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Mary Ray
NJ Commission on Spinal Cord Research
P.O. Box 360
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4 March 2008

Mary,
Enclosed please find the original plus 5 copies of my Final Narrative Report for NJCSCR Grant # 05-3047-SCR-E-0, including appendices.

Sincerely,

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Final Narrative Report, Grant # 05-3047-SCR-E-0

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2. Organization:
UMDNJ-Robert Wood Johnson Medical School
675 Hoes Lane, S-225, Piscataway NJ 08854

3. Grant Title: *Netrin directed glial migration*

4. Grant Number: # 05-3047-SCR-E-0

5. Grant Period Covered by Report: 06/2005-06/2007

6. Date of Submission of Report: March 4, 2008

II. Narrative Report:

1. **Original aims** of the project, summarized from the original proposal:

Our specific aims focus on netrin as a directional cue for oligodendrocyte progenitor cells, and address whether netrin promotes OPC attraction or repulsion.

Specific Aim 1. To examine the expression of netrin receptors unc5, DCC and neogenin in spinal cord OPCs in vitro and in vivo.

Specific Aim 2. To address the hypothesis that the repulsive netrin receptor unc5 controls directional OPC migration.

The immediate results of these studies will define the receptors present on OPCs in the rat spinal cord and the competence for netrin directed migration. The long term consequences will direct efforts to modulate glial environment in the injured spinal cord.

2. **Project successes**:

We have made tremendous progress in this research program. Progress will be briefly detailed (below) for each of the individual manuscripts (4) and funded grant applications (3) that arose directly from studies funded by 05-3047-SCR.

This grant provided ongoing support for the completion of four manuscripts. Two of these focus on Netrin signaling (Labrador et al., Current Biology 2005; Reilly et al., in prep.), while the remaining two focus on stem cell derived glia for therapeutic remyelination (Chen et al., Stem Cell Reviews 2007; Kiel et al., submitted). Copies of these manuscripts are included in the appendix of this current report.

Work supported by this grant has been reported by our staff and students in 9 abstracts and accompanying posters at scientific meetings throughout the US.

The PI has also presented these results in lecture format at 5 national and international meetings, including a talk in the inaugural session of a very prestigious meeting in Cold Spring Harbor NY, and at an international conference at McGill University, Canada.

Finally, the studies resulting from this NJCSCR award formed the basis for grant proposals submitted to the NJCSCR (declined) and the NJCST Stem Cell Research (funded). Our Stem Cell grants include studies on manipulating ES cells for (1) netrin and (2) PDGF-directed glial migration in vivo, as well as (3) studies on human ES cells in vitro.

As a direct consequence of this award we now have a concrete understanding of how netrin directs glial migration in development. We have also made great strides towards our longer term goal of using netrin receptors to direct the migration of glial cell transplants into CNS lesions in vivo. Thus the studies initiated in 05-3047-SCR continue to move forward.

3. **Project challenges**: We were faced with neither technical nor interpretive obsticles, and our results led to an elegant and simple (Polar Receptor) model to explain how netrin dictates both attraction and repulsion (Reilly et al., in prep). This also led us to a testable hypothesis on how to use netrin receptors to direct glial cell transplants.

We were quite dissappointed with the outcome of our NJCSCR submission to continue this research. Basically, the reviewers appreciated the model but questioned our ability to bring a project such as this through completion. Fortunately for us we found an alternative source of support, and we have redirected our efforts towards non-SCI repair models. We remain optimistic that the NJCSCR reviewers will recognize our significant progress and remain open to future funding requests.

4. **Implications** for future research, clinical treatment.

We believe we now understand how netrin coincindently attracts and repels glial cells in order to control their migration in neural development. Thus we are now positioned to test whether we can manipulate their response and direct glial migration in vivo. Preclinical tests of the Polar Receptor model are currently in progress, as per the general outline and anticipated time line submitted of our original NJCSCR research proposal.

5. **Plans** to continue research:

We are continuing with our overall goal of using netrin to direct the migration of transplanted glial cells, with a current focus on neural degeneration.

5a. **Applications** submitted to other sources for ongoing support.

Active:

Source: New Jersey Commission on Stem Cell Research
Title: "Stem cell therapeutics: PDGF-directed glial migration"
Dates: 6/1/07 - 5/31/09
PI: R.D. McKinnon, 25% effort
Direct indirect total Costs: $ 260,870; $ 39,130; $ 300,000

Source: NJ Commission on Stem Cell Research, CORE Facility Grant
Title: "Bioengineering of human embryonic stem cells"
Dates: 6/1/07 - 5/31/09
Co-PIs: R. McKinnon, P. Casaccia, M. Roth, V. Pirrotta
Direct, indirect, total: $ 2,189,513; $ 329,344; $ 2,518,857
Project 3: hES derived oligodendrocytes: Netrin directed migration;
R McKinnon, PI (25% effort)
Direct indirect total Costs: $ 260,870; $ 39,130; $ 300,000
Core A: Tissue culture and gene transfer facility
RD McKinnon, PI (2% effort)
Direct, indirect, total: $ 633,389; $ 95,068; $ 728,457
Core C: Confocal imaging
RD McKinnon, PI (2% effort)
Direct, indirect, total: $ 113,808; $ 17,071; $ 130,879

Pending:

Source: NJ Commission on Spinal Cord Research, Graduate Fellowship
Title: "*Induced pluripotential stem cells for spinal cord repair*"
Dates: 06/08-06/10
PI: R.D. McKinnon (Dorota Sadowski, PhD candidate)
Direct indirect total Costs: $ 60,000; $ 60,000; $ 60,000

6. **Publications arising from this research**.

