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Differentiation state determines neural effects on microvascular endothelial cells

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Abstract

Growing evidence indicates that nerves and capillaries interact paracrinely in uninjured skin and cutaneous wounds. Although mature neurons are the predominant neural cell in the skin, neural progenitor cells have also been detected in uninjured adult skin. The aim of this study was to characterize differential paracrine effects of neural progenitor cells and mature sensory neurons on dermal microvascular endothelial cells. Our results suggest that neural progenitor cells and mature sensory neurons have unique secretory profiles and distinct effects on dermal microvascular endothelial cell proliferation, migration, and nitric oxide production. Neural progenitor cells and dorsal root ganglion neurons secrete different proteins related to angiogenesis. Specific to neural progenitor cells were dipeptidyl peptidase-4, IGFBP-2, pentraxin-3, serpin f1, TIMP-1, TIMP-4 and VEGF. In contrast, endostatin, FGF-1, MCP-1 and thrombospondin-2 were specific to dorsal root ganglion neurons. Microvascular endothelial cell proliferation was inhibited by dorsal root ganglion neurons but unaffected by neural progenitor cells. In contrast, microvascular endothelial cell migration in a scratch wound assay was inhibited by neural progenitor cells and unaffected by dorsal root ganglion neurons. In addition, nitric oxide production by microvascular endothelial cells was increased by dorsal root ganglion neurons but unaffected by neural progenitor cells.

Keywords

Wound repair; Neural progenitor cells; Dorsal root ganglion neurons; Microvascular endothelial cells; Paracrine interactions; Angiogenesis

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Introduction

Crosstalk between the nervous and vascular systems is important during development, tissue homeostasis and repair [1]. The anatomical proximity of nerve fibers and capillaries facilitates paracrine signaling that mediates angiogenesis, neurogenesis and nerve regeneration. Nerve fibers release soluble factors that regulate vascular development, angiogenesis and endothelial cell survival [1-5] and endothelial cells secrete soluble factors that mediate neuronal cell migration and axon guidance [1, 6]. Nerve-derived factors have also been implicated in the mobilization of endothelial progenitor cells [7] and endothelial cell-derived factors have been reported to regulate neural stem cell self-renewal, neurogenesis and migration [8-11].

Growing evidence indicates that nerves and capillaries interact paracrinely in uninjured skin and cutaneous wounds. During development, sensory nerve secretion of VEGF regulates blood vessel branching and arterial differentiation in the skin [12-13]. Angiogenic factors also affect neurogenesis in uninjured adult skin. Transgenic mice overexpressing VEGF and/or angiopoietin-1 in the skin have increased numbers of cutaneous nerves [14]. During normal wound repair, angiogenesis typically begins within the first few days after injury and gradually subsides as the wound matures; sensory nerve fibers regenerate in human partial thickness wounds at around 14 days following injury [15]. Impaired healing impacts the number of nerves and capillaries in the wound. In diabetic wounds, there is reduced numbers of nerves and capillaries [16]. In contrast, hypertrophic scars have increased density of both nerves and capillaries [17]. Furthermore, treatment of wounds with nerve-derived factors affects angiogenic response to injury. Administration of nerve growth factor to wounds in a mouse model of type 1 diabetes enhanced angiogenesis [18] and the neuropeptide substance P increased angiogenesis in a sponge implantation model of wound repair [19]. Interestingly, a recent study showed that a dopamine antagonist induced angiogenesis in murine excisional wounds [20], which suggests that neurotransmitters may also be negative regulators of angiogenesis during wound repair.

A limited number of *in vitro* studies have also investigated paracrine interactions between nerves and dermal microvascular endothelial cells, the predominant type of endothelial cell in the skin. Substance P induces dermal microvascular endothelial cell proliferation and migration [21]. It also induces dermal microvascular endothelial cells to up-regulate expression of the adhesion molecules, VCAM and ICAM [22-23]. Similarly, nerve growth factor induces dermal microvascular endothelial cells to proliferate and up-regulate ICAM expression [24]. Collectively these *in vitro* and *in vivo* studies suggest that paracrine interactions between nerves and capillaries are necessary for angiogenesis and neurogenesis during cutaneous wound repair.

