The Drug Test for Neuron Degenerative Disease

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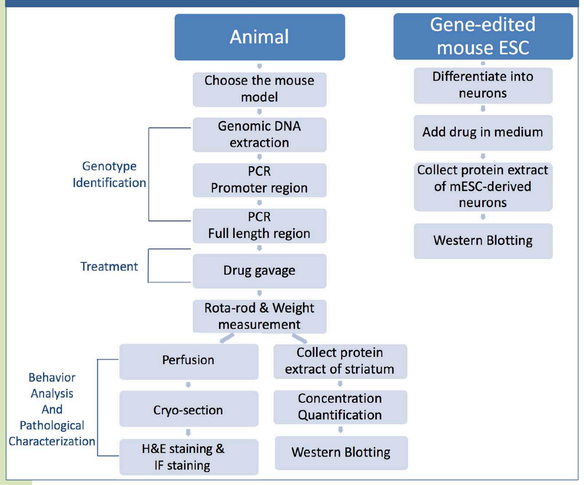
Introduction

Neuron degenerative diseases (NDDs) are a miscellaneous group of disorders, which have been characterized by the progressive breakdown in many aspects of the coordination in the CNS and PNS. The NDDs worldwide such as Alzheimer's disease and Parkinson's disease resulted from a variety of progressive degeneration or death of neurons are presumed as long-term disorders, have mental and physical issues, and are incurable nowadays.

The current circumstance shows the desperation and possibility of prevent or curing therapeutics for NDDs. Here, we undergo a series of experiments to test the function of a specific drug on several NDDs, and discover the pharmacological mechanisms and cross-interaction might be different in distinct NDDs. With the promising future, we aims to confront the growing challenge posed by NDDs, cooperating researchers and gathering existing research evidence to investigate the key research questions and barriers to progress in this area.

Outline



Results

Figure 2.

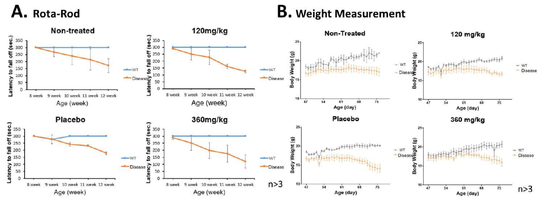


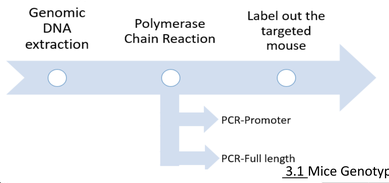
Figure 2. Physical condition of motility and weight of mice. (Conducted by Jeremy)
A. Analysis of rota-rod test. These mice were performed rota-rod test once a week from the age of 8 weeks to 12 weeks. The mice were separated into 4 groups, non-treated control, placebo control, 120 mg/kg and 360 mg/kg dosage of drug, respectively. The result indicated NDDs mice (DIII) is no improvement in the dosage of 120 mg/kg comparing to non-treated. Moreover, 360 mg/kg dosage of drug apparently performed even worse than 120 mg/kg dosage of drug. **B.** Body weight measurement. These mice were weight daily from the age of 8 weeks to 12 weeks. The mice were separated into 4 groups, non-treated control, placebo control, 120 mg/kg and 360 mg/kg dosage of drug, respectively. The result indicates that the body weight of DIII mice were treated by drug which were lighter than the wild-type. n>3 for each test.

Results

Figure 1.

A.

