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14. ABSTRACT

Peripheral neuropathy is an incredibly common and debilitating disorder. For most forms of peripheral neuropathy, developing an understanding of pathogenesis, identifying targets for treatment, and testing of new therapeutic approaches have been severely hampered by a lack of adequate models to address these questions. The purpose of this project is to create a new humanized mouse for the study of peripheral neuropathies.

15. SUBJECT TERMS

NONE LISTED

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INTRODUCTION

Subject: We propose to create a new humanized mouse model in which the endogenous mouse Schwann cells will be replaced by human Schwann cells derived from human patients.

Purpose: Peripheral neuropathy is an incredibly common and debilitating disorder. For most forms of peripheral neuropathy, developing an understanding of pathogenesis, identifying targets for treatment, and testing of new therapeutic approaches have been severely hampered by a lack of adequate models to address these questions. The purpose of this project is to create a new humanized mouse for the study of peripheral neuropathies.

Scope of the research:

Aim 1. To establish a partially humanized mouse model of peripheral neuropathy. In this aim we will genetically ablate the mouse SCs and replace them with human SCs. The *ROSA26-eGFP-DTA* DTA [7] mouse contains a suppressed Diphtheria toxin A gene (DTA). CRE recombinase expression leads to de-repression. This allows for ablation of any genetically distinct populations of cells in which CRE recombinase can be selectively expressed. Crossing *ROSA26-eGFP-DTA* mice with the Tg(Mpz-cre/ERT2) mouse [8] creates a mouse where Schwann cells can be selectively ablated by treating the mice with Tamoxifen. We will then inject promyelinating human IPSCs into the sciatic nerves of these mice to reconstitute the Schwann cell population. Use of hIPSCs tagged with dTomato will allow for in vivo tracking (IVIS Spectrum) of engraftment and migration of these SCs along the sciatic nerve.

Aim 2. To characterize the chimeric nature and integrity of the partially humanized mouse model. This aim uses electrophysiological, transcriptomic, immunohistochemical (IHC) and morphological studies to characterize the nature and integrity of this model.

Aim 3. To create a partially humanized mouse model of the human inherited peripheral neuropathy CMT1X. To demonstrate the applicability of our method, we will create a new model of CMT1X, a common inherited neuropathy in patient with mutations in *GJB1*, the gene encoding connexin 32 (Cx32) using Schwann cells derived from human patients with pathogenic mutations in GJB1. We have knockin mice expressing the Cx32 p.Arg75Trp mutation; by three months these mice show neuropathy by both electrophysiology and pathology. Thus, we will leverage the New York Stem Cell Foundation's stem cell bank which houses hiPSCs from a patient harboring the p.Arg75Trp mutation to produce a humanized model of CMT1X.

KEYWORDS

Charcot Marie-Tooth disease (CMT), CMT1X, connexin 32 (Cx32, also known as GJB1), Schwann cells, peripheral neuropathy, human inducible pluripotent stem cells (hiPSCs).

ACCOMPLISHMENTS

What were the major goals of the project?

	Timeline	Percentage of completion
Specific Aim 1: To establish a partially humanized mouse model of peripheral neuropathy.	Months	
Subtask 1 Re- expand and confirm characterization of wild type human Schwann cell differentiated iPSCs	1-6	90%
Subtask 2 IACUC Approval (within 2 months), Maintain, characterize and expand the p. R75W mouse line to be used in Task 3.	1-12	100%
Subtask 3 IACUC Approval (within 2 months), Maintain, characterize and expand the MPZ-CreErT -DTA mouse line.	1-6	100%
Milestone(s) Achieved: Establishing P0-CreERt-DTA mice, including adjusting the tamoxifen and immunosuppression administration protocol to reduce lethality, while maximizing SC ablation.		
Specific aim 2 To characterize the chimeric nature and integrity of the partially humanized mouse model.		
Subtask 1 To inject promyelinating human IPSCs into the sciatic nerves of the DTA mice to reconstitute the Schwann cell population.	3-6	80%
Subtask 2 To use hIPSCs tagged with dTomato to allow for in vivo tracking (IVIS Spectrum) of engraftment and migration of these SCs along the sciatic nerve.	7-9	0%
Subtask 3 To characterize the chimeric nature and integrity of the partially humanized mouse model using transcriptomic and morphological studies.	8-12	40%
Milestone(s) Achieved: Establishing the humanized mouse model in which human derived SCs myelinate large caliber axons and/or acquire non- myelination SCs phenotype, in vivo, and documenting its integrity and chimeric nature.		

