THESIS OF MASTER DEGREE

A proteomic study of the analysis of differentially expressed proteins in the hippocampus of the Niemann−Pick Disease, type C mouse

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A proteomic study of the analysis of differentially expressed proteins in the hippocampus of the Niemann−Pick Disease, type C mouse

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프로테오믹스를 이용한 니이만픽 타입 C 생쥐해마의 단백질 발현 변화에 관한 연구

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ABSTRACT

Niemann-Pick disease type C (NPC) is a fatal autosomal recessive cholesterol disorder characterized by severe, progressive neurodegeneration. In 1997, the causative gene, NPC1, was identified, however; the mechanism by which mutations in this gene lead to neurodegeneration remains elusive. In order to determine the mechanism of neurodegeneration, candidate proteins were identified through a proteomics approach. Two-dimensional electrophoresis (2-DE) was utilized to resolve the hippocampal protein expression profiles of 4-week and 8-week old NPC +/+ and -/- mice. Spots located only on the NPC -/- gel or only on the NPC +/+ gel were screened and characterized by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and database searching. At 4 weeks of age, there were no significant protein expression differences between NPC +/+ and -/- mice. However, by the age of 8 weeks, NPC +/+ and -/- mice showed marked differences in their expression profiles. Hopefully, this data will not only provide insight into how cells handle this type of pathological state but will also provide new directions for treatment-centered research.

Keywords: Niemann-Pick disease type C, NPC1, hippocampus, proteome analysis, neurodegeneration, cholesterol

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Niemann-Pick Disease is actually a term for a group of diseaes which affect metabolism and which are caused specific genetic mutations. The three most commonly recognized forms of the disease are Types A, B and C. Type C, although similar in name to Type A and B, is very different at the biochemical and genetic level.

Patients diagnosed with Niemann-Pick disease, type C show symptoms ranging from ataxia and dystonia to dementia. Histopathological studies show an abnormal accumulation of cholesterol in late endosomal and lysosomal compartments, ballooned neurons, and massive neuronal loss (Vanier and Suzuki, 1998). It was recently discovered that NPC is caused by alterations of either of two genes: NPC1 (Carstea *et al*., 1997), mutated in about 95% of patients with NPC, and HE1 (Naureckiene *et al*., 2000), mutated in the remaining 5%. Both genes encode for proteins that influence the intracellular transport of cholesterol. Mutations in these NPC genes result in massive and a diagnostically useful accumulation of cholesterol in non-brain tissue (eg spleen and fibroblasts). Originally, repeated efforts to demonstrate cholesterol accumulation convincingly in brain tissue had failed using biochemical methods (Vanier, 1999; Tint *et al*.,1998; Xie *et al*., 1999). However, research has recently revealed a massive accumulation of intracellular cholesterol in human and murine NPC brains using filipin histochemistry (Sawamura et al., 2001; Zervas *et al*., 2001).

The BALB/c-npc1nih mouse model for Niemann-Pick type C has facilitated closer examination of this disorder. As in the human phenotype, central neurological symptoms such as ataxia and hind limb paresis dominate the clinical

picture and abnormal cholesterol storage and neuronal loss in the central nervous system dominate cellularly (German *et al*., 2002). However, a strong correlation between cholesterol accumulation and neurodegeneration remains elusive.

Two-dimensional gel electrophoresis (2-DE) was introduced in 1975 and still the most commonly used technique for monitoring changes in the expression of protein mixtures. Many technical issues that initially limited performance have been addressed over the past few years and optimized protocols have resulted in small-scale gels that can resolve 2,000 to 3,000 proteins and large-scale gel that can resolve up to 10,000 proteins. Important technologic advances include the use of immobilized pH gradients for isoelectric focusing and fluorescent stains for protein imaging. Similarly, sophisticated and higher throughput procedures have been developed for protein identification. Peptide mass fingerprinting by mass spectrometry (MS) and tandem MS (MS/MS) produce partial amino acid sequences of target proteins that can be used to identify protein spots from 2D gels through database searching of the genome. These techniques can be further optimized by focusing on a specific pH or size range (zoom gels) and preseparating proteins of interest from bulk proteins.