(a) Procedure of Genotype Identification



(b)

1 Reagents

a. Tail digestion buffer
—1M Tris HCl (pH8)
—0.5M EDTA (pH8)
—20% SDS
—5M NaCl
—ddH2O
—Proteinase K

b. 5M Potassium acetate

2 PCR-Primer Design

a. Primer length
—15-30 nucleotide bases
b. Optimal G-C content
—range between 40-60%
c. 3' end of primer
—contain a G or C
—include a plus strand primer and a minus strand primer, should not be complementary to each other
d. Melting temperatures for primers
—45-65 ˚C
e. Di-nucleotide repeats or single base runs should be avoided

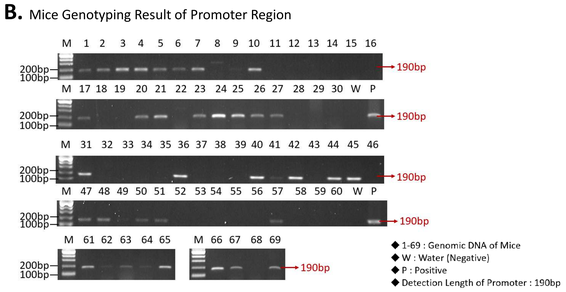
3.1 Mice Genotyping of Promoter

Primer	Primer Forward
	Primer Reverse
Enzymes	2X Dream Taq
Template	95˚C 5'
	95˚C 40"
	65˚C 30"
	72˚C 30"
	31 cycles
	70˚C 7'
	10˚C 10'

3.2 Mice Genotyping of Full Length Fragment

Primer	Primer Forward
	Primer Reverse
Enzymes	DNA Polymerase Taq
Template	95˚C 5'
	95˚C 1'
	65˚C 1'
	72˚C 1' 30"
	39 cycles
	72˚C 7'
	10˚C 10'
Other working reagents	Taq polymerase buffer 10X
	$MgCl_2$
	Betaine
	dNTP

B. Mice Genotyping Result of Promoter Region



C. Mice Genotyping Result of Full Length Fragment

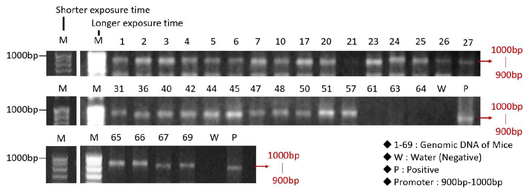


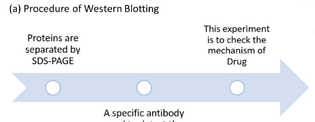
Figure 1. Identification of NDDs mice
A. Materials and methods of genotyping PCR. (a) Disease Mouse Model Identification is presented by flowchart. (b) 1 The reagents of genomic DNA (gDNA) extraction is for lysising 0.5 centimeter mouse toes and tails. (b) 2 Primer Design condition is for avoiding formation of primer dimers and hairpin loop structures. (b) 3.1 Mice Genotyping replicated specific DNA on promoter region. (b) 3.2 The template of mice genotyping detected full length fragment of mutant genes. **B.** The result of promoter region PCR. Note there are 34 mice carrying the promoter of the mutant gene, including 1,2,3,4,5,6,7,10,17,20,21,23,24,25,26,27,31,36,40,42,44,45,47,48, 50,51,57,61,63,64,65,66,67 and 69. **C.** Genotyping PCR of full length fragments. The gDNA samples were referred from these 34 candidates in B.

Results

Figure 3.

A.

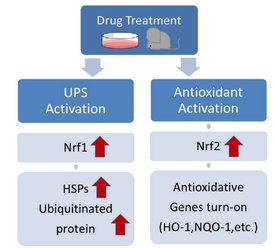
(a) Procedure of Western Blotting



(b) Reagents of Protein Extraction Preparation

1 Chaotropic agents
Urea/thiourea
Disrupting hydrogen bonding between amino acids
Detergents
The hydrophobic end binds to the hydrophobic portions of proteins
2 Protease inhibitors
—Avoidance of Proteases released after lysis resulting in target protein degradation
3 Reductant
—Disrupt disulfide bonds so the proteins become monomeric form