In the field of wound repair, almost all neuroendothelial studies to date have focused on the paracrine effect of mature neurons on dermal microvascular endothelial cells [21-24]. Although mature neurons are the predominant neural cell in the skin, neural progenitor cells have also been detected in uninjured adult skin [25-27]. However it remains to be determined whether neural progenitor cells secrete soluble factors that regulate endothelial cell responses to injury. The aim of our study was to characterize differential paracrine effects of neural progenitor cells and mature sensory neurons on dermal microvascular endothelial cells. Our results suggest that neural progenitor cells and mature sensory neurons have unique secretory profiles and distinct effects on dermal microvascular endothelial cell proliferation, migration, and nitric oxide production.

Materials and Methods

Microvascular endothelial cells

Primary human adult dermal microvascular endothelial cells were obtained commercially and expanded using Medium 131 supplemented with 5% Microvascular Growth Supplement, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA). Microvascular endothelial cells were maintained at 37°C in 5% CO₂ and passages 5-9 were used for all experiments.

Neural Progenitor Cells

The ENStem-A™ human neural progenitor cells derived from NIH approved H9 human embryonic stem cells were obtained commercially (Millipore, Billerica, MA). These cells have the capacity to differentiate into neuronal subtypes and express high levels of nestin and SOX2 and low levels of Oct-4. We confirmed the SOX2 expression by immunocytochemistry. The neural progenitor cells were maintained in the recommended ENStem-A™ Neural Expansion Medium (Millipore, Billerica, MA) supplemented with FGF-2 (20 ng/ml) (Millipore, Billerica, MA), 2mM L-Glutamine and Penicillin-Streptomycin (50 units/ml penicillin and 50 µg/ml streptomycin; Invitrogen/Gibco, Carlsbad, CA). The neural progenitor cells were always grown on tissue culture plastic or insert membrane coated with poly-L-ornithine and laminin at 37°C in 5% CO₂. Cell passages 4-9 were used for experiments.

Co-culture of microvascular endothelial cells and neural progenitor cells

Human dermal microvascular endothelial cells were co-cultured with human neural progenitor cells grown in cell culture inserts with 0.4 µm pore size (BD Falcon, Franklin Lakes, NJ). The medium used for the co-culture system was as follows: the microvascular endothelial cells were cultured in Medium 131 with a reduced amount of Microvascular Growth Supplement (2.5% instead of 5% used for maintenance cultures) and the neural progenitor cells in the insert were in ENStem-A™ medium with a reduced amount of FGF-2 (10 ng/ml instead of 20 ng/ml used for maintenance cultures). Control microvascular endothelial cells were cultured in Medium 131 supplemented with 2.5% Microvascular Growth Supplement with an empty insert containing ENStem-A™ supplemented with 10 ng/ml FGF-2. In the co-culture system, the amounts of FGF-2 and Microvascular Growth Supplement were reduced in order to limit their effects on endothelial cell proliferation, migration, angiogenesis and gene expression. However, these growth factors could not be completely omitted from the co-culture medium because FGF-2 inhibits neural progenitor cell differentiation and Microvascular Growth Supplement promotes endothelial cell viability.