In order to determine a possible link between cholesterol accumulation and neuronal degeneration, we performed a hippocampal proteomic study using the BALB/c-npc1nih mouse model. By screening proteomic profiles at pre- and post-symptomatic ages for changes in protein expression levels, we believe neurodegeneration-inducing candidate proteins can be identified.

MATERIALS and METHOD

1. Animal

A colony of BALB/c-npc1 mice (BALB/c mice containing the NPC1 mutation) is currently being maintained by the interbreeding of heterozygous mice. For the current study, wild-type +/+ and homozygous -/- NPC mice at 4 and 8 weeks of age were used from both sexes. The genotypes of the mice were determined from genomic DNA isolated from tail-snip DNA using a polymerase chain reaction (PCR)-based method described previously. The NPC animals were allowed to take food and water ad libitum.

2. Filipin staining

Briefly, 30 μm coronal sections of the hippocampus were made with a freezing microtome and sequentially collected in 6-well culture dishes. The free floating slices were incubated for 3 hours under agitation at room temperature in the dark with 50 ug of filipin complex (sigma, F9765) per ml phosphate buffer, (pH 7.4 PB). Cholesterol was visualized in the individual neurons by using an inverted microscope (Olympus IX70) suitable for ultraviolet light, 360 nm excitation, and filter MBA 510 for filipin. The sections were photographed with a CoolsnapPro (Media Cybernetics Co.) camera immediately after the cells were exposed to the UV light.

3. Stereological cell counting

One well tissue sections from 6 well culture dish were processed for cresyl violet staining to mark all cells. Tissues were mounted on the gelatin coated slides and dried for overnight. Then tissues were rehydrated with PBS and stained with cresyl violet solution for 5 minutes. Slides were dehydrated, cleared and mounted with permount. Cresyl violet staining was used to mark all cells. Cell number was calculated under a CAST stereological system with an Olympus BX-51 microscope. CAST software was used for stereological neuronal cell counting. An unbiased stereological estimation of the total cell number was made with the optical fractionator. All hippocampi were outlined from front to back. The cells were counted inall regions outlined. Sampling was done using the Olympus CAST (Computer Assisted Stereological Toolbox) system version 2.1.0 (Olympus Denmark A/S, Albertslund, Denmark). A counting frame (2025 $\mu m2$) was placed randomly on the first counting area and systematically moved through all counting areas until the entire delineated area was sampled. The sampling frequency was chosen so that roughly 140 cells were counted in each specimen. Average thickness of the sections was12-17μm. A neuron was defined as a cell with a clearly visible cell body within the 10 μm Z-plane of the counting frame using a 100 X oil immersion objective (numerical aperture, 1.35). An upper guard zone of 2 μm was used, so the cells were excluded from both surfaces to avoid the problem of the lost caps, and only the profiles that came into focus within the counting volume were counted. The estimate of the total number of the neurons was calculated according to the optical fractionator formula.

4. Electron microscopy

NPC +/+ and -/- mice at 4 weeks and 8 weeks of age were deeply anesthetized and intracardially perfused with 10 ml of 0.1 M sodium phosphate buffer (pH=7.4) followed by 50 ml of 0.1 M sodium phosphate buffer containing 0.5 % glutaraldehyde and 2 % paraformaldehyde. The brains were removed and hippocampi were collected and postfixed in 2% osmium tetroxide for 60 minutes at 4 °C. After being dehydrated with graded ethanol and cleared with propylene oxide, the tissues were embedded in an Epon-812-Araldite mixture and polymerized at 60°C for 72 hours. 70 nm sections were cut coronally and mounted on one-hole copper grids. The sections were stained with 1% uranyl acetate and lead citrate and examined under a JEOL JEM1200EX electron microscope at 80 KV.