B. Known of Molecular Mechanism for the drug



C. Western Blotting Result of protein extract from striatum of DIII mice

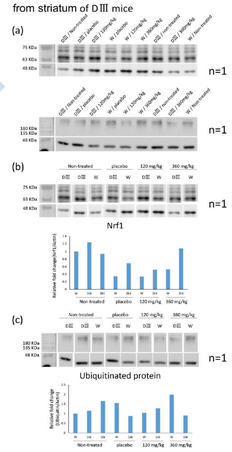


Figure 3. The expression level change of the drug's target protein in the striatum of DIII mice
A. (a) Procedure of Western Blot. (b) The reagents of Protein Extraction Preparation. **B.** Known of the molecular mechanism for the drug. Two signal pathways could be affected by the drug : the ubiquitin–proteasome system (UPS) and the antioxidant pathway. The drug will activate Nrf1 (Nuclear respiratory factor 1) which regulates several HSPs genes turn-on, and further increase degradation of misfolded proteins by ubiquitinoylation. The antioxidant pathway has been shown to be induced by the drug via up-regulation of Nrf2, which plays a role in mediating genes of antioxidant enzymes, such as HO-1, Nqo1, Gclc and catalase. **C.** (a) Western Blotting Result of protein extract from striatum of DIII mice. n=1. (b) (c) is rearranged panel by grouped into the same treatment as , non-treated, placebo, 120 mg/kg and 360 mg/kg dosage of drug respectively. DIII is our mice disease model, and W is the wild type of disease mice strain. (Conducted by Jeremy)

Summary

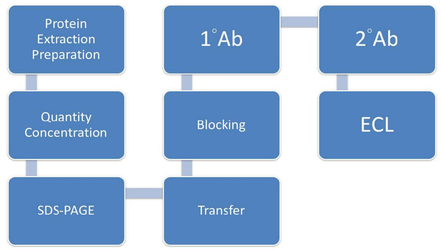
1. I learned that genotyping PCR is an excellent fundamental tool for various applications, such as identification of gene mutation, paternity test, detection of microbes, etc.
2. Although the drug has been reported it might rescue another kind of NDD, it shows no significant improvement in behavior tests of our NDD mouse models (DIII) with the same or higher dosage treatments.
3. The fold changes of the drug's target protein (e.g. Nrf1) increased after drug treatment indicating the activity of this drug is normal; however the expression level of down stream proteins (e.g. ubiquitinated protein) is varied in DIII mice. It might imply the reason why there were no improvement in DIII mice after drug treatment.
4. The Nrf1 protein expression is decreased in DI and DII cell models indicating that the molecular mechanism of the drug might be different in different cell-types / NDD mouse models.

Conclusion & Discussion

The results of this drug treatment indicate that the drug might be not curable for our NDD models. This is different from our expected result; however, the repeat number is only one so that we need more experimental repeats to validate data's reproducibility. Although the treatment of NDDs often encounters many obstacles, we must maintain a positive attitude and apply the knowledge and technology. One day, neuron degenerative diseases will be cured and bring the perspective of neuron research to the next level.

Figure 4.

A Western Blot Flowchart



B Quantification of Protein Extract from mESC-derived neurons

sample title	Conc.(ug/ul)	sample volume(ul)	lysis buffer(ul)	5x SB(ul)	total volume(ul)	final loading Conc.(ug/ul)
WT-mock	7.74	19.4	20.6	10	50	3
WT-drug	6.38	23.5	16.5	10	50	3
Disease I -mock	5.55	27	13	10	50	3
Disease I -drug	4.94	30.3	9.7	10	50	3
Disease-IImock	4.94	30.4	9.6	10	50	3
DiseaseII-drug	4.69	32.1	7.9	10	50	3

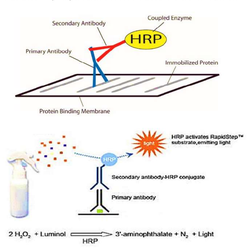
C

(a)SDS-PAGE

Stacking gel	
Solution component	Component volume of 3mL
5%	
H2O	2.100
30% acrylamide	0.500
1.0M Tris pH6.8	0.380
10% SDS	0.030
10% APS	0.030
TEMED	0.003