Dorsal Root Ganglion Neuron-Conditioned Medium

The rat dorsal root ganglion neurons were obtained commercially (Lonza, Walkersville, MD) and grown on tissue culture plastic coated with poly-D-lysine and laminin. These primary neurons were cultured in Primary Neuron Basal Medium supplemented with 2% Neural Survival Factor 1, 2mM L-Glutamine, 300 µg/ml Gentamicin and 150 ng/ml Amphotericin (Lonza, Walkersville, MD). The mitotic inhibitors, 5-fluoro-2'-deoxyuridine and uridine (Sigma-Aldrich Corp, St Louis, MO), were also included to inhibit growth of Schwann cells. These mitotic inhibitors were removed prior to the collection of the conditioned medium. For all experiments, the dorsal root ganglion neuron-conditioned medium was mixed with Medium 131 supplemented with 2.5% Microvascular Growth Supplement, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA) at a ratio of 2:1. Control conditioned medium consisted of

Primary Neuron Basal Medium supplemented with 2% Neural Survival Factor 1, 2mM L-Glutamine, 50 µg/ml Gentamicin and 37 ng/ml Amphotericin (Lonza, Walkersville, MD) mixed with Medium 131 supplemented with 2.5% Microvascular Growth Supplement, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA) at a ratio of 2:1.

Cell proliferation assay

Microvascular endothelial cells were either co-cultured with neural progenitor cells or treated with dorsal root ganglion neuron-conditioned medium for 48 hours and then trypsinized and counted using a Vicell Cell Viability Analyzer. For the neural progenitor cell experiments, the microvascular endothelial cells were seeded at 4.8×10^4 cells/well in a 6-well plate and neural progenitor cells were seeded at 3.15×10^5 cells/insert. Both cell types were grown separately for one day prior to co-culture. The co-culture was then initiated by switching both cell types to their respective co-culture medium after washing twice with PBS. The neural progenitor insert or empty control insert was then placed in the microvascular endothelial cell well. For the dorsal root ganglion neuron experiments, microvascular endothelial cells were also seeded at 4.8×10^4 cells/well in a 6-well plate and grown for one day prior to treatment with dorsal root ganglion neuron-conditioned medium.

Scratch wound closure assay

The effect of neural progenitor cells and dorsal root ganglion neurons on endothelial cell migration was measured using a scratch wound closure as previously described [28]. Microvascular endothelial cells were seeded at 4.8×10^4 cells/well in a 6-well plate and grown for 4 days to achieve a confluent monolayer. The microvascular endothelial cell monolayer was then scratched using a sterile wooden stick. This action resulted in an uniform 1.8 mm scratch running the entire length of the well between guidelines that had been previously etched on the underside of the well. Each well was then washed with PBS to remove cell debris. The scratched microvascular endothelial cells were then either co-cultured with a neural progenitor cell insert or incubated with dorsal root ganglion neuron conditioned-medium. For the neural progenitor cell insert, the neural progenitor cells were seeded at 2.1×10^5 cells/insert and grown for 3 days prior to co-culture.

In order to record endothelial cell migration, photos were taken at multiple locations immediately after scratching and every 24 hours until the scratch wound was closed. Phase contrast images (4× magnification) were captured with a Nikon Eclipse TE300 inverted microscope equipped with a QImaging QICAM Fast 1394 Monochrome 12-bit cooled digital camera. Images were then acquired digitally using QImaging's QCapture software, saved as TIFF files, and imported into Adobe Photoshop CS2 for analysis. The perimeter of the cell free space was traced and filled with black and the area was measured using the image analysis plug-in Fovea Pro version 4.0 (Reindeer Graphics, Asheville, NC). Each time point was normalized to the post-scratch area. All data were expressed as percent area closed.

Tube formation assay

Microvascular endothelial cells grown on Matrigel™ (BD Biosciences, Bedford, MA) were either co-cultured with neural progenitor cells or treated with dorsal root ganglion neuron-conditioned medium. The microvascular endothelial cells were seeded at 5×10^4 cells/well on 300 µl Matrigel/well. For the neural progenitor cell insert, the neural progenitor cells were seeded at 2.48×10^4 cells/insert and grown for 1 day prior to co-culture. Phase contrast images (4× magnification) were captured at 24 and 48 hours with a Nikon Eclipse TE300 inverted microscope equipped with a QImaging QICAM Fast 1394 Monochrome 12-bit cooled digital camera. Images were then acquired digitally using QImaging's QCapture

software, save as TIFF files, and imported into Adobe Photoshop CS2 for analysis. Images were converted to a black and white binary image and skeletonized in order to determine total branch points and total line length, which are both accepted measures of tube formation. Tube formation assays were also performed using Geltrex™ LDEV-free Growth Factor Reduced Basement Membrane Matrix (Invitrogen, Carlsbad, CA).