5. 2-Dimensional Electrophoresis

The hippocampi of the NPC +/+ and -/- mice were dissolved in a Tris-base and sonicated at low power in liquid nitrogen. The tissues werelysed in thiurea and lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris-HCl, 20 mM DTT, 2% IPGphor buffer). The lysates were maintained for 1 hr in ice and centrifuged at 16,000 g for 30 min at 4 °C. Protein concentrations were estimated by the Bradford protein assay using Bovine Serum Albumin as a standard. All reagents used in 2-DE were obtained from Amersham Biosciences (Seoul, Korea). Immobilized Dry-Strips (24 cm, pH 4-7; Amersham Biosciences) and IPGphor (Amersham Biosciences) were used for first dimension IEF. A Hoeffer 2-DE

system was used for second dimension SDS-PAGE (11% gel). IEF was carried out using a 300V gradient for 3 hours, and 3,500-95,000 Vh. Second dimensional electrophoresis was conducted at 100 V in running buffer (25 mM Tris-HCl, pH 8.8, 198 mM glycine, and 0.1 % SDS). Following protein separation via electrophoresis, the protein spots were visualized with Coomassie Blue and scanned using a Bio-Rad Scanner 800 (Bio-Rad, Hercules, CA, USA). Differential protein expression was determined using the Image Master Platinum 5.0 software (Amersham Biosciences).

6. MALDI-TOF analysis

Protein spots were excised from gels with a sterile scalpel and placed into Eppendorf tubes. Proteins were digested using trypsin (Promega, Madison, WI, USA) as previously described (Choi *et al*., 2003). For MALDI-ToF MS analysis, the tryptic peptides were concentrated by a POROS R2 , Oligo R3 column (Applied Biosystems, Fostercity, CA, USA) (Lee *et al*., 2003). After washing the column with 40% methanol, 100% acetonitrile and then 50mM ammonium bicarbonate, samples were applied to the R2, R3 column and eluted in 2µL αµ -cyano-4-hydroxycinamic acid (CHCA) dissolved in 70% acetonitrile and 0.1% TFA before MALDI-ToF analysis. Peptide mass fingerprinting was performed using a PerkinElmer/PerSeptive Biosystems Voyager DE-PRO MALDI-TOF mass spectrometer, operating in delayed reflector mode at an accelerating voltage of 20 kV. Protein database searching was performed with the MS-Fit program (http://prospecto-r.ucsf.edu) using monoisotopic peaks. Resulting peptide masses were searched through the database managed by SWISS-PROT (http://kr.espas-y

.org) and the National Center for Biotechnology Information (http://www. ncbi .nlm.nih.gov).

RESULTS

1. NPC -/- hippocampal cells accumulate cholesterol.

At 4 weeks of age, the hippocampus of both the NPC +/+ and NPC -/-mice showed only a faint level of filipin staining (data not shown). At 8 weeks of age, filipin staining of the NPC (+/+) hippocampus remained faint whereas, the intensity of the NPC (-/-) hippocampus showed a pronounced increase.

2. NPC -/- hippocampal neurons show signs of neurodegeneration.

At 4 weeks and 8 weeks of age both NPC +/+ and NPC -/-mice showed no significant differences in stereological cell counts of cresyl stained cells in the CA1, CA2/3 and dentate gyrus regions of the hippocampus (Fig.1).

