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Profiling Changes in Mouse Brain Protein Abundance in a Neurotoxin-Induced Parkinson's Disease Model

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Comparative analyses of four regions of mouse brain

Overview

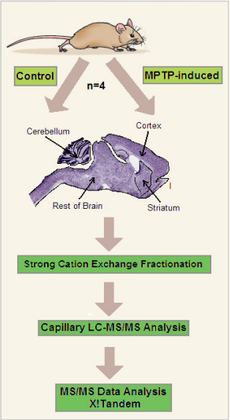
- Neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced Parkinson's Disease (PD) Mouse Model
- Four regions of mouse brain: striatum, cortex, cerebellum, and the rest of the brain region were pooled from four mice for each condition
- Global profiling of proteomic changes using LC/LC-MS/MS
- Extensive proteome coverage achieved with ~4000 proteins identified in each region
- MPTP-induced pathways such as mitochondrial dysfunction, apoptosis, and dopamine receptor pathway were revealed

Introduction

- Parkinson's disease (PD) is a chronic, progressive, degenerative disorder of the central nervous system.
- MPTP is an exogenous neurotoxin that induces PD in mice, humans, and various animals, but the mechanism underlying PD is not well understood.
- Proteomics is attractive for investigating this mechanism, as it directly measures the abundances of gene products including post-translational modification rather than gene transcripts that may or may not be translated into proteins.
- We compared proteomic profiles of four regions of mouse brain generated from 2D LC-MS/MS analyses of samples obtained from untreated mice and mice treated with MPTP. Protein abundance changes as well as changes in methionine (Met) oxidation were evaluated.

Methods

Strategy



Experimental

- **Mouse brain samples**: C57BL/6J mice received four intraperitoneal injections of MPTP-HCl in saline (15 mg/kg) at 2 h intervals; control mice received saline only. Six days later, 4 control and 4 MPTP-mice were sacrificed.
- **Sample preparation:** All samples were homogenized and digested with trypsin under identical conditions, and then further fractionated into 25 fractions by strong cation exchange (SCX) chromatography (Polysulfoethyl A 200 mm x 2.1 mm (5 µm, 300 Å) column).
- **LC-MS/MS analysis:** Each fraction was analyzed by an automated capillary HPLC system coupled online with an LTQ ion trap mass spectrometer. Column: 75 µm id x 65 cm capillary packed with 3 µm Jupiter C18.
- **Data analysis**: The MS/MS data were searched using X!Tandem against the mouse International Protein Index database.

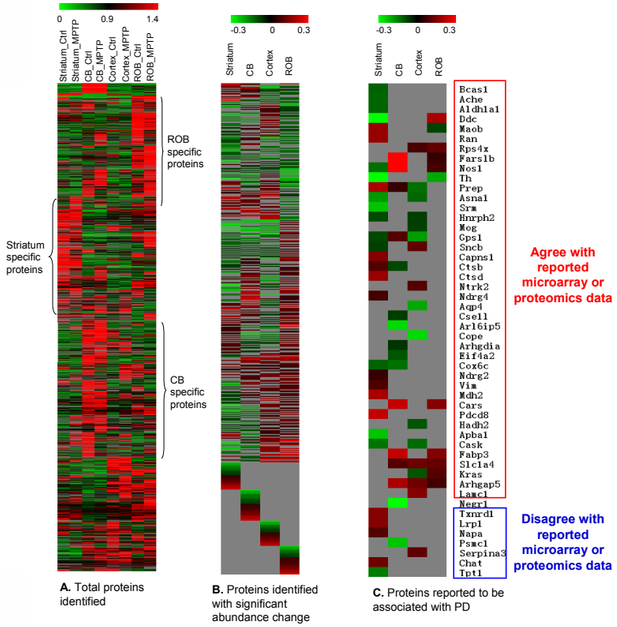
Results

Proteome Coverage from 2D LC-MS/MS

Table 1. Number of different peptide and protein identifications from four brain regions

	Unique peptides	Unique proteins	Proteins with significant changes in abundance	Unique Met-modified peptides
Striatum (S)	24,646	4161	366	1077
Cerebellum (CB)	24,791	4761	350	1684
Cortex	23,658	4377	340	1575
Rest of Brain (ROB)	26,557	4754	392	1972

