Developing Tools to Discover New Therapeutic Agents

Roger A. Ashmus Laboratory of Chemical Glycobiology Simon Fraser University

Parkinson Society British Columbia Webinar 2019-July-16

First part: develop probe for live cell imaging of GCase



Second part: use probe for drug discovery



Images modified from Castaldi Annual Rep. Med. Chem. 2017, 50, 335.

Key researchers for the work presented:



Dr. Samy Cecioni University of Montreal



Dr. Christina Gros MSFHR/PSBC Trainee



Matthew Deen

Classical hallmarks of PD





Loss of dark pigmentation in substantia nigra

Presence of protein aggregates (Lewy bodies) in substantia nigra

Summary of some PD-related genes discovered

Genetic Discovery to Therapeutic Target

By J. Follet and E. Gustavsson, UBC

https://www.youtube.com/watch?v=Z65bI2KvJnM&feature=youtu.be



Genes encode for proteins/enzymes

PD is complex and why researchers focus on α Syn



Cherubini and Wade-Martins Cell Tissue Res. 2018, 373, 79-90.

Lysosomes are important for removing waste



Mutations in *GBA1*: significant genetic risk factor for Parkinson Disease (PD)

The NEW ENGLAND JOURNAL of MEDICINE

N ENGLJ MED 361;17 NEJM.ORG OCTOBER 22, 2009

ORIGINAL ARTICLE

Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease

E. Sidransky, M.A. Nalls, J.O. Aasly, J. Aharon-Peretz, G. Annesi, E.R. Barbosa,
A. Bar-Shira, D. Berg, J. Bras, A. Brice, C.-M. Chen, L.N. Clark, C. Condroyer,
E.V. De Marco, A. Dürr, M.J. Eblan, S. Fahn, M.J. Farrer, H.-C. Fung,

Carrier risk factor for PD (5-10 fold):

Earlier age of onset and greater severity

GBA1 encodes for the enzyme Glucocerebrosidase (GCase)

GCase reduction increases αSyn in cells

GCase knockdown leads to reduction in GCase levels and decreased activity



Decreased GCase activity leads to increased α Syn levels



Mazzuli et al. Cell 2011, 146, 37.

GCase reduction and inhibition increases αSyn in mouse models

Gaucher disease (GD) \rightarrow GCase dysfunction (little to no enzyme activity)



Inhibitor induced GCase mouse model



4L, no-CBE

4L, 24 CBE injections



4L, 36 CBE injections

4L, 24 CBE injections

Postmortem analysis of GD-associated brain lysate show elevated toxic αSyn levels



Increasing GCase activity could prevent or delay onset of PD



GCase is a lysosomal glycoside hydrolase that hydrolyzes glucosylceramide



How to monitor GCase activity for cells?

Common protocol for measuring GCase activity is performed on tissue lysate is limiting



Solution: Develop biochemical tool to monitor GCase activity in live cells



Benefits:

- Direct readout (no lysis or dilution step)
- Native cellular environment



Live Cell Imaging

Idea: Use fluorescence-quenched (FQ) probe concept to monitor GCase in cells



GBA-FQ1 detects GCase in patient fibroblasts, but...







Yadav et al. J. Am. Chem. Soc. 2015, 137, 1181-1189

Synthetic effort toward building better FQ probes





Is new FQ probe design appropriate for GCase?



Synthesis of GBA-FQ4



Synthetic effort toward building better FQ probes



GBA-FQ5 presents imaging problems



Still images from video



Issue: Fluorophore is rapidly diffusing outside the lysosomes and cells



GBA-FQ6 seems suitable for live cell imaging



Still images from video



Control: Colocalization experiments shows GCase activity is within lysosomes



Control: Inhibition shows GCase is targeted enzyme

Inhibitor: AT3375



Provisional Patent Application

Development of probe summary

 Successfully developed a fluorescence-quenched probe for monitoring GCase activity within the lysosome of live cells



GBA-FQ6

Recently developed an even better FQ probe



Provisional Patent Application

GBA1 Probes in High-Throughput Screen for Drug Discovery

Drug discovery general approaches





• Rational design of small molecules



- Screening campaign
 - Library of >5000 compounds and extracts usually from a natural source

Drug Discovery Process Overview



Drug Discovery Process - basic research overview

Basic Research	Drug Discovery	Preclinical
Target Identification	Probe Development	HTS Assay Development

In vitro



1 Protein/Enzyme

VS



GCase







>20k Proteins/Enzymes Cellular compartments

Drug Discovery Process – assay development

Basic Research	Drug Discovery	Preclinical
Target Identification	Probe Development	HTS Assay Development
со	ntrol compounds fo	or screen control
384 well plate		33

