3047.5 (3)

The Aresty Research Center for Undergraduates
Second Annual Undergraduate Research Symposium
April 21, 2006

Title: Netrin-Mediated Guidance of Spinal Cord Oligodendrocyte Precursor Cells

Student Information

Name: James E. Reilly
Major: Cell Biology & Neuroscience

Advisor Information

Name: Randall D. McKinnon, PhD
Address: Surgery (Neurosurgery)
Robert Wood Johnson Medical School
675 Hoes Lane S-225, Piscataway NJ 08854

Abstract

Understanding what controls cell migration during central nervous system (CNS) development may prove helpful in the search for therapies for diseases and injuries in the adult CNS. In mammals, oligodendrocyte precursor cells (OPCs) migrate throughout the CNS then differentiate into myelin-producing oligodendrocytes. Netrin-1, a protein secreted by CNS midline cells, binds to receptors on neurons and OPCs and can mediate attractive and repulsive migratory signals. In neurons the receptor Unc5b mediates short-range repulsion while another, DCC, mediates long-range attraction. Together, Unc5b and DCC mediate long-range repulsion. Yet another receptor, Neogenin, is thought to act like DCC.

In this study we examined expression of Netrin-1 receptors on OPCs to determine whether their function is similar to that on neurons. We examined OPCs *in vitro* and *in vivo* using fluorescently labeled antibodies targeted to Netrin-1 receptors as well as OPC specific markers (Olig2, NG2, PDGFRa). In the spinal column, we found Unc5b (repulsive) localized to lagging edges (nearest to the Netrin-1 source) while DCC and Neogenin (both attractive) were localized to leading edges (furthest from the source). The optic nerve presented a more complex situation that may involve the positions of OPCs relative to a Netrin-1 "collar" which we found near the optic chiasm.

Our results to date suggest that a polarized pattern of Netrin-1 receptor localization could allow OPCs to orient their migration relative to a Netrin-1 source. Ongoing studies will focus on how this pattern forms and whether OPCs use the pattern to orient their migration relative to Netrin-1.

Key Words: oligodendrocyte precursor / progenitor cells (OPCs), netrin, cell migration

Presentation Preference: Oral (Will need laptop projector.)

Abstract Category: Natural and physical sciences

Asymmetric localization of Netrin receptors on migrating glial cells during neural development.

James E. Reilly, Mary E. Kiel and R.D. McKinnon

Rutgers University Honors Thesis Symposium, Piscataway, Spring 2006

In the central nervous system (CNS), the secreted protein Netrin-1 binds to receptors on neural cells and can mediate attractive and repulsive migratory signals. In this study, we examined the Netrin receptors Unc5b, DCC (deleted in colorectal cancer), and Neogenin on migrating oligodendrocyte progenitor cells (OPCs) both *in vitro* using cell cultures and *in vivo* using rodent models and compared their function on OPCs to that on axons. We examined the OPCs using fluorescently labeled antibodies targeted to Netrin receptors as well as OPC specific markers (Olig2, NG2, PDGF α-receptor). In cell cultures, we found Unc5b and Neogenin both localized to the cell body while Unc5b but not Neogenin was more prevalent in the processes. In the spinal cord, we found Unc5b (repulsive) localized to ventral regions of the cord and to OPCs' lagging edges. Further, we found DCC and Neogenin (both attractive) localized to more dorsal regions of the cord and, more specifically, to OPCs' leading edges. These results demonstrate an asymmetric distribution of Netrin receptors on OPCs. In the optic nerve, we were presented with a more complex situation that may involve the positions of OPCs relative to a Netrin-1 "collar" where the optic nerve exits the brain.

(5)

Glia in Health & Disease **McKinnon**
Oral

NETRIN-DIRECTED GLAL MIGRATION

James E. Reilly, Katherine Chen, Mary Kiel and Randall D. McKinnon
Neurosurgery, UMDNJ-R.W.Johnson Med School, Piscataway NJ 08854.