Specific aim 3: to create a partially humanized mouse model of the human inherited peripheral neuropathy CMT1X		
Subtask 1 To obtain patient derived iPSCs form New York Stem Cell foundation.	6-15	20%

Differentiate into Schwann cells. Characterize and expand.		
Subtask 2 To characterize the chimeric nature and integrity of the partially humanized mouse model expressing Arg75Trp using transcriptomic and morphological studies.	12-24	0%
Subtask 3 To continue to breed p.Arg75TRP mice, and harvest tissues for comparison with humanized mouse expressing human CMT1X p.Arg75TRP mutation	12-24	60%
Milestone(s) Achieved: Establishing the humanized mouse model that recapitulates in vivo the inherited peripheral neuropathy CMT1X.		

What was accomplished under these goals?

Major activities:

Aim 1: We have re- expanded wild type human Schwann cell differentiated iPSCs and have enough frozen cells for all the anticipated experiments. RNA-derived from these cells was submitted for RNA-seq analysis.

In addition, we have established the P0-CreERt-DTA mice model; we have adjusted the tamoxifen regime and characterized the clinical score of the mice over time, to identify the best time-point for human cells injection.

Aim 2: Over the last year we have injected promyelinating human IPSCs into the sciatic nerves of the DTA mice at the peaks of endogenous Schwann cells loss, and harvested the sciatic nerve at one, two and three weeks post human cell injection. The tissues were harvested and are currently under analysis by IHC.

Aim 3: Ove the last year we have harvested tissues from the p.Arg75TRP mice for further comparison with humanized mouse expressing human CMT1X p.Arg75TRP mutation.

What opportunities for training and professional development has the project provided?

- *"Nothing to Report."*

How were the results disseminated to communities of interest?

- *"Nothing to Report."*

What do you plan to do during the next reporting period to accomplish the goals?

Aim 1: Over the next year the ongoing transcriptomic studies will be completed.

Aim 2: Over the last year we have injected promyelinating human IPSCs into the sciatic nerves of the DTA mice at the peaks of endogenous Schwann cells loss and harvested the sciatic nerve for further analysis. Over the next year the samples will be studied by IHC and by RNA-seq, for transcriptomic studies. The transcriptomic studies will start after confirming human cells integration into the mouse nerve by IHC. In the transcriptomic studies we will compare the transcriptome of the human IPSC derived Schwann cells in vitro, and in vivo.

In the currently ongoing IHC studies, we will identify the engrafted human-derived cells in the mouse tissue by an antibody that recognizes only human cells. In parallel, over the next year we will transfect the cell with an expression vector encoding for the fluorescent protein dTomato. We will use the in vivo tracking (IVIS Spectrum) to track over time the engraftment and migration of these SCs along the sciatic nerve.

Aim 3: Over the last year we have harvested tissues from the p.Arg75TRP mice for further comparison with humanized mouse expressing human CMT1X p.Arg75TRP mutation. Over the next year these samples will be analyzed by IHC and by RNA-seq.

We have initiated the process of getting the iPSCs from huma patients harboring the CMT1X causing mutation. We will get these cells from the iPSCs collection of the New York Stem Cell foundation. Over the next year we will differentiate these cells into promyelinating Schwann cells and use them to generate and characterize a humanized mouse with human Schwann cells harboring the CMT1X causing p.Arg75TRP mutation.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

- *"Nothing to Report."*

What was the impact on other disciplines?

- *"Nothing to Report."*

What was the impact on technology transfer?

- *"Nothing to Report."*

What was the impact on society beyond science and technology?

- *"Nothing to Report."*

CHANGES/PROBLEMS

This project continues as planned, and there are no planned changes in the scope and the approach of the project.

PRODUCTS:

"Nothing to Report."

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Benayahu Elbaz-Eilon
Project Role:	PI
Research Identifier:	0000-0001-5202-4598
Nearest person month Worked:	O.48
Contribution to the project:	Dr. Elbaz coordinates the project, performs some of the experiment, and analyzes the results.
Funding Support:	RO1 grant from the NIH

Name:	Charles Abrams
Project Role:	Collaborating PI
Research Identifier:	0000-0002-1272-9320
Nearest person month Worked:	0.24
Contribution to the project:	Dr. Abrams coordinates the project, performs some of the experiments, and analyze the results.
Funding Support:	

Name:	Tanya Klein
Project Role:	Research Technician
Research Identifier:	NA

Nearest person month Worked:	12
Contribution to the project:	Tanya maintains the mouse colony, performs the mouse injections, harvests tissues for IHC and for RNA-seq, and analyzes the data.
Funding Support:	

Name:	Mona Freidin
Project Role:	Post-Doctoral fellow
Research Identifier:	0000-0002-3162-4858
Nearest person month Worked:	2
Contribution to the project:	Dr. Freidin performs the tissue culture work associated with this project.
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

- **Organization Name:** University of Illinois at Chicago
- **Location of Organization:** Department of Neurology and Rehabilitation, University of Illinois at Chicago, Chicago, IL, USA.
- **Partner's contribution to the projec**t
  - **Collaboration**- This project is done in collaboration with Dr. Charles Abrams at University of Illinois, Chicago.