Nitric oxide assay

Medium was collected from microvascular endothelial cells either co-cultured with neural progenitor cells or treated with dorsal root ganglion neuron-conditioned medium for 48 hours. Total nitric oxide levels in the medium were measured using the total nitric oxide assay kit (Assay Designs, Inc., Ann Arbor, MI). This colorimetric assay is based on the conversion of nitrate to nitrite and the subsequent interaction of nitrite with the Griess reagent. A Spectramax plus plate reader (Molecular Devices, Sunnyvale, CA, USA) was used to measure absorbance of the purple azo derivative at 540 nm. Data were normalized to endothelial cell number.

Proteome profiling

The neural progenitor cell and dorsal root ganglion neuron proteomes were analyzed using Proteome Profiler™ Arrays (R&D Systems, Inc, Minneapolis, MN). The Human Angiogenesis Array was used to detect the expression of 55 angiogenesis related proteins in the culture supernatant of human neural progenitor cells. The Mouse Angiogenesis Array was used to detect the expression of 53 angiogenesis related proteins in the culture supernatant of rat dorsal root ganglion neurons. These Proteome Profiler Arrays were performed according to the manufacturer's protocol. In brief, the culture supernatant was diluted and mixed with a cocktail of biotinylated detection antibodies and then incubated with a nitrocellulose membrane spotted with capture antibodies in duplicate. Protein-detection antibodies bound to the capture antibody were detected using Streptavidin-HRP and chemiluminescent detection reagents.

Statistical analyses

All data were reproducible in a minimum of three independent experiments. In each experiment, we included six replicates for both the control and the experimental conditions. For each figure, a single representative experiment is shown with all data expressed as a mean±standard deviation (N=6). The Student's *t*-test was used to determine statistically significant differences between experimental and control samples. A *P*-value less than or equal to 0.05 was considered significant.

Results

This study compared the paracrine effects of neural progenitor cells (Figure 1A) and dorsal root ganglion neurons (Figure 1B) on dermal microvascular endothelial cell responses to injury. Primary adult human dermal microvascular endothelial cells were cultured with either human neural progenitor cells grown in inserts or conditioned medium from dorsal root ganglion neurons isolated from rat brain. Two controls were needed because of the different base medium requirements for neural progenitor cells and dorsal root ganglion neurons. The control for the neural progenitor cell experiments consisted of microvascular endothelial cells cultured with empty inserts in neural progenitor cell specific medium. For the dorsal root ganglion neuron experiments, the control microvascular endothelial cells were grown in base medium specific for dorsal root ganglion neurons.

Dorsal root ganglion neurons, not neural progenitor cells, regulate microvascular endothelial cell proliferation

Neural progenitor cells had no significant effect on the proliferation of dermal microvascular endothelial cells after 48 hours in co-culture (Figure 2A). In contrast, dorsal root ganglion neuron-conditioned medium inhibited microvascular endothelial proliferation with a significant decrease in viable endothelial cell number observed at 48 hours (Figure 2B, $P=0.0029$).

