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TITLE: Identification of Receptors for TDP-43 That Mediate Cellular Uptake and Neurotoxicity

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CONTRACTING ORGANIZATION: Trustees of Boston University, BUMC, Boston, MA

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6. AUTHOR(S)

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5e. TASK NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this project is to 1) identify receptors at the cell surface that mediate entry of pathological TDP-43 protein aggregates into the cell and 2) determine the role of these receptors in the subsequently observed neurotoxicity of these TDP-43 protein aggregates. This will be accomplished through the use of two varied but complementary techniques: CRISPR interference screening and a novel microenvironment mapping technique (MicroMap). Top hits will be confirmed and further investigated using biochemical and cell biological techniques. During the period of time covered by this report, we have obtained a number of crucial reagents, established and/or made more efficient a number of required techniques, and sought regulatory approval for a subset of the required experiments. These efforts have put us in a position to more efficiently complete the experiments outlined in the approved Statement of Work.

15. SUBJECT TERMS

TDP-43, Neurodegeneration, Prion, Receptors, Neurotoxicity

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
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1. INTRODUCTION:

Work in recent years has uncovered the self-templated misfolding of disease causing proteins in a number of neurodegenerative diseases. These findings have earned these protein aggregates the designation of "prion-like", as this mechanism of spread has been appreciated in the prototypical prion diseases for more than two decades. Mounting evidence suggests that transactive response DNA-binding protein 43 (TDP-43), which forms pathological aggregates in many subsets of frontotemporal lobar degeneration (FTLD), also spreads from cell-to-cell in this way. Because TDP-43 aggregates are found intracellularly, there must be receptors at the cell surface that mediate entry of these protein assemblies into the cell. Further, these receptors may also be involved in neurotoxicity. This project aims to identify these receptor(s) through the use of two complimentary approaches: i) a CRISPRi screen will be used to identify gene products whose loss reduces a cells ability to uptake fluorescently labeled TDP-43 aggregates, ii) a novel microenvironment mapping technique (MicroMap), capable of labeling cellular factors in proximity to exogenously applied TDP-43 aggregates with an unprecedented resolution. Molecules confirmed to interact with and mediate TDP-43 uptake will be assessed for their role in neuronal toxicity using TDP-43 derived from the brains of FTLD patients. These findings will open the door to a wealth of future studies, providing novel targets for therapeutic intervention and a deeper understanding of the role played by the prion-like spread of TDP-43.

2. KEYWORDS:

TDP-43, Neurodegeneration, Prion, Receptors, Neurotoxicity

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Specific Aim 1: Identification of candidate cellular receptors for pathological TDP-43	**Timeline Months (proposed)**	**% Completion**
Major Task 1: Preparation of labeled TDP-43 aggregates		
Expression of recombinant protein	2	100
Labeling with fluorescent dye and photocatalyst	1	10
Aggregation and characterization of protein populations	0.5	25
Major Task 2: Identification of cellular receptors		
CRISPRi screen/FACS analysis	2-4	10
Next generation sequencing/analysis	1	0
Microenvironment mapping/analysis	3	20
Specific Aim 2: Validation of the role of candidate receptors in the uptake of, and neurotoxicity induced by, TDP-43 aggregates		
Major Task 1: Genetic manipulation of cells		
CRISPR/Cas9 mediated gene disruption of H4, SH-SY5Y and human iPSC	2	0
Lentiviral mediated over expression of hits	2	0
FACS analysis	2	0
Major Task 2: Biochemical assays		
Pull down and analysis of candidate interactors with	2	0

recombinant TDP-43 aggregates		
Major Task 3: Neurotoxicity assays		
Enrichment of TDP-43 aggregates from patient samples	2	0
Differentiation of iPSC to cortical neurons	9	0
Confocal microscopy	3	0

- **What was accomplished under these goals?**

  Over the period covered by this report:

  1. We have successfully expressed recombinant TDP-43 and are prepared to begin labeling it with Alexa Fluor 488, a fluorescent dye, and a photocatalyst. Full-length TDP-43 is enriched following bacterial expression through size exclusion chromatography; dialysis is used for buffer exchange and to remove unbound dye. The Photocatalyst is covalently linked to functionalized protein through CLICK chemistry. We have established a relationship with The Structural Electron Microscope Facility at Boston University Medical School, and have already characterized the ultrastructure of fibrils of Aβ, α-synuclein, and the prion protein. We will perform these same characterizations using recombinant TDP-43 aggregates (**Aim 1: Major task 1**).

