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DNA Microarray Analysis of Gene Expression Profiles in Aging process of Mouse Brain

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Abstract

In order to investigate the molecular basis of the aging process in brain, we have employed high-density oligonucleotide microarrays providing data on 10,108 gene clusters to define transcriptional patterns in three brain regions, cerebral cortex, cerebellum, and hippocampus. Comparison of the expression patterns between young (6-week-old) and aged (17-month-old) C57BL/6 male micerevealed that about ten percent (1098) of the genes showed a significant change in the expression level in at least one of the three tissues. Among them, 23 genes were upregulated and 62 genes were downregulated in all three tissues of the old mice. The number of genes upregulated exclusively in hippocampus (337) was much larger compared to other tissues. Gene ontology-based analysis showed the genes related with signal transduction or molecular transports are more likely to be upregulated than downregulated in the aging process of hippocampus. These data may provide some useful means for elucidating the molecular aspect of aging in hippocampus and other regions in brain.

Keywords: microarray, aging, brain, cerebral cortex, cerebellum, hippocampus

Introduction

With increased life span resulting from the improved socioeconomic status and the progress of medical techniques, the aging process becomes one of the most important research subjects in both social and biomedical sciences. One of the major problems in the elderly is the cognitive decline, which is related with the aging process in the brain (Yankner, 2000). Despite a lot of research, themolecular basis of brain aging still remains unclear, in part because we lack a large number of biomarkers for aging process in the brain.

DNA microarray technology is expected to revolutionize the biomedical research field through the simultaneous analysis of gene expression patterns in the whole genome scale (Lander, 1999). There were several studies about microarray analysis of the aged brain in mouse, rat, or even human (Prolla, 2002; Blalock *et al*., 2003; Lu *et al*., 2004; Erraji-Benchekroun *et al*., 2005). However, analysis with only one region of the brain has a serious limitation for the complete understatingof the molecular networks involved in the brain aging processes, which are enormously complex phenomena related with