Signals that regulate CNS development are conserved, and we show that Netrin directs glial migration in both vertebrate and invertebrates. Netrins control neural assembly by attracting growing axons towards the ventral midline via trans-membrane receptors DCC, and repelling axons via the repulsive receptor UNC5. In rodents, glial precursors of oligodendrocytes (OPCs) also express the Netrin receptors DCC, Neogenin and UNC5b. In vitro, OPCs are repelled from Netrin sources including HEK293-Netrin, but not HEK293 control cells. Emigration was blocked by UNC5-specific RNA interference using expression cassettes assembled with a novel PCR amplification strategy. In vivo, OPCs have an intrinsic polarity in developing spinal cord and optic nerve; DCC and Neogenin are on leading edges while UNC5b is on trailing edges. This polarity directs OPC migration through an undulating gradient of Netrin in the optic chiasm; OPCs first migrate towards then away from a 'collar' of Netrin-1 expressing cells which we identified at the optic nerve exit points.

In *Drosophila*, UNC5 is expressed by exit glia which emigrate along motor neuron axons during embryogenesis. We isolated a P-element insertion into the single *Drosophila unc5* locus then generated an *unc5* null allele by imprecise excision. In the absence of UNC5 the exit glia stalled at the PNS boundary. Exit glial migration was also affected by deficiencies of Netrin [*Df(1)NP5*] and of the attractive receptor DCC (*frazzled*). Gain of function studies [*gcm::Unc5; fra3/fra4*] further revealed that UNC5 was sufficient while Fra was dispensable for short range repulsion of midline glia. Thus UNC5 cooperates with Fra for long but not short range repulsion from the *Drosophila* midline.

Together these studies suggest a model in which signaling downstream of UNC5 and DCC/*fra* function independently to interpret a Netrin gradient that directs long range glial migration.

Supported by the NIH (MH54652), the NJ Commission on Science and Technology (05-3047-SCR), and an Aresty Award (Rutgers U.) to JER.

HASAN, OMAR (RWJMS 2010)

NETRIN DIRECTED MIGRATION OF ENDOGENOUS OLIGODENDROCYTE PRECURSORS IN ADULT CNS

RD McKinnon, Ph.D., Neurosurgery and David Crockett, Ph.D., Neurosci. Cell Biology

Oligodendrocytes (OL) function as supportive cells in the propagation of neural signals. However, they also produce inhibitors blocking axon regrowth after spinal cord injury. Inducing migration of OLs and their immediate precursors, OL progenitor cells (OPCs) may promote an environment at the site of spinal cord injury to facilitate repair. The ability to induce OPC/OL migration away from an injury could allow axon regeneration, and directing their subsequent migration back to the site would allow these cells to resume their supportive functions.

Netrin is a signaling molecule used in the embryonic spinal cord to direct axons and OPCs during development. Our studies were designed to test the hypothesis that Nerin could also direct OPC migration in the adult spinal cord. We used a stereotactic approach to precisely deliver aliquots of recombinant Netrin directly into the spinal cord of adult mice. A dorsal thoracic laminectomy (approximately T9-T10) was performed on adult wild type mice, with delivery via glass pipette into the left gracile fasiculus of 1 μl (10 ng) recombinant Netrin plus charcoal to mark the injection site. Experimental groups included sham controls and Netrin injections, with harvest times of either 3 or 6 days. The controls received 1 μl of phosphate buffered saline (PBS). For analysis, pups were perfused with a 4% paraformaldehyde solution to fix the tissue then the spinal cord was removed and cryoprotected in 20% sucrose, frozen, and 20 μm thin sections were prepared by cryostat. These were mounted on slides then stained with antibodies to identify NG2-positive OPCs and visualized using confocal microscopy. Analysis was done by comparing the numbers of NG2-positive cells in 100 μ radial bins around the injection site to determine whether Netrin induced the migration of NG2-cells.