When observed under the electron microscope at 4 weeks and 8 weeks, NPC +/+ hippocampal neurons showed no signs of neurodegeneration. However, hippocampal neurons of the NPC-/- mouse containeda few intracellular inclusions at 4 weeks of age and by 8 weeks of age showed neurodegenerative changes as well as a heavy accumulation of intracellular inclusion bodies (Fig. 2). In all the cases, no signs of neurodegeneration were observed in the synapses and distal axons of the hippocampal cells (Fig. 2).
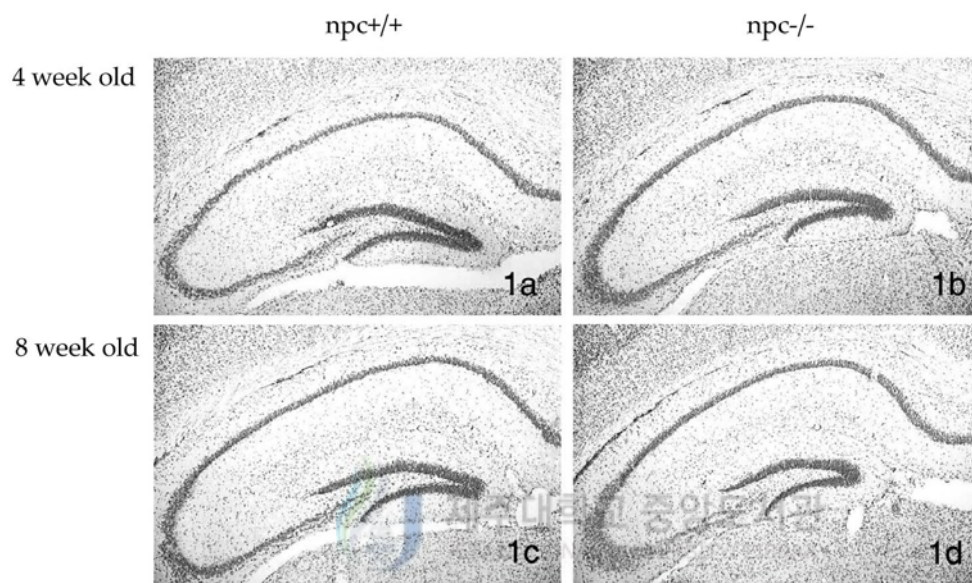
Fig. 1. Coronal sections of the hippocampus stained with cresyl violet (X40): the (a) 4 week old NPC +/+ mouse, (b) 4 week old NPC -/- mouse, (c)8 week old NPC +/+ mouse, and (d) 8 week old NPC -/- mouse. No differences in cell numbers were seen between wild type and knockout mice at either 4 weeks or 8 weeks under cresyl violet staining.
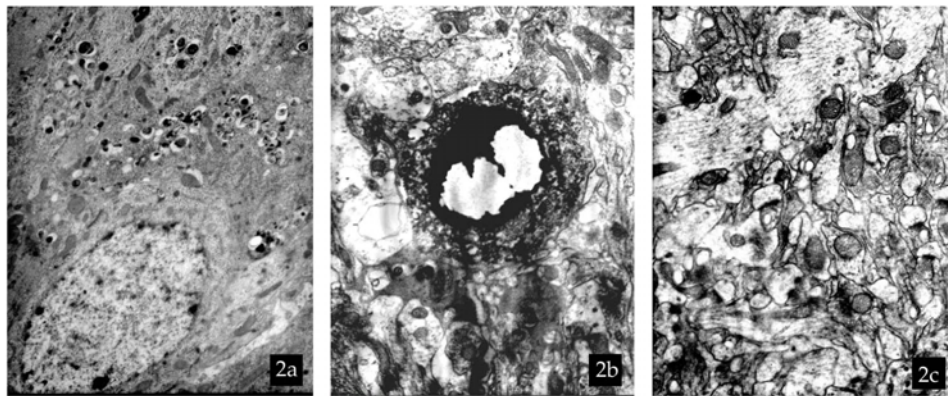
Fig. 2. Electron microscopic pictures of the neuronsin the hippocampus of the NPC -/- mouse (X12,000). There are some inclusions in the neurons of the 4 week old mouse but overall, the neurons look healthy (a). In the 8 week old mouse, cells show apoptotic changes with abundant inclusions (b) but overall, the synapses look intact (c).

3. NPC -/- hippocampus undergo a marked change in their protein expression profiles.

There were no differences in hippocampal protein expression levels between 4-week-old NPC +/+ and NPC -/- mice (data not shown). In In 8-week-old mice, there were prominent differences in protein expression profiles between NPC +/+ and NPC -/- hippocampi. Most of the spots were located between pH 4 and pH 8(Fig. 3). Using MALDI-TOF MS, thirty-six unique spots were analyzed from the NPC -/- gels and eleven unique spots were analyzed from the NPC +/+ gels. Using the SWISS-PROT database, these spots were identified and mapped on the gel images as shown in Fig. 3 and described in Table 1(a,b) and Table 2.
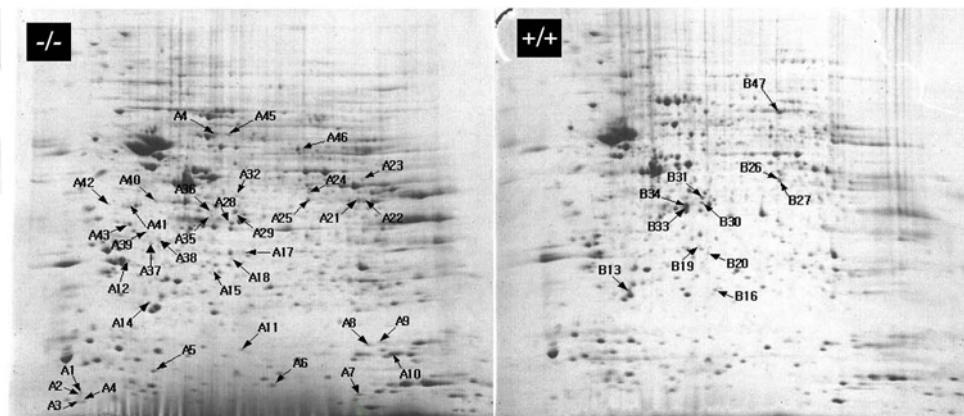
Fig. 3. Comparison of 2DE maps from the hippocampi of NPC -/- (a) and NPC +/+ mice (b). Proteins were prepared and separated on pH 3-10 linear immobilized pH gradient strips and then by 12.5% SDS-PAGE. Gels were stained with CBB R-250.

