NanoPro™ Assay: Mitofusin 1 (Mfn1)

SUMMARY

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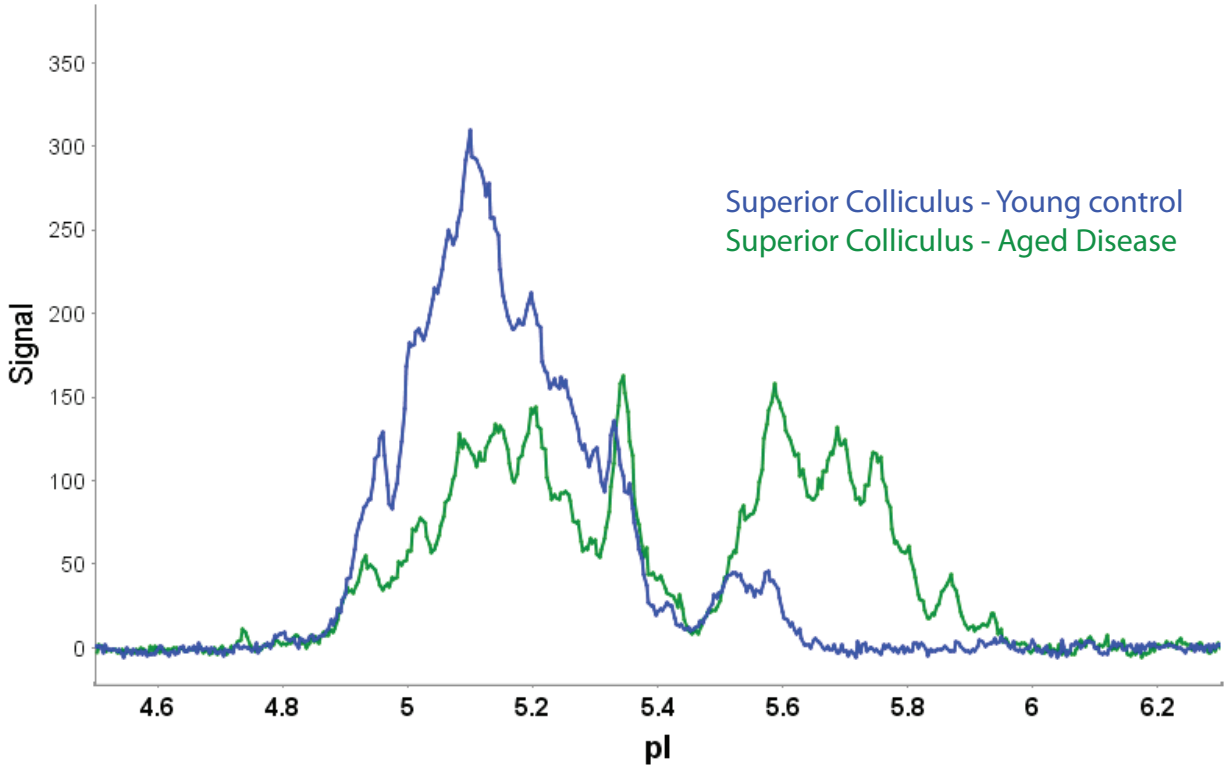
Primary Antibody: Anti-Mfn1 (Santa Cruz Biotechnology, cat# sc50330)
Detection Antibody: Amplified Rabbit Secondary Antibody Detection Kit (ProteinSimple, p/n 041-126)

Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) are two highly homologous mitochondrial outer membrane proteins necessary for mitochondrial fusion. Mitochondrial fusion is necessary to maintain mitochondrial health and function, and mitochondrial dysfunction is implicated in diseases such as Charcot-Marie-Tooth 2A and dominant optic atrophy, as well as in multiple tissue types. Phosphorylation of these proteins has not been widely studied. Our data show detectoin of Mfn1 protein in three neuronal tissues: retina, optic nerve, and the superior colliculus region of the brain in a mouse model, DBA/2J, a widely accepted model for glaucoma.

RESULTS



DETECTION OF MFN1 IN SUPERIOR COLLICULUS TISSUE SAMPLES

Mfn1's two major peak clusters show a shift occurring over age and disease in the superior colliculus. The shift of peak area to the right is present in young diseased animals (green e-gram trace), while the size of the peak area to the left decreases with age (blue e-gram trace). Similar types of peak shifts occur in retinal and optic nerve tissue.

Data shown for superior colliculus. Other tissue results not shown. Please contact author for details.

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PROTOCOL

CELL PREPARATION

Cell harvest:	Retina, optic nerve and superior colliculus were removed from mice that had been lethally anesthetized. Tissue samples were immediately placed into tubes being stored on dry ice. Tubes were weighed prior to and after the addition of the tissues to determine tissue weight, and maintained on dry ice. For every mg of tissue, 5-10 µL of ice-cold lysis buffer (with protease inhibitors and DMSO added) is added to the tube. *NOTE: for tissue harvest details, please contact author at mnivison@uw.edu* Determine lysate concentration using BCA assay. Snap freeze on dry ice.
Storage:	-80 °C
Animal age and control definitions:	Young animals are 3 months old, with no outward disease manifestation; old animals are 9-12 months of age, by which age disease will have manifest itself in the DBA/2J strain, but not in the controls. This allows age-matched comparisons to differentiate changes due to aging vs. changes due to disease. The DBA/2J strain (diseased) have two naturally occurring mutations that cause them to develop glaucoma naturally as they age, while the control animals have only one mutation and do not develop disease.

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ASSAY REAGENTS

Protein concentration:	0.05 mg/mL final in capillary by BCA assay
Sample diluent:	Bicine/CHAPS Lysis Buffer plus 1x DMSO Inhibitor Mix
Ampholyte premix:	Ampholyte-free Premix G2 (ProteinSimple, p/n 040-967) containing 8% v/v of pH 4-7 Servalyt Ampholyte (Helix Technologies, p/n 42948.01)
pI standards:	pI Standards 4.4, 6.4 and 7.0 (ProteinSimple, p/n 040-026, 040-030 and 040-031)
Wash:	Wash Buffer Concentrate (ProteinSimple, p/n 041-108)
Primary Antibody:	Anti-Mfn1 (Santa Cruz Biotechnology, cat# sc50330), 1:300
Detection antibody:	Amplified Secondary Antibody Detection Kit (ProteinSimple, p/n 041-126), 1:100
Anolyte:	Phosphoric Acid, 10 mM (ProteinSimple, p/n 040-650)
Catholyte:	Sodium Hydroxide, 100 mM (ProteinSimple, p/n 040-651)
Luminol/peroxide:	Mixed 1:1 (ProteinSimple, p/n 040-652 and 041-084)

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ASSAY CONDITIONS

System:	NanoPro 1000
Sample loading time:	25 seconds
Focus conditions:	21000 µW, 40 minutes
Immobilization:	80 seconds
Wash 1:	2 x 150 seconds (default)
Primary antibody incubation:	240 minutes
Wash 2:	2 x 150 seconds (default)
Detection antibody incubation:	120 minutes
Chemiluminescence exposure:	120, 240, 480 and 960 seconds

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