Protein Abundance Changes



A. Total proteins identified

B. Proteins identified with significant abundance change

C. Proteins reported to be associated with PD

Figure 1. Heat map of identified proteins based on observation peptide counts. **A)** Protein vs. peptide counts/average of peptide counts; **B and C)** Proteins vs. Log2 ratio of [peptide counts of MPTP]/[peptide counts of Ctrl] for each protein

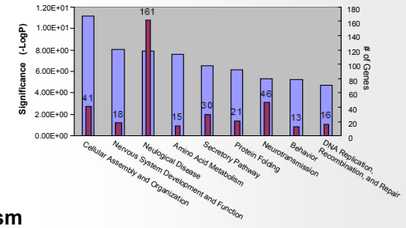
Protein Function Analysis

Table 2. Observation peptide counts of selective proteins (Ctrl/MPTP)

Protein name	Gene Code	Striatum	CB	Cortex	ROB	Protein Function
TYROSINE 3-MONOOXYGENASE	Th	17/2			4/1	Synthesis of Dopamine
AROMATIC-L-AMINO-ACID DECARBOXYLASE	Ddc	11/1			1/4	Synthesis of Dopamine
AMINE OXIDASE [FLAVIN-CONTAINING] B	Maob	2/7			7/4	Catabolism of Dopamine
RETINAL DEHYDROGENASE 1	Aldh1a1	16/7				Amino acid metabolism
ISOFORM N-NOS-1 OF NITRIC-OXIDE SYNTHASE, BRAIN	NOS1	5/2	1/9		5/9	Amino acid metabolism
CRL-1722 L5178Y-R CDNA, RIKEN FULL-LENGTH ENRICHED	Farslb		1/8		5/8	Aminoacyl-tRNA biosynthesis
VACUOLAR ATP SYNTHASE SUBUNIT D	Atp6v1d	11/7		11/17		Mitochondrial dysfunction
NADH DEHYDROGENASE [UBIQUINONE] 1 BETA SUBCOMPLEX	Ndufb5	9/5		3/7	10/6	Mitochondrial dysfunction
CYTOCHROME C OXIDASE POLYPEPTIDE VIC.	Cox6c	7/3				Mitochondrial dysfunction
NEURONAL PENTRAXIN-1 PRECURSOR	Nptx1	6/1			12/6	unknown
CATHEPSIN D PRECURSOR	Ctsd	4/14				Pro-apoptosis
ISOFORM 1 OF COP9 SIGNALOSOME COMPLEX SUBUNIT 1	Gps1	9/5	4/8	11/3		GTPase inhibitor

Red: ↑ in MPTP Blue: ↓ in MPTP

Figure 3. Top protein functional categories and the number of genes identified



MPTP Interaction Mechanism

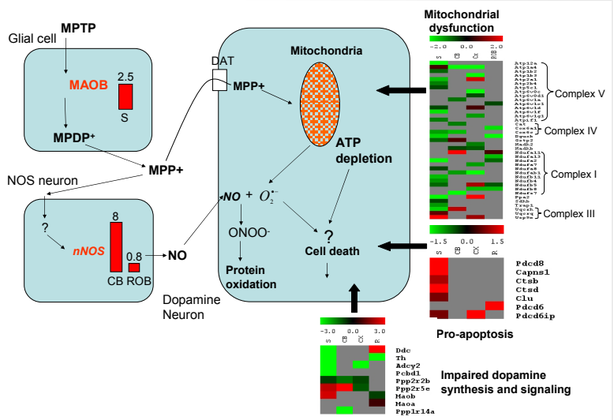


Figure 3. Schematic representation of the mechanism of MPTP action in the brain system.

Conclusions

- 46,731 peptides corresponding to 6918 nonredundant protein groups were confidently identified.
- In MPTP-treated mice, 366, 350, 340 and 392 proteins displayed significant changes in relative abundance between the striatum, cortex, cerebellum, "rest of brain" region, respectively.
- Functional analysis of proteins showing significant changes in abundance showed they belonged to number of biological processes, including mitochondrial dysfunction, oxidative stress response, and apoptosis.
- A number of proteins were identified that constitute potential biomarkers of the disease and/or new drug targets for therapeutic intervention.
- Selected proteins will be orthogonally validated by microarray analysis, e.g., Western blot.

Acknowledgements

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