Final Report

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Date: 8-11- 2019

Title of Project: *A pre-clinical model of human iPSC-derived neurons to reveal neurobiological mechanisms of Parkinson's disease*

***Summary**: (approximately 1,000 words)*
Our aim was "to generate an experimental platform in vitro to study the neurobiology of familial and sporadic Parkinson's disease with patient-derived neurons, and screen the efficacy of prospective treatments before clinical trials". We have successfully established such platform, with the support of this grant. We have thoroughly validated the quality of the reprogram human neuronal tissue with Patch-clamping analysis, calcium imaging, transcriptomics and imaging assays. We are currently using this platform in multiple projects all aiming to improve treatments of neurological disorders.

Some of our work supported by this grant has been or will be reported in scientific articles (two published, two in revision, three in final preparation for submission):

Van den Hurk M, Bardy C: Single-cell multimodal transcriptomics to study neuronal diversity in human stem cell-derived brain tissue and organoid models Journal of Neuroscience Methods 325: 108350, 2019
ABSTRACT: Advances in human cell reprogramming and induced pluripotent stem cell technologies generate tremendous potential for neuroscience studies in health and disease, while the neuroscientist toolbox for engineering a range of brain tissues and neuronal cell types is rapidly expanding. Here, we discuss how the emergence of new single- cell genomics methods may help benchmarking and optimizing the tissue engineering process. The inherent heterogeneity and variability of reprogrammed brain tissue may conceal important disease mechanisms if not accounted for by rigorous experimental design. Single-cell genomics methods may address this technical chal- lenge and ultimately improve the development of new therapeutics for neurological and psychiatric disorders.

Van den Hurk M, Erwin JA, Yeo GW, Gage FH, Bardy C: Patch-Seq Protocol to Analyze the Electrophysiology, Morphology and Transcriptome of Whole Single Neurons Derived From Human Pluripotent Stem Cells Front Mol Neurosci 11: 376, 2018
ABSTRACT: The human brain is composed of a complex assembly of about 171 billion heterogeneous cellular units (86 billion neurons and 85 billion non-neuronal glia cells). A comprehensive description of brain cells is necessary to understand the nervous system in health and disease. Recently, advances in genomics have permitted the accurate analysis of the full transcriptome of single cells (scRNA-seq). We have built upon such technical progress to combine scRNA-seq with patch-clamping electrophysiological recording and morphological analysis of single human

neurons in vitro. This new powerful method, referred to as Patch-seq, enables a thorough, multimodal profiling of neurons and permits us to expose the links between functional properties, morphology, and gene expression. Here, we present a detailed Patch-seq protocol for isolating single neurons from in vitro neuronal cultures. We have validated the Patch-seq whole-transcriptome profiling method with human neurons generated from embryonic and induced pluripotent stem cells (ESCs/iPSCs) derived from healthy subjects, but the procedure may be applied to any kind of cell type in vitro. Patch-seq may be used on neurons in vitro to profile cell types and states in depth to unravel the human molecular basis of neuronal diversity and investigate the cellular mechanisms underlying brain disorders.

Tran J, Anastacio H, Bardy C: Genetic predispositions of Parkinson's disease revealed in patient-derived brain cells.
ABSTRACT: Parkinson's disease (PD) is the second most prevalent neurological disorder and has been the focus of intense investigations to understand its etiology and progression, but it still lacks a cure. Modeling diseases of the central nervous system in vitro with human induced pluripotent stem cells (hiPSC) is still in its infancy but has the potential to expedite the discovery and validation of new treatments. Here we discuss the interplay between genetic predispositions in people living with PD and iPSC-derived brain cell functions. We first summarize the prevalence of causal Parkinson's genes and risk factors reported in 74 epidemiological and genomic studies. We then present a meta-analysis of 287 hiPSC-derived neuronal lines from 51 recent independent original research articles, which point towards specific impairments in neurons from Parkinson's patients, within the context of genetic predispositions. Despite the heterogeneous nature of the disease, current iPSC models reveal converging molecular pathways underlying neurodegeneration in a range of familial and sporadic forms of Parkinson's disease. Altogether, consolidating our understanding of robust cellular phenotypes across genetic cohorts of Parkinson's patients may guide future personalized drug screens in pre-clinical research. *In revision for publication.*

Shani Stern, Andreea Manole, Shong Lau, Kile Mangan, Simon Schafer, Sara Linker, Krishna Vadodaria, Ana Mendes Diniz, Thao Nguyen, Aidan Aicher, Alexis Brice, Juergen Winkler, Beatriz C. Freitas, Eugenia Jones, Cedric Bardy, Carol M. Marchetto, Fred H. Gage: Reduced synaptic transmission is an early mark of alpha synuclein-associated Parkinson's disease in dopaminergic neurons derived from human subjects. *In revision for publication.*
ABSTRACT: α-Synuclein mutations cause a severe, highly penetrant and dominantly inherited form of Parkinson's disease (PD). Patients show motor deficits as early as in their 30s, with a very rapid deterioration. Inducing these mutations in mice and rats causes an almost immediate change in synaptic transmission that is followed many weeks later by the main phenotype of PD, motor deficits. Induced pluripotent stem cell (iPSC) technology allows for the study of a large number of juvenile disorders but is considered less useful for studying neurodegenerative diseases because it erases epigenetic modifications and produces rejuvenated, young neurons. However, here we show that iPSC-derived dopaminergic neurons from patients with mutations and copy number variations of their SNCA gene show a very early phenotype of reduced synaptic transmission. These neurons also exhibit misfolded protein aggregates that are trapped in the soma, as well as swollen and dystrophic neurites. RNA sequencing reveals changes in expression of pre- and post-synaptic proteins and a reduction in

the expression of HSF1, along with changes in many other genes related to PD. These findings of alterations in dopaminergic young neurons imply that some forms of PD can and should be studied in young human-derived neurons. The neurodegeneration process can be followed step by step in these models to recapitulate the early changes that occur in PD patients, likely long before they are diagnosed.

Hypothesis vs Findings

Our findings so far support the overarching Hypothesis proposed: “Distinct genotypes of sPD and fPD patients converge to common downstream molecular perturbations. In turn, such cell-autonomous factors cause abnormal neuronal phenotypes, and elevate the probability of cell death.”

Unanswered Questions

We are finishing the RNAseq analysis of iPSC-redrived neurons from Parkinson’s patients and matched controls. We have collected PatchSeq data from PD and control cell lines, we have also obtained bulk RNAseq data from our collaborators overseas (Fred H Gage). We are finishing to prepare this work for publication.

What these research outcomes mean

These research outcomes are important to better understand the dysfunction of human brain cells in the context of neurological disorders and in particular Parkinson’s disease. Although, much more work is needed to reach our ultimate objective of finding a cure for Parkinson’s, we believe that these research outcomes contribute to this goal.

We have also filed a new provisional patent in which we describe a new method to improve the long-term culture of human brain cells in vitro. When commercialised these new product will benefits other researchers to improve neurological models in vitro to study a range of brain disorders.

Please include any appropriate photos or diagrams.

Please see full list of publications http://www.bardylab.com/publications.html