Table 1-a. Up-regulated proteins identified by MALDI-TOF analysis of the NPC -/- hippocampus

No.	MOWSE Score	Masses Matchde	% Cov	% TIC	Mean Err ppm	Data Tol ppm	MS-Digest Index#	Protein MW (Da)/pI	Accession #	Protein Name
Up-regulated protein										
A2	1.954e+04	6(13)	11.0	13.3	29.5	195	107262	93205/5.8	O35136	Neural cell adhesion molecule 2 precursor(N-CAM 2)
A3	6.590e+04	5(38)	24.0	38.5	-7.57	1645	47648	15531/9.2	P21460	Cystain C precusor(Cystatin 3)
A4	3.419e+04	9(31)	59.0	31.0	-39.1	38.4	21365	17972/7.7	Q8R4I1	Peptidyl-prolyl cis-trans isomerase A (PPIase)(Cyclophilin A)
A5	1262	5(13)	31.0	13.9	-11.1	108	123211	32542/7.6	P49070	Calcium-signal modulating cyclophilin ligand(CAML)
A6	2.352e+04	5(18)	17.0	18.5	-32.7	73.3	36700	54528/6.0	Q9QZ04	MAGE-like protein 2(Protein nS7)
A7	175	5(38)	31.0	38.5	-2.29	43.2	61172	10963/7.9	Q64433	10 kDa heat shock protein, mitochondrial(Hsp10)
A8	736	3(15)	17.0	15.0	-33.2	52.6	31167	26498/9.0	Q9R0Q9	Mannose-P-dolichol utilization defect 1 protein
A10	2390	6(31)	43.0	31.6	-43.7	27.3	21365	17972/7.7	P17742	Peptidyl-Prolyl cis-trans isomerase A(PPIase)(Cyclophilin A)
A11	7672	4(9)	27.0	9.8	146	138	126721	22705/5.1	Q9R0Q3	Cop-coated vesicl membrane protein p24 precursor(p24A)
A12	6648	5(10)	10.0	10.6	17.9	142	131118	47396/4.8	O70477	Homeobox protein PKNOX1 (PBX/knotted homeobox 1)
A14	6.430e+04	8(36)	26.0	36.4	11.7	43.2	16585	25425/5.0	Q60692	Proteasome subunit beta type 6 precursor(Proteasome delta chain)
A15	606	3(60)	12.0	60.0	48.4	933	112806	24753/8.5	Q91VU0	Protein FAM3C precursor

Table 1-b. Up-regulated proteins identified by MALDI-TOF analysis of the NPC −/− hippocampus