Neural progenitor cells, not dorsal root ganglion neurons, regulate microvascular endothelial cell migration

Neural progenitor cells inhibit microvascular endothelial cell migration in a scratch wound assay whereas dorsal root ganglion neurons had no significant effect (Figure 3). Images of the scratch wounds at 24, 48, 72, and 96 hours after scratching show that endothelial cell migration into the scratch wound area was delayed in the presence of neural progenitor cells (Figure 3A). At 96 hours, the scratch wound was still evident for microvascular endothelial cells co-cultured with neural progenitor cells; in contrast the scratch wound was completely closed in the control microvascular endothelial cell cultured with empty insert. Quantification of the percentage of scratch wound closure using image analysis confirmed a significant delay in the rate of microvascular endothelial cell migration when co-cultured with neural progenitor cells at 24, 48, 72 and 96 hours (Figure 3C; $P<0.05$ for all time points). Dorsal root ganglion neuron-conditioned medium had no significant effect on the rate of microvascular endothelial cell migration at 24, 48, 72 and 96 hours (Figures 3B and 3D). Similar to the control, microvascular endothelial cells treated with dorsal root ganglion neuron-conditioned medium had completed wound closure by 96 hours.

Neural progenitor cells and dorsal root ganglion neurons do not affect microvascular endothelial tube formation

Neural progenitor cells and dorsal root ganglion neuron-conditioned medium had no significant effect on the ability of dermal microvascular endothelial cells to form tubes when cultured on Matrigel™ (Figure 4). Compared to controls, there was no significant difference in endothelial tube length for endothelial cells either co-cultured with neural progenitor cells or treated with dorsal root ganglion neuron-conditioned medium (Figures 4A and 4C). In addition, the total branch point number was unaffected by the presence of either neural progenitor cells or dorsal root ganglion neuron-conditioned medium (Figures 4B and 4D). Similar results were observed when the tube formation assay was performed using growth factor reduced-basement membrane extract (data not shown).

Dorsal root ganglion neurons, not neural progenitor cells, regulate microvascular endothelial cell production of nitric oxide

Dorsal root ganglion neurons induced dermal microvascular endothelial cells to increase production of nitric oxide whereas neural progenitor cells showed no effect (Figure 5). Nitric oxide levels were higher in endothelial cells treated with dorsal root ganglion neuron-conditioned medium compared to control microvascular endothelial cells (Figure 5B; $P=0.0031$).

Neural progenitor cells and dorsal root ganglion neurons have different secretory profiles for angiogenic mediators

The secretory profiles of neural progenitor cells and dorsal root ganglion neurons were determined in order to identify potential mediators for the effects on the dermal microvascular endothelial cells. Conditioned medium collected from neural progenitor cells and dorsal root ganglion neurons was analyzed using antibody arrays to detect angiogenesis-

related proteins. There were seven proteins unique to the neural progenitor cells and four proteins unique to the dorsal root ganglion neurons (Figure 6). The following proteins were specific to neural progenitor cells: dipeptidyl peptidase-4, IGFBP-2, pentraxin-3, serpin f1, TIMP-1, TIMP-4 and VEGF. In contrast, endostatin, FGF-1, MCP-1 and thrombospondin-2 were specific to dorsal root ganglion neurons. Both neural cell types secreted IGFBP-3 and serpin E1.

Discussion

In this study, we have determined that neural progenitor cells and mature neurons have distinct paracrine effects on dermal microvascular endothelial cells. Neural progenitor cells regulate endothelial cell migration whereas mature neurons regulate endothelial cell proliferation and nitric oxide production. Neural progenitor cells and dorsal root ganglion neurons also have different effects on dermal microvascular endothelial cell expression of genes involved in angiogenesis. Further analysis indicated that these differences are likely due to neural progenitor cells and dorsal root ganglion neurons having distinct secretory profiles for mediators of angiogenesis.

