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Directed Migration of Neural Stem Cells by Angiogenic Factors

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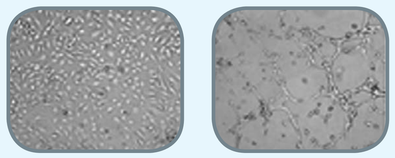
Abstract and Introduction

Neural stem cells (NSCs) of the central nervous system (CNS) are highly mobile and have been shown to migrate towards gliomas and sites of cellular damage. Tumors and damaged tissues are highly vascularized and are sites of active angiogenesis. While recent studies have implicated the role of endothelial cells in the creation of a vascular niche that stimulates the proliferation and neuronal differentiation of NSCs, the mechanisms for the preferential migration of neural stem cells to damaged tissues remain unknown.

To investigate the role of angiogenesis in neural stem cell recruitment to sites of injury, we examined the effects of various states of endothelial activation on NSCs migration. Using a co-culture system, we show that soluble factors released from activated tube-forming endothelial cells direct the migration of both human- and rat-derived NSCs. In contrast, monolayer cultures of endothelial cells did not elicit a migratory response from either human or rat NSCs. Soluble factors from non-activated and activated tube-forming endothelial cells were also compared for their capacity to differentially influence the proliferation and differentiation of NSCs. These results suggest that an important function of endothelial cells may be to help direct the replacement of damaged or lost cells in the CNS.

Assay Overview

Day 0 Plate human umbilical vein endothelial cells (HUVECs) onto fibrin coated 24-well plates under non-inducing and inducing conditions.



Uninduced Monolayer | Induced Tubular Networks

Day 1 Wash wells 3 times with NSC Basal Medium. Overlay wells with new NSC Basal Medium for conditioning.

Day 2 Collect conditioned medium. Assess effects on NSC migration, proliferation and differentiation.

Migration	**Proliferation**	**Differentiation**
Millicell 8 µM inserts in boyden chamber	MTT Cell Proliferation Assay	5-day differentiation protocol

Results

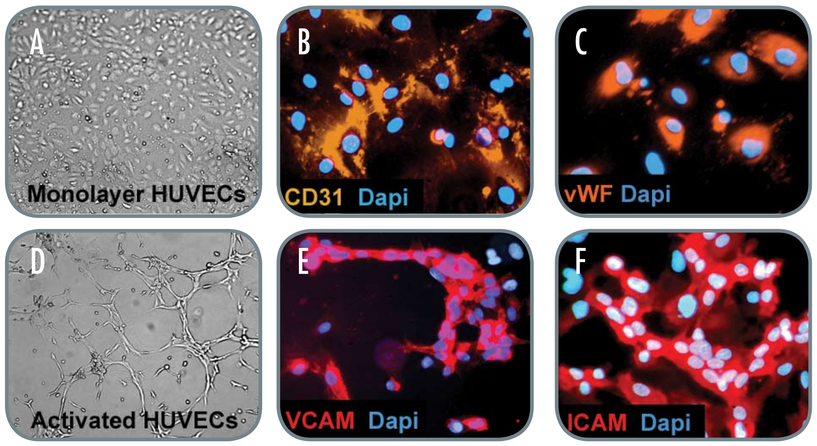


Figure 1. Human umbilical vein endothelial cells (HUVECs) grow as a monolayer when cultured on fibrin gels in basal medium for two days (A). Uninduced monolayer cultures of HUVECs stain for constitutive markers, CD31 (B, yellow), and von Willibrand Factor (vWF) (C, orange). Activated HUVECs form tubular networks when grown on fibrin gels for two days in the presence of bFGF (40 ng/mL), VEGF (40 ng/mL), and PMA (50 ng/mL) (D). Activated tube-forming HUVECs upregulate the expression of VCAM (E, red), and ICAM (F, red). HUVECs were seeded (75,000 cells/well) on fibrin coated 24-well culture plate and allowed to adhere overnight in EGM basal medium (Cambrex) (non-activated condition), or EGM basal medium supplemented with bFGF, VEGF and PMA (activated condition). The following day, HUVECs were washed with NSC Basal Medium three times to remove residual growth factors followed by overnight incubation with fresh NSC Basal Medium to obtain conditioned medium.

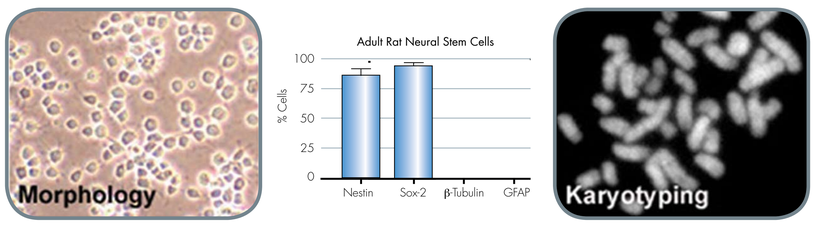


Figure 2. Adult Rat Hippocampal NSCs (Cat. No. SCR021, SCR022) are grown as monolayers, have a normal karyotype, express NSC markers and are negative for neuronal and astrocyte lineage markers.