No.	MOWSE Score	Masses Matchde	% Cov	% TIC	Mean Err ppm	Data Tol ppm	MS-Digest Index#	Protein MW (Da)/pI	Accession #	Protein Name
Up-regulated protein										
A21	2574	4(16)	17.0	16.0	−36.5	278	73397	46403/5.3	P10630	Eukaryotic initiation factor 4A-II(eIF4A-II)(eIF-4A-II)
A23	2923	6(26)	22.0	26.1	8.86	37.4	7725	46661/6.5	O88844	Isocitrate dehydrogenase [NADP] cyptoplasmic
A29	2.793e+04	6(21)	20.0	21.4	−35.2	326	46159	47141/6.4	P17182	Alpha enolase(Non-neural enolase)(NNE)(Enolase 1)
A32	8.920e+04	6(42)	21.0	42.9	31.0	239	77159	38732/5.7	P54314	Guanine nucleotide-binding protein beta subunit 5
A35	5.124e+04	8(50)	22.0	50.0	22.8	238	119619	36461/5.4	Q9QZD9	Eukaryotic translation initiation factor 3 subunit 2(eIF-3beta)
A37	183	5(20)	29.0	20.8	−22.6	34.7	55751	26791/5.0	Q9D6Y0	Swiprosin
A38	2.727e+04	7(17)	11.0	17.1	−37.5	111	104514	98663/7.1	P23819	Glutamate receptor 2 precusor(GluR-2)(GluR-B)(GluR-K2)
A39	233	3(42)	15.0	42.9	180	412	44228	35753/4.8	P48036	Annexin A5(Annexin V)
A41	3.680e+04	8(42)	26.0	42.1	−19.3	38.7	51420	40301/4.9	P51863	Vacuolar ATP synthase subunit d(V-ATPase d subunit)
A43	4.437e+04	9(40)	30.0	40.9	0.0810	0.592	227180	30997/4.8	32484336	Otub 1 protein
A44	537	5(25)	9.0	25.0	0.531	80.0	1481789	76762/6.6	55132M	Protein kinase C
A45	1.671e+04	8(33)	22.0	33.3	−22.0	190	49747	60956/5.9	P19226	60 kDa heat shock protein, mitochondrial precursor(Hsp60)
A46	4.804e+05	10(22)	25.0	22.2	−10.4	43.3	70703	57478/6.0	P80314	T-complex protein 1, beta subunit(TCP -1-beta)(CCT-beta)

Table 2. Down-regulated proteins identified by MALDI−TOF analysis of the NPC −/− hippocampus

No.	MOWSE Score	Masses Matchde	% Cov	% TIC	Mean Err ppm	Data Tol ppm	MS−Digest Index#	Protein MW (Da)/pI	Accession #	Protein Name
Down-regulated proteins										
B13	360	5(18)	38.0	18.5	−20.0	41.7	95447	20831/5.2	P70296	Phosphatidylethanolamine-binding protein(PEBP)(HCNPpp)
										[Contains: Hippocampal choinergic neurostimulating peptide(HCNP)]
B16	3558	7(17)	33.0	17.9	33.2	30.7	90380	22166/5.7	Q9DBP5	UMP-CMP kinase(Cytidylate kinase)(Deoxycytidylate kinase)
B19	574	4(11)	22.0	11.8	79.8	121	114106	41526/6.1	P42208	Septin 2(NEDD5 protein)
B20	6659	5(35)	13.0	35.7	0.319	1.38	73139	58629/9.1	P70327	T-box transcription factor TBX6(T-box protein 6)
B26	1.857e+04	6(13)	31.0	14.0	−3.80	74.2	66286	40604/7.6	Q99LC3	NADH-ubiquinone oxidoreductase 42 kDa subunit
B27	1.474e+08	13(37)	47.0	37.1	−51.3	65.0	66286	40604/7.6	Q99LC3	NADH-ubiquinone oxidoreductase 42 kDa subunit
B30	352	4(16)	7.0	16.7	18.50	97	48209	72680/6.0	Q8CIG8	Protein arginine N-methyltransferase 5
B31	5.833e+08	12(37)	34.0	37.5	34.4	22.7	46159	47141/6.4	P17182	Alpha enolase(Non-neural enolase)(NNE)(Enolase 1)
B33	2.503e+04	10(22)	35.0	22.7	−6.80	58.4	1165259	37333/5.6	984551	G protein beta 2 subunit
B34	7136	5(10)	7.0	10.9	−32.6	109	132524	68568/9.5	Q99N48	Synaptotagmin-like protein 3(Exophilin 6)
B47	6.923e+09	14(53)	31.0	53.8	40.6	293	104012	62171/6.0	O08553	Dihydropyrimidinase related protein 2(DPR-2)

DISCUSSION

Cholesterol accumulation has been a hallmark of the NPC1 disease but the relationship between this accumulation and neurodegeneration still remains a mystery. Our data showed that at four weeks of age, neither NPC +/+ nor NPC -/- mice exhibita high level of filipin staining and all mice were pre-symptomatic. However, cholesterol accumulation showeda marked increase between the ages of 4 weeks and 8 weeks in NPC -/- mice, which correlates strongly with the development of symptoms. These findings were same to the results of others(Tint *et al*., 1998; Xie *et al*., 1999; Zervas *et al*., 2001).