We successfully visualized NG2-positive cells on a non-injected spinal cord. We also successfully delivered a bolus of Netrin into the spinal cord of and adult mouse, allow it to incubate for 6 days and then visualize NG2 cells at the injection site by confocal microscopy. The initial results suggested Netrin may have had an effect on NG2-positive OPCs in the adult spinal cord, as we observed a large number of NG2-positive cells around the injection site. However, further analysis will be needed to determine if this is significant. Sham controls were not analyzed due to technical difficulties with the cryostat and preparing the samples. Thus our results were incomplete, and the analysis will require a proper sham control before an accurate comparison can be made.

Our study suggests further technical approaches to improve the analysis. One possible idea to differentiate the migration of NG2-positive cells from other possible effects, such as reactive gliosis, would be to inject BrdU immediately before sacrifice to see which cells are actively replicating versus migrating from different parts of the spinal cord. Also, varying the amount of Netrin injected could produce varying levels of migration and these should be optimized. In short, Netrin-induced migration of OPC's could be an important first step in a model for spinal cord injury repair.

Control/Tracking Number: 2007-A-43303-SfN
Activity: Scientific Abstract
Current Date/Time: 5/15/2007 12:57:46 PM

Asymmetry of attractive and repulsive Netrin receptors on migrating glial progenitor cells.

AUTHOR BLOCK
: **M. E. KIEL**, *R. D. MCKINNON;
Dept Neurosurgery S-225, RW Johnson Med. Sch. UMDNJ, Piscataway, NJ

Abstract:
Cell migration and process extension are central to metazoan development, and molecules involved have conserved roles amongst distinct organ systems and between phyla. Netrin, a laminin-related protein secreted from ventral midline cells during neural tube development, is critical for axon path finding. It attracts axons via high affinity receptors DCC/Fazzled/Unc40 and repels axons via Unc5. These receptors signal independently, as axon attraction requires only DCC/Fra while repulsion requires only Unc5, although some axons require an interaction between DCC and Unc5 for repulsion. At present it is unclear what regulates Netrin signaling to enable midline crossing axons to be first attracted toward then repelled away from Netrin.
We demonstrate that Netrin also directs the migration of glial cells in both invertebrates and vertebrates. Loss of function studies in *Drosophila* demonstrate that Unc5 and Fra are required for Netrin-directed long range exit glia repulsion, and gain of function studies demonstrate that DCC/Fra is not required for short range repulsion. Unc5 is also required for repulsion of vertebrate oligodendroglial progenitor cells (OPCs). OPCs are repelled from Netrin in vitro and using RNA interference we show that UNC5 is necessary. Finally we demonstrate an asymmetric distribution of Netrin receptors Unc5 and DCC on OPCs which suggests a simple mechanism to explain the dual response paradox (attraction then repulsion) of cells to a chemotactic gradient. In the spinal cord Unc5 segregates to OPC soma proximal to midline Netrin, while DCC is on processes extending away from the midline. Thus Unc5 on the trailing process can signal repulsion from Netrin while DCC on the leading process can signal attraction to an intermediate target. Consistent with this we find a focal 'collar' of Netrin expressing cells in the optic chiasm, at an intermediate target for OPC migration into the optic nerve. Thus Netrin receptors direct glial migration in both invertebrates and vertebrates, and for vertebrate OPCs their asymmetric distribution establishes a polarity to their response. Supported by grants from the NIH (MH54652) and the New Jersey Commission on Spinal Cord Research (05-3047).
:

Theme and Topic (Complete): A.02.e. Glial differentiation; A.02.c. Cell migration

Keyword (Complete):

DEVELOPMENT; OLIGODENDROCYTE; MIGRATION; SIGNALING; GUIDANCE

Presentation Preference (Complete): Poster Preferred

Support (Complete):

Support : Yes

Grant/Other Support: : NIH Grant MH54652

Grant/Other Support: : NJ Commission Spinal Cord Research, 05-3047