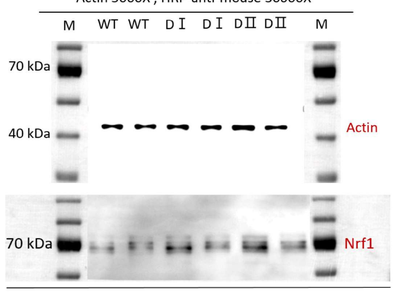
Seperative gel	
Solution component	Component volume of 20mL
10%	
H2O	7.900
30% acrylamide	6.700
1.5M Tris pH8.8	5.000
10% SDS	0.200
10% APS	0.200
TEMED	0.008

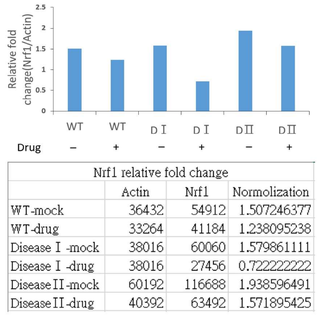
(b) Antibody hybridization and ECL reaction



1˚Ab	2˚Ab
Mouse- α Actin	HRP-anti-mouse
Mouse- α Ubiquitin	HRP-anti-mouse
Rabbit- α Nrf 1	HRP-anti-rabbit

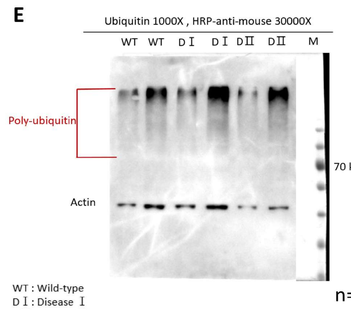
D

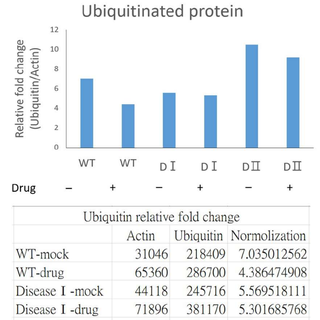




Nrf1 relative fold change			
	Actin	Nrf1	Normolization
WT-mock	36432	54912	1.507246377
WT-drug	33264	41184	1.238095238
Disease I -mock	38016	60060	1.579861111
Disease I -drug	38016	27456	0.722222222
Disease II-mock	60192	116688	1.938596491
Disease II-drug	40392	63492	1.571895425

E





Ubiquitin relative fold change			
	Actin	Ubiquitin	Normolization
WT-mock	31046	218409	7.035012562
WT-drug	65360	286700	4.386474908
Disease I -mock	44118	245716	5.569518111
Disease I -drug	71896	381170	5.301685768
Disease II -mock	22059	231334	10.48705744
Disease II -drug	35948	328624	9.141649049

Figure 4. The protein level change of mESC-derived neuron after drug treatment
A. Western Blot flowchart. **B.** Quantification of protein extract from mESC-derived neurons carrying different gene mutations with/without drug treatment. **C.** (a) The recipe of stacking and seperative gel in SDS-PAGE. This protein were separated by their molecular weight. (b) Antibody hybridization and ECL reaction. The primary antibodies and the secondary antibodies as shown in the lower chart. The interaction between antibodies and ECL are shown in the figure. **D.** Western Blotting result of Nrf1. n=1. M : marker, WT : wild-type, D I : Disease I and D II : Disease II . The western blotting data was shown as the left panel. The molecular weight were labeled by marker. Actin is internal control. The statistic data was presented on the right upper panel. The Nrf1 relative fold change was calculated by Alphalmager 2200. **E.** Western Blotting result of Ubiquitin. n=1. M : marker, WT : wild-type, D I : Disease I and D II : Disease II . The western blotting data was shown as the left figure. The molecular weight were labeled by marker. The statistic data was presented on the right upper panel. Relative fold change of relative fold change was calculated by Alphalmager 2200 and normalized by actin.