Drug Discovery Process – begin live cell screening

Basic Research	Drug Discovery	Preclinical
Screening	Lead Identification	Lead Optimization
HOUGH CURRENT CONTRACTOR CONTRACT		

~70,000 compounds

* 0000000000000000000000000000000000000
000000000000000000000000000000000000000
+ 000000000000000000000000000000000000
* 000000000000000000000000000000000000
(* * *********************************

214 x 384 well plates

Workflow of GCase live cell screening assay



MJFF - 16536: High content live cell image-based screening and validation of GCase enhancers

Screening is automated by High Throughput **Chemical Biology (HTCB) screening facility**



Fully automated: Cell culture \rightarrow Microscopy \rightarrow Data analysis / Handling

CORE PLATFORM:

Thermo [™] Momentum Integration/Scheduling DFMZ, Inc. Full HEPA-Filtered Enclosure University of Michigan, MScreen LIMS Software

PLATE HANDLING:

Thermo[™] Spinnaker[™] 4-Axis SCARA-type Robot Thermo[™] 120 (8x15) Slot Plate Storage Carousel Agilent[™] VSpin Microplate Centrifuge Brooks XPeel® Automated Microplate Seal Removal Agilent[™] PlateLoc Thermal Microplate Sealer Thermo[™] Cytomat 2C4 Automated Cell Incubator

LIQUID HANDLING:

Tecan[™] Freedom EVO® 100 Liquid Handler MultiChannel Arm[™] (MCA) [96 channel head / 384 pintool] Robotic Manipulator Arm (RoMa) BioShake Module / Pin Dryer Module BioTek[™] EL406 Washer Dispenser **Thermo[™]** Multidrop[™] Combi Reagent Dispenser Thermo[™] Multidrop[™] Combi Reagent Dispenser

PLATE READING:

Molecular Devices ImageXpress® Micro XLS High-Content Microscope Molecular Devices[™] SpectraMax® i3x Platereader 36

HTS facility in action

Screening facility in action: <u>https://www.sfu-htcb.ca/</u>

Still images from video







What we are looking for in screen...

Basic Research	Drug Discovery	Preclinical
Screening	Lead Identification	Lead Optimization
μμμ <td< th=""><th>DMSO 1% (100%)</th><th>Inhibitors (chaperones)</th></td<>	DMSO 1% (100%)	Inhibitors (chaperones)
214 x 384 well plates	Control DMSO 1% (100%)	Representative example Activators

Live cell GCase screening campaign: data analysis

Current data set: 208 plates (6 plates failed) Current data analysis ongoing: – 20K compounds analyzed



HTS sample screen for 'activators'



Future work:



Summary of Webinar

- Hope our research can lead to the discovery of new compounds and/or discovery of new protein targets for PD therapy
- Provide some new information about PD
- Share some of the ongoing research being conducted to target PD
 - Focusing on GCase as a therapeutic target



Acknowledgments



Prof. David Vocadlo

Canada Research Chair, Chem. Biol. Prof., Dept. of Chemistry Prof., Dept. Mol. Biol. & Biochem. Co-Director, HTCB Centre CSO, Alectos Therapeutics Technicians/Staff

- David Shen
- Zarina Madden
- Cameron Proceviat

Students

- Hong-Yee Tan
- Matt Alteen
- Matt Deen (co)
- Jessica Miller (co)
- Jesus Serrano
- Peter Sidhu (co)
- Viktor Holicek
- Ben Tiet
- Jil Busman
- Hanna Beverly
- Helana Ryan

PDFs/Research Associates

- Dr. Xiaoyang Shan
- Dr. Yanping Zhu
- Dr. Tai-Wei Liu
- Dr. Sandeep Bhosale (co)
- Dr. Dustin King
- Dr. Christina Gros
- Dr. Sachin Kandalkar (co)
- Dr. Sha Zhu
- Dr. Omid Tavassoly
- Dr. Daniela Salas (co)
- Dr. Pierre Gilormini

Previous Group Members

- Dr. Samy Cecioni (UofM)
- Evan Perley-Robertson









Information:

General consensus for PD prevention: Exercise, Healthy diet, and Sleep

Want to help?

Online resources to participate:

<u>https://www.parkinson.bc.ca/resources-services/</u> <u>http://www.can.ubc.ca/the-patient-factor/patient-participation/</u> Vocadlo Lab is seeking blood donors to test a new method for measuring GCase activity in peripheral blood mononuclear cells

Contact me: rashmus@sfu.ca

Information on ketogenic diet and intermittent fasting for PD: <u>https://www.michaeljfox.org/news/ask-md-whats-best-diet-parkinsons</u> <u>https://www.michaeljfox.org/grant/effect-dietary-ketosis-alpha-synuclein-accumulation?grant_id=1385</u>