     - The preparation and thorough characterization of this reagent is crucial for the CRISPRi screen (Alexa Fluor 488) and micromapping experiments (**Aim 1: Major Task 2**) as well as subsequent validation of identified receptor molecules (**Aim 2: Major Task 1 & 2**).

  2. We have established the correct conditions for FACS analysis, which will allow us to isolate TDP-43 uptake deficient cell populations for subsequent identification of the expressed sgRNAs (**Aim 1: Major Task 2**).

  3. Significant progress has been made in the adaptation of the MicroMap technique, originally developed using cells grown in suspension (lymphocytes), for use with adherent cells (**Aim 1: Major Task 2**). We have successfully used this technique to identify interactors of other neurodegenerative disease-causing proteins, namely Aβ oligomers and the cellular prion protein. These experiments have been carried out using immortalized mouse cell lines and primary neuronal cultures, demonstrating the amenability of this method for use with adherent cells. We are primed to begin these experiments with TDP-43 in the near future.

  4. We have significantly decreased the time required to achieve gene knockout using CRISPR/Cas9 by adopting a methodology whereby ribonucleoprotein particles (RNPs) formed between recombinant Cas9 and chemically stabilized sgRNAs are delivered to cells through electroporation. This will significantly increase the speed of hit confirmation (**Aim 2: Major Task 1**)

  5. We have recently adopted a new method of differentiating iPSC into cortical neurons that utilizes the direct overexpression of neurogenin-2 (a transcription factor directing neuronal fate) using a piggyBac transposase system. This approach allows us to differentiate iPSC into mature neurons in one-third of the time of traditional methods using growth factors. This will significantly hasten the time required to perform the outlined neurotoxicity assays (**Aim 2: Major Task 3**)

  6. We have received approval from the Animal Care and Use Review Office (ACURO) to perform experiments using primary neuronal cultures obtained from mouse pups (**Aim 2: Major Task 3**).

7. We have obtained a designation of “not human research” from the Boston University Institutional Review Board and have submitted these documents to the Human Research Protection Office (HRPO) for review (**Aim 2: Major Task 3**).

Stated goals not met:

We are behind on the timelines outlined in the approved SOW (Timeline months (proposed) in the above table). The realities of performing research during the COVID-19 pandemic were not fully appreciated at the time of submission of the grant. The change in months worked towards these aims by Robert C.C. Mercer, combined with additional research staff who act in a supporting role funded from other sources (or potentially this grant; see below), is predicted to result in a significant increase in productivity towards achieving the goals outlined in the SOW.

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

During the period covered by this report:

Robert C.C. Mercer has gained additional proficiency in techniques used to enrich protein populations, namely in Fast Protein Liquid Chromatography (FPLC), and has deepened his knowledge of TDP-43 biology through individual study.

- **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?**

The recent relaxation of pandemic-related restrictions and an increase in time dedicated to this project by Robert C.C. Mercer will have a major impact on the completion time of the aims outlined in the SOW. The new techniques that we have adopted will also have a positive impact on the speed at which these experiments will be completed. The possibility of adding a portion of a technician's salary to the budget will be discussed with the assigned science officer (Kathryn J Argue, PhD).

4. **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

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
  Nothing to Report

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  Nothing to Report

- **Changes that had a significant impact on expenditures**
  Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  Nothing to Report

- **Significant changes in use or care of human subjects**

Nothing to Report

- **Significant changes in use or care of vertebrate animals**
  Nothing to Report

- **Significant changes in use of biohazards and/or select agents**
  Nothing to Report

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**

     1. **Journal publications.**
        Nothing to Report

     2. **Books or other non-periodical, one-time publications**
        Nothing to Report

     3. **Other publications, conference papers, and presentations.**
        Nothing to Report

   - **Website(s) or other Internet site(s)**
     Nothing to Report

   - **Technologies or techniques**
     Nothing to Report

   - **Inventions, patent applications, and/or licenses**
     Nothing to Report

   - **Other Products**
     Nothing to Report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name:	*Robert Mercer*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	0000-0001-6029-511X
Nearest person month worked:	6
Contribution to Project:	*Dr. Mercer has overseen the project and prepared the reagents required.*
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?**

     For W81XWH-21-1-0141:

     - Robert C.C. Mercer had previously been supported at 50% salary/year and has changed to 99% salary support/year

    - Andrew Emili had previously been supported at 3% salary/year but will be removed from support as of 3/31/2022

- **What other organizations were involved as partners?**
  Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS**
   None

9. **APPENDICES:**
   None