation assay (H,I). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. NSCs; $^{\&\&\&}p < 0.001$ vs. ET-1. SCMECs, spinal cord microvascular endothelial cells; NSCs, neural stem cells; GPNMB, glycoprotein nonmetastatic B; ET-1, endothelin 1; Ab, antibody. n = 3.

High Concentration of ET-1 in NSCs Promoted Viability, Migration, Invasion, and Angiogenesis of SCMECs by Upregulating GPNMB

Next, NSCs were cocultured with SCMECs to evaluate the role of ET-1 and we found that NSCs upregulated GPNMB expression in SCMECs ($p < 0.001$; Fig. 3A,B), and ET-1 treated NSCs further upregulated GPNMB expression in SCMECs ($p < 0.001$; Fig. 3A,B). Next, SCMEC viability was evaluated. As shown in Fig. 3C, NSCs increased the cell viability of SCMECs ($p < 0.05$), NSCs-treated with ET-1 further increased the cell viability of SCMECs ($p < 0.05$), while the GPNMB Ab abolished the role of NSCs-treated with ET-1 ($p < 0.001$). The ability of SCMECs to migrate (Fig. 3D,E), invade (Fig. 3F,G), and create new blood vessels (Fig. 3H,I) was promoted by NSCs ($p < 0.001$), and further enhanced by ET-1 treated NSCs ($p < 0.05$), while the GPNMB Ab abolished these changes ($p < 0.001$). These phenomena suggest that high concentrations of ET-1 had a promoting effect on SCMECs by upregulating GPNMB.

Discussion

In this study, we cultured primary mouse SCMECs and NSCs and further treated NSCs with ET-1 to discover that ET-1 promotes viability, proliferation, as well as secretion of GPNMB by NSCs. It was also observed that NSCs, along with ET-1-treated NSCs, promoted the expression of GPNMB in SCMECs, the migration and invasion of SCMECs, and angiogenesis. Our research verified that ET-1-treated NSCs promoted the secretion of GPNMB, which enhanced the angiogenesis of SCMECs, providing novel biomarkers and therapeutic targets for SCI. The following are the specific results of this study.

SCMECs are damaged after SCI, and the feeble plasticity of SCMECs is one of the major causes of the exacerbation of SCI. Therefore, the functional recovery of SCMECs is critical for the treatment of individuals with SCI [19]. To identify novel ways to enhance SCMEC function, mouse SCMECs were isolated and confirmed to express CD146, CD144, and CD31, which are the surface markers of SCMECs [3]. NSCs, the most promising cells for the treatment of certain neurological conditions, were also isolated and confirmed to express nestin, which is the surface marker of NSCs [20]. The role of NSCs in the nervous system is due to their ability to differentiate into astrocytes, oligodendrocytes, and neurons under different culture conditions [21], which we evaluated in this study through the use of different culture media. Astrocytes are star-shaped with many short protrusions, abundant cytoplasm, and large nuclei, and GFAP is a surface marker of astrocytes. Oligodendrocytes are smaller than astroglia, with fewer and shorter protrusions and round, small, and dense nuclei. Olig2 is the surface marker of oligodendrocytes. Neurons are fusiform, small and round, with smooth edges and 2–3 long protrusions, and the β-III tubulin is the surface marker of NSCs [3]. We isolated NSCs to confirm that they could differentiate into astrocytes, oligodendrocytes, and neurons, thereby verifying that NSCs were isolated and cultured.

ET-1 plays a regulatory role in diseases such as diabetes, oral squamous cell carcinoma, osteoporosis, and arrhythmia [22–25]. In addition, ET-1 is upregulated in the plasma of patients with SCI [26]. Downregulation of ET-1 ameliorates spinal cord ischemia and then inhibits apoptosis and oxidative stress, indicating it has a negative effect on ET-1 [27]. ET-1 can also promote NSC proliferation via the Ednrb receptor directly on oligodendrocyte progenitor cells [16]. Similarly, we also discovered that ET-1 enhanced the viability and proliferation of NSCs, indicating ET-1 acts on NSCs; however, further studies are needed to understand the specific regulatory mechanism.

ET-1 stimulates GPNMB expression, an endogenous glycoprotein [28]. Consistently, the upregulated GPNMB expression and enhanced proliferation of NSCs after ET-1 treatment were also discovered in this research. The role of GPNMB in certain diseases, including cancers, chronic obstructive pulmonary disease, obesity-related metabolic disorders, hyperthyroidism, and bone loss has been widely reported [29–33]. GPNMB has a regulatory effect on the nervous system, that is, it can mitigate the inflammatory response of astrocytes via the CD44 receptor [34]. Furthermore, GPNMB attenuates Alzheimer's disease and promotes autophagy by inhibiting mTOR signaling [35], and it also has neuroprotective effects [12]. In addition, GPNMB expression is abnormal in spinal cord tissues of NSCs transplanted into SCI mice. As expected, the migration and invasion of SCMECs, along with angiogenesis, were enhanced by NSCs and ET-1-treated NSCs. Furthermore, the role of NSCs and ET-1-treated NSCs in the context of SCMECs was abolished by the GPNMB Ab. However, the effects of different concentrations of ET-1-treated NSCs on SCMECs have not been compared, which is one of the limitations of our study and will be investigated in the future.

Conclusion

In conclusion, we first found that ET-1 can promote the proliferation of NSCs, stimulate NSCs to secret GPNMB and upregulate GPNMB expression, thereby augmenting the migration, invasion, and angiogenesis of SCMECs. These findings suggest that ET-1 and GPNMB may be novel biomarkers for SCI treatment.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Substantial contributions to conception and design: ZJD. Data acquisition, data analysis and interpretation: BW, JH, JMY. Drafting the article or critically revising it for important intellectual content: All authors. Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: All authors.

Ethics Approval and Consent to Participate

Experiments involved in mice were authorized by the China Council on Animal Care and Use and was approved by the Committee of Experimental Animals of Zhejiang Baiyue Biotech Co., Ltd. (approval number: ZJBYLA-IACUC-20230208).

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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