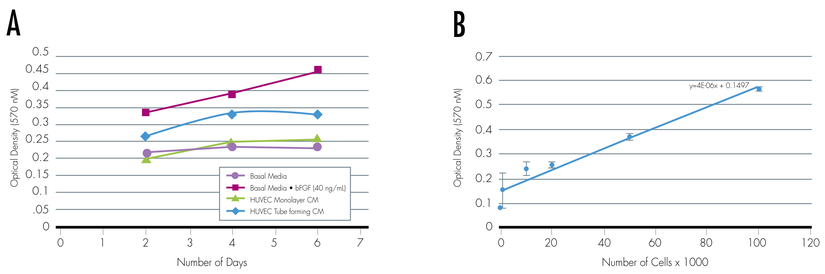


Figure 3. Secreted angiogenic factors increase rat NSC proliferation *in vitro*. Rat NSCs were seeded (20K/well) onto poly-L-ornithine and laminin coated 96 well plates. Cells were incubated in the following medium: NSC Basal Medium, NSC Basal Medium with 40 ng/mL FGF, conditioned medium (CM) from monolayer cultures of HUVECs and CM from activated tube-forming cultures of HUVECs. Cell proliferation was monitored on day 2, 4, 6 following Millipore's MTT Cell Proliferation Assay (Cat. No. CT02) (A). Standard curve for Rat NSC using MTT Cell Proliferation Assay (B).

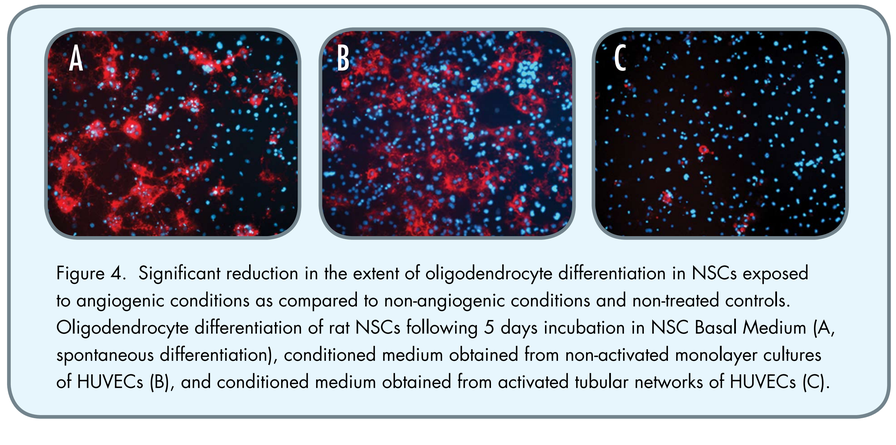


Figure 4. Significant reduction in the extent of oligodendrocyte differentiation in NSCs exposed to angiogenic conditions as compared to non-angiogenic conditions and non-treated controls. Oligodendrocyte differentiation of rat NSCs following 5 days incubation in NSC Basal Medium (A, spontaneous differentiation), conditioned medium obtained from non-activated monolayer cultures of HUVECs (B), and conditioned medium obtained from activated tubular networks of HUVECs (C).

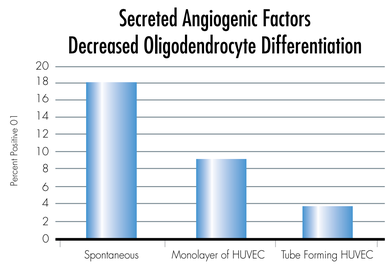


Figure 5. Secreted angiogenic factors negatively regulate the differentiation of NSCs to oligodendrocytes. Rat NSCs were seeded (100K/well) onto poly-L-ornithine and laminin coated 24-well plates. NSCs were incubated for five days in NSC Basal Medium and conditioned medium (CM) from either monolayer or activated tube-forming cultures of endothelial cells (HUVECs). The percentage of O1- positive cells was manually quantitated by averaging the percentage of O1-positive cells versus total Dapi cells from ten random fields.

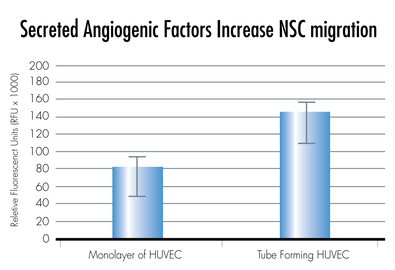


Figure 6. Secreted angiogenic factors increase rat NSC migration *in vitro*. Rat NSCs were seeded 100K cells/well onto a laminin (5 µg/mL) coated 24-well Millipore millicell insert (8 µm pore size). Rat NSCs were incubated for 4 hours in either conditioned medium (CM) obtained from non-activated monolayer cultures of HUVECs or tubular networks of activated endothelial cells.

Summary

1. Factors secreted from activated tubular networks of endothelial cells significantly increased NSCs migration (1.8 fold) and proliferation compared to factors secreted from non-activated HUVECs.

2. Multilineage differentiation of NSCs to neurons and astrocytes was not observed in either conditions using a short 5-day differentiation protocol.

3. Significant reduction in the extent of oligodendrocyte differentiation (4-fold versus 2-fold) was observed in NSCs exposed to angiogenic and non-angiogenic conditions, respectively, as compared to non-treated controls.

Our results are consistent with the hypothesis that the angiogenic niche supplies secreted factors that aid in the maintenance of the stem cell state by simultaneously increasing proliferation and inhibiting differentiation. Because these secreted factors also increase the migratory behavior of NSCs, future studies are aimed at interrogating the effects of the angiogenic niche in enabling and accelerating the conversion of normal stem cells to cancer stem cells.

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