Labrador, J.P., D. O'Keefe, S .Yoshikawa, **R.D. McKinnon**, J.B. Thomas and G. Bashaw (2005). *The homeobox transcription factor even-skipped regulates Netrin-receptor expression to control dorsal motor-axon projections in Drosophila*. ***Current Biol.*** 15(15):1413-1419. This paper identifies even-skipped as a critical regulator of netrin signaling in fly development. Our netrin project started in Drosophila axon path finding, and the PI isolated the Unc5 (netrin receptor)-null allele that formed the starting point for this manuscript.

C.P. Chen, M.E. Kiel, D. Sadowski and **R.D. McKinnon** (2007). *From Stem Cells to Oligodendrocytes: Prospects for Brain Therapy.* **Stem Cell Reviews** 3(4): 280-288. The NJCSCR project was designed around an ES-derived glial transplant model. This review outlines the basis for this therapeutic approach in SCI and demyelinating diseases.

M.E. Kiel, C.P. Chen, D. Sadowski and **R.D. McKinnon**. *Stem cell derived therapeutic myelin repair requires 7% cell replacement* (submitted). In parallel to our netrin studies, ongoing work asked whether ES-derived glial grafts could repair a dysmyelinated brain. This study describes how much engraftment is necessary and sets an attainable threshold for brain repair.

J.E.Reilly, A.Gordon, M.Kiel, S.Yoshikawa, J.B.Thomas and **R.D.McKinnon**. *Independent roles for UNC5 and DCC in dictating polarity of netrin-directed glial migration* (in prep). This is a very significant study and the major outcome of 05-3047-SCR. It describes the "Polar Receptor" model which explains coincident attraction (DCC) and repulsion (Unc5) via netrin receptors. We believe this resolves several controversies in the field, and it also suggests how we may use these receptors for homing ES-derived glial cells in vivo. We are currently resolving one issue (*fra* expression in fly glia) before submitting this for peer review.

Abstracts:

1. James E. Reilly, Mary E. Kiel and R.D. McKinnon (2006). Asymmetric localization of Netrin receptors on migrating glial cells. Research Day, RW Johnson Med School.

2. R.D. McKinnon, A. Chang, Alan Gordon and Mirat Shah (2006). Control of glial position via the repulsive netrin receptor Unc5. Research Day, RW Johnson Medical School.

3. James E. Reilly, Mary E. Kiel and R.D. McKinnon (2006). Asymmetric localization of Netrin receptors on migrating glial cells during neural development. Aresty Research Symposium, Rutgers University New Brunswick NJ.

4. James E. Reilly, Mary E. Kiel and R.D. McKinnon (2006). Asymmetric localization of Netrin receptors on migrating glial cells during neural development. Rutgers University Honors Thesis Symposium, Piscataway.

5. James E. Reilly, Katherine Chen, Mary Kiel and R.D. McKinnon (2006). Netrin-directed glial migration. Cold Spring Harbor, Glia in Health & Disease, CSH NY

6. Nayak Natasha, Katherine Chen and R.D. McKinnon (2006). Netrin signaling and glial migration. Biology Research Symposia, The College of New Jersey, Ewing NJ.

7. Omar Hasan, David Crockett and R.D. McKinnon (2007). Netrin directed migration of endogenous oligodendrocyte progenitors in the adult CNS. RWJMS Medical Student Symposia.

8. Mary E. Kiel and R.D. McKinnon (2007). Asymmetry of attractive and repulsive Netrin receptors on migrating glial progenitor cells. 37th Soc Neurosciences #A43303, San Diego CA.

9. Cui Ping Chen, Dorota Sadowski, Mary E. Kiel and R.D. McKinnon (2007). Minimal chimerism for phenotypic rescue of dysmyelination by stem cell therapy. 37th Soc Neurosciences #114674, San Diego CA.

Invited Lectures (RMcK):

Celgene Cellular Therapeutics, Summit NJ, 18 April 2006.
"*From Stem Cells to Oligodendrocytes: Prospects for Brain Therapy*"
Cold Spring Harbor Symposia, Glia in Health and Disease, CSH NY, July 2006
"*Netrin-directed glial migration*"
Neuroscience Lecture Series, UMDNJ Newark, Nov. 2006
"*Glial cell migration: Netrin and PDGF signaling targets for Stem Cell Therapeutics*"
Quebec/New Jersey Stem Cell Workshop, Montreal Neurological Institute, June 2007
"*Stem Cell Therapeutics for Myelin Repair*"
Celgene Cellular Therapeutics, Warren NJ, Oct 2007.