At 4 weeks and 8 weeks of age both NPC +/+ andNPC -/- mice showed no significant differences in stereological cell counts of cresyl stained cells in the CA1, CA2/3 and dentate gyrus regions of the hippocampus. However, it should be noted that cresyl violet stains all cells that contain cellular components regardless of their state of health. Cellular differences in pathological states are better illustrated under an electronmicroscope.

When observed under the electron microscope at 4 weeks and 8 weeks, NPC +/+ hippocampal neurons showed no signs of neurodegeneration. However, hippocampal neurons of the NPC-/- mouse contained a few intracellular inclusions at 4 weeks of age and by 8 weeks of age showed neurodegenerative changes as well as a heavy accumulation of intracellular inclusion bodies. In all the cases, no signs of neurodegeneration were observed in the synapses and distal axons of the hippocampal cells. These age-related changes aresimilar to the findings of the filipin staining.

In order to identify proteins that could be responsible for the pathological

changes seen in NPC -/- cells, we conducted a proteomic analysis of the hippocampus of NPC +/+ and -/- mice. There were no differences in hippocampal protein expression levels between 4-week-old NPC +/+ and NPC -/- mice (data not shown). In In 8-week-old mice, there were marked differences in protein expression profiles between NPC +/+ and NPC -/- hippocampi. Most of the spots were located between pH 4 and pH 8. Using MALDI-TOF MS, 36 unique spots were analyzed from the NPC -/- gels and 11unique spots were analyzed from the NPC +/+ gels. Using the SWISS-PROT database, these spots were identified and mapped on the gel images as shown in Fig. 3 and described in Table 1(a, b) and Table 2. Caution must be taken when interpreting this number due to protein modifications, which could shift the location of the protein spot during the 2-DE analysis and result in an apparent loss of one spot and gain of another.

Of the 34 identified proteins sixteen have been implicated in neurodegeneration: the cytosolic form of NADP(+)-dependent isocitrate dehydrogenase (IDPc) (Lee *et al*., 2002), neural cell adhesion molecule 2 (N-CAM 2) (Pedersen *et al*., 2004), calcium-signal modulating cyclophilin ligand (CAML) (Feng *et al*., 2002), mage-like protein 2 (Barker and Salehi, 2002), 10 kDa heat shock protein, mitochondrial (Hsp10) (Takayama *et al*., 2003), 60 kDa heat shock protein, mitochondrial precursor (Hsp60) (Takayama *et al*., 2003), T-complex protein 1, beta subunit (TCP-1-beta)(Takayama *et al*., 2003), cyclophilin A (Cande *et al*., 2004), protein kinase C (Gutcher *et al*., 2003), proteasome subunit beta type 6 precursor (Wojcik, 2002),alpha enolase (Magi *et al*., 2004), glutamate receptor 2 precursor (GluR-2) (Lu *et al*., 2003), Otubain 1 (Wojcik, 2002; Kim *et al*., 2003), NADH-ubiquinone oxidoreductase 42 kDa subunit, mitochondrial [Precursor] (Kim *et al*., 2001), cystatin C [Precursor] (Nishio *et al*., 2000), and annexin V

(Gidon-Jeangirard *et al*., 1999; Hawkins *et al*., 2002).

Further studies will be needed to determine the influence of these candidate proteins on the development of pathological conditions and clinical symptoms. Of these sixteen candidate proteins our primary focus for further research will include the down-regulated NADH-ubiquinone oxidoreductase 42 kDa subunit and the up-regulated annexin V protein. Both have been strongly implicated in neurodegeneration in previous studies.

CONCLUSION

A proteomic approach has enabled us to identify a number of uniquely expressed proteins in the hippocampus of the NPC -/- mice at 8 weeks of age, a time when the degenerative changes in the hippocampus and severe symptoms occur. Our data suggests these proteins in NPC -/- mice may be involved in the phenomena related to neurodegeneration and/or altered cholesterol metabolism. Because the number of proteins, which have been reported to play a role in the pathogenesis of NPC, is limited, our findings open a new way of studying the link between proteomic aspects and gene mutation in the NPC disease.

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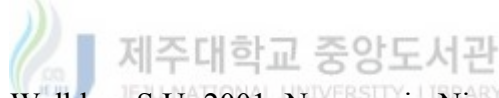
ABSTRACT IN KOREAN

프로테오믹스를 이용한
니이만픽 타입 C 생쥐해마의 단백질 발현 변화에 관한 연구

김 재 우

Niemann Pick disease type C(NPC)는 열성 유전 콜레스테롤 질환으로 진행성 신경 퇴화를 나타낸다. NPC의 원인이 되는 유전자 NPC1과 HE1은 확인되었지만 신경 퇴화로 진행되는 이 유전자의 돌연변이에 의한 메카니즘은 아직까지 이해하기 어려운 채 남아있다. 본 연구는 이런 신경퇴화의 메카니즘을 확인하기 위한 후보 단백질들을 프로테오믹스 연구를 통하여 확인하고자 하였다.

2차원 전기영동기법을 통하여 4주와 8주된 NPC +/+와 NPC-/- 쥐들의 해마 속 단백질을 분리해 낼 수 있었다. 메트릭스상 레이저 탈리 이온화와 비행시간차 분석기 조합(MALDI-TOF)을 이용하여 분석 결과 얻어진 단백질 정보를 바탕으로 펩티드 질량 지문 측정용 데이터베이스 서버에서 단백질들을 규명할 수 있었다. 4주된 NPC +/+와 NPC -/-쥐들 사이에는 주목할 만한 단백질의 발현 차이가 없었으나 8주된NPC +/+와 NPC -/- 쥐들에서는 발현프로필이 두드러진 차이를 보였다.

이 데이터는 NPC 질환의 병리기전과 치료의 새로운 방향을 제시해 줄 수 있는 기초 자료가 될 것이라고 사료된다.

주요어: Niemann-Pick disease type C, NPC1, 해마, 프로테오믹스, 신경퇴화, 콜레스테롤

감사의 글

2년 동안 대학원 생활을 하면서 좋은 일도 많았고 힘든 일도 많았지만 모두 배움의 과정이었다는 생각을 하며, 아직도 배워야 할 것들이 많다는 것을 느낍니다.

본 논문이 완성되기까지 저에게 도움을 주신 많은 분들께 이 지면을 통해서 감사드리고자 합니다.

우선, 부족한 저를 제자로 받아주시고 항상 지켜봐 주신 이봉희 교수님께 감사드립니다. 항상 건강하십시오. 또한 논문의 부족한 부분을 지적해 주시고 완성까지 이끌어 주신 조문제 교수님과 김명주 교수님께도 감사의 말씀을 드립니다. 바쁘신 가운데에도 대학원 과정동안 학문의 길로 이끌어 주신 이영기 교수님, 박덕배 교수님, 정영배 교수님, 강현욱 교수님, 현진원 교수님, 고영상 교수님, 강희경 교수님, 유은숙 교수님, 신경수 교수님께 감사드립니다. 특히 대학원 생활동안 많은 실험을 같이 한 변경희, 강혜정, 고선영에게 고마운 마음을 전합니다. 힘들때마다 다독여주고 용기를 준 송지훈, 윤원종 대학원생에게도 감사의 맘을 전합니다. 그리고 강신해 선생님과 박수영 선생님, 김영미, 임희경, 강윤석, 윤지현, 이정희, 김은희 선생님과 약리학 교실 이혜자, 현재희, 현은아, 김상철 기생충학 교실 김지영 대학원생, 대학원 동기인 강경아, 최수길에게도 감사드립니다.

끝으로, 끊임없는 사랑으로 항상 지켜봐 주시는 부모님과 어려울때 힘이 되어준 형님, 그리고 형수님께 고마움을 전하며 이 논문을 드리고 싶습니다. 감사합니다.