A number of recent studies have investigated interactions between neural progenitor cells and endothelial cells, however the majority of these have focused on the effect of endothelial cells on the progenitor cells [8-11]. The limited number of studies that have investigated neural progenitor cell signaling to endothelial cells used endothelial cells isolated from brain. Neural progenitor cells had no effect on proliferation of brain microvascular endothelial cells [29-30], which is consistent with our results using dermal microvascular endothelial cells. In contrast to our results, neural progenitor cells induced brain microvascular endothelial cells to increase tube formation [5, 29]. The lack of effect on dermal microvascular endothelial cell tube formation that we observed may be due tissue specificity. It is also possible that these conflicting results are due to the use of neural progenitor cells derived from human embryonic stem cells [31-32] rather than neural progenitor/stem cells isolated from the brain [5, 29]. Both neural progenitor cell populations express cell surface markers characteristic of neural progenitors and are able to differentiate into different neuronal subtypes [5, 29, 31-32]. Furthermore, neural progenitor cells derived from human embryonic stem cells increased proliferation in the presence of dermal microvascular endothelial cells (data not shown), which is consistent with previous studies reporting that endothelial cells increased proliferation of neural progenitor/stem cells isolated from the brain [8, 11].

The effect of neural progenitor cells on microvascular endothelial cell migration has not been previously described. It is interesting to speculate that inhibition of endothelial cell migration may be mediated by TIMP-1 and serpin f1, which were unique to the neural progenitor secretory profile. Directly relevant to our study is that tissue inhibitor of matrix metalloproteinase-1, TIMP-1, has been reported to inhibit migration of dermal microvascular endothelial cells [33]. In addition, Serpin f1 also known as pigment epithelium derived factor (PEDF), is also a potent inhibitor of endothelial cell migration [34-35]. It is clear that further investigation is required to identify which factors secreted by neural progenitor cells are responsible for the inhibition of endothelial cell migration.

To our knowledge our study is the first to investigate the effect of dorsal root ganglion neurons on dermal microvascular endothelial cells. Other *in vitro* studies investigating nerve and dermal microvascular endothelial cell interactions have determined the effect of specific individual neuropeptides but have not expanded to other nerve-derived mediators [21-24]. In our study dorsal root ganglion neuron-conditioned medium inhibited dermal microvascular endothelial cells proliferation. It also induced the endothelial cells to increase production of

nitric oxide production. These effects were specific to the dorsal root ganglion neurons and were not observed in endothelial cells co-cultured with neural progenitor cells. Given that endostatin, FGF-1, MCP-1 and thrombospondin-2 were unique to the dorsal root ganglion neuron-conditioned medium, it is likely that these proteins mediate in part the effects on endothelial cell proliferation and nitric oxide production. Endostatin and thrombospondin-2 have been shown to inhibit proliferation of dermal microvascular endothelial cells [36]. Interestingly, acute exposure to endostatin also induces endothelial cell release of nitric oxide [37-38]. A review of the current literature indicates that almost nothing is known about the effect of FGF-1 and MCP-1 on endothelial cell proliferation and nitric oxide production. Loss-of-function experiments are necessary to determine whether endostatin and thrombospondin-2 are required to mediate the effects of dorsal root ganglion neurons on dermal microvascular endothelial cells. In our study the analyses of the secretomes of neural progenitor cells and dorsal root ganglion neurons were restricted to known regulators of angiogenesis. The antibody array did not include neuropeptides and neurotrophic factors known to mediate endothelial cell angiogenesis [2-4, 15, 19-20]. Determining whether there are differences in these nerve-derived factors between the neural progenitor cells and dorsal root ganglion neurons is therefore an important future direction for this study.

A limitation of this study is that the dorsal root ganglion neurons were isolated from rat in contrast to the neural progenitor cells and dermal microvascular endothelial cells, which were derived from human tissue. This species heterology was due to the difficulty in obtaining dorsal root ganglion neurons from humans. As a consequence, species differences must be considered for data interpretation because regulation of angiogenesis during wound repair may be different between humans and rodents. Differences between mouse and human immunology underscore that rodents and humans do for some biological processes use different receptors, signaling molecules and downstream effectors. [39]. Also relevant is that wound repair is different between humans and loose-skinned mouse and rats. In humans, wound closure is due to epithelialization and granulation tissue formation whereas in rodent wounds heal primarily by contraction [40-41]. It is clear therefore that future experiments using cells from the same species are important to confirm that differentiation state determines neural effects on dermal microvascular endothelial cells.

This study has implications for nerve regulation of angiogenesis during cutaneous wound repair. Wound angiogenesis is initiated by the innate immune response and occurs through sprouting of capillaries from existing blood vessels and mobilization of bone marrow-derived endothelial cell progenitors [42]. Sprouting of capillaries requires endothelial cells to degrade extracellular matrix, detach from basement membrane, migrate, proliferate and form tubules that then connect to form capillary networks [43]. In normal wound repair, angiogenesis is completed with scar formation, which is characterized by regression of capillaries and differentiation into mature blood vessels [42]. Our results suggest that both neural progenitor cells and mature neurons are involved in limiting wound angiogenesis with neural progenitor cells inhibiting endothelial cell migration and mature neurons inhibiting endothelial cell proliferation. Interestingly, mature neurons may also have a role in the early steps of wound angiogenesis given their positive effect on endothelial cell nitric oxide production. Nitric oxide has been reported to mediate angiogenesis induced by either substance P [44] or monocytes [45].

In conclusion, this study shows that neural progenitor cells and mature neurons have different effects on dermal microvascular endothelial cells. Neural progenitor cells secrete soluble factors that inhibit endothelial cell migration whereas paracrine signaling from mature neurons inhibits endothelial cell proliferation while increasing nitric oxide production.

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Abbreviations

DRG-CM	dorsal root ganglion neuron-conditioned medium
FGF	fibroblast growth factor
ICAM	intercellular adhesion molecule
IGFBP	insulin-like growth factor binding-protein
MCP	monocyte chemotactic protein
MEC	microvascular endothelial cells
NPC	neural progenitor cells
TIMP	tissue inhibitor of metalloproteinases
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

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Highlights

- Dorsal root ganglion neurons, not neural progenitor cells, regulate microvascular endothelial cell proliferation
- Neural progenitor cells, not dorsal root ganglion neurons, regulate microvascular endothelial cell migration
- Neural progenitor cells and dorsal root ganglion neurons do not effect microvascular endothelial tube formation
- Dorsal root ganglion neurons, not neural progenitor cells, regulate microvascular endothelial cell production of nitric oxide
- Neural progenitor cells and dorsal root ganglion neurons have different secretory profiles for angiogenic mediators

Figure 1. Human neural progenitor cells (A) and rat dorsal root ganglion neurons (B) at low magnification (10 $\times$). These are representative bright field/phase contrast images with the magnification bar = 100 μ m.

Figure 2.

Effect of neural progenitor cells and dorsal root ganglion neurons on dermal microvascular endothelial cell proliferation. (A) Dermal microvascular endothelial cells were co-cultured with neural progenitor cells (MEC+NPC) or with an empty insert (MEC with NPC base medium) for 48 hours. There was no significant difference in the number of viable dermal microvascular endothelial cells between MEC+NPC and the control MEC with NPC base medium. (B) Dermal microvascular endothelial cells were incubated in dorsal root ganglion neuron-conditioned medium (MEC+DRG-CM) or in DRG base medium (MEC with DRG base medium) for 48 hours. Dorsal root ganglion neuron-conditioned medium significantly reduced the number of viable microvascular endothelial cells (MEC+DRG-CM) compared to control MEC with DRG base medium (* $P=0.0029$). Data shown represent mean $\pm$ SD of a single representative experiment with $N=6$ for each condition.

Figure 3.

Effect of neural progenitor cells and dorsal root ganglion neurons on migration of dermal microvascular endothelial cells in a wound “scratch” assay. (A) Scratched dermal microvascular endothelial cells were co-cultured with neural progenitor cells (MEC+NPC) or with an empty insert (MEC with NPC base medium). Images were captured pre-scratch, immediately post scratch, 24, 48, 72 and 96 hours. (B) Scratched dermal microvascular endothelial cells were dorsal root ganglion neuron-conditioned medium (MEC+DRG-CM) or in DRG base medium (MEC with DRG base medium). Images were captured pre-scratch, immediately post scratch, 24, 48, 72 and 96 hours. (C and D) In order to determine the percentage scratch closure, the perimeter of each scratch was traced and area of cell free space was measured. Each time point was normalized to day 0 image area and reported as percent closure. (C) Neural progenitor cells inhibited significantly the rate of migration of dermal microvascular endothelial cells (MEC+NPC) compared to the control MEC with NPC base medium at 24, 48, 72 and 96 hours (All $*P < 0.05$). (D) Dorsal root ganglion neuron-conditioned medium had no significant effect on migration of dermal microvascular endothelial cells (MEC+DRG-CM) compared to control MEC with DRG base medium. Data shown represent mean $\pm$ SD of a single representative experiment with N=6 for each condition. The magnification bar = 500 μ m.

Figure 4.

Effect of neural progenitor cells and dorsal root ganglion neurons on dermal microvascular endothelial cell tube formation. Neural progenitor cells (NPC; A and B) and dorsal root ganglion neuron-conditioned medium (DRG-CM; C and D) had no significant effect on the ability of dermal microvascular endothelial cells (MEC) to form tubes when cultured on Matrigel™. There was no significant difference in total line length (A) and branch point number (B) between dermal microvascular endothelial cells co-cultured with neural progenitor cells (MEC+NPC) and control endothelial cells in NPC base medium (MEC with NPC base medium). There was also no significant difference in total line length (C) and branch point number (D) between dermal microvascular endothelial cells incubated in dorsal root ganglion neuron-conditioned medium (MEC+DRG-CM) and endothelial cells incubated in DRG base medium (MEC with DRG base medium). Data shown represent mean $\pm$ SD of a single representative experiment with N=6 for each condition.

Figure 5.

Effect of neural progenitor cells and dorsal root ganglion neurons on dermal microvascular endothelial cell production of nitric oxide. (A) Dermal microvascular endothelial cells were co-cultured with neural progenitor cells (MEC+NPC) or with an empty insert (MEC with NPC base medium) for 48 hours. There was no significant difference in the total nitric oxide in the culture supernatant between MEC+NPC and the control MEC with NPC base medium. (B) Dermal microvascular endothelial cells were incubated in dorsal root ganglion neuron-conditioned medium (MEC+DRG-CM) or in DRG base medium (MEC with DRG base medium) for 48 hours. Dorsal root ganglion neuron-conditioned medium induced microvascular endothelial cells to significantly increase production of nitric oxide (* $P=0.0031$). Data were normalized to cell number and are expressed as $\mu\text{mol/L per } 10^6$ cells. Data shown represent mean $\pm$ SD of a single representative experiment with $N=6$ for each condition.

Figure 6.

Secretory profiles for neural progenitor cells and dorsal root ganglion neurons. (A) Human neural progenitor cell-conditioned (NPC)-medium was analyzed using a proteome profiler human angiogenesis antibody array. The solid white circles indicate proteins secreted by the neural progenitor cells. The dashed white circles indicate proteins secreted by the dorsal root ganglion neurons but absent in the NPC-conditioned medium. (B) Rat dorsal root ganglion neuron (DRG)-conditioned medium was analyzed using a proteome profiler mouse angiogenesis antibody array. The solid white circles indicate proteins secreted by the dorsal root ganglion neurons. The dashed white circles indicate proteins secreted by the neural progenitor cells but absent in the DRG-conditioned medium. (C) Summary of results from A and B; a grey box indicates presence in the conditioned medium and a white box indicates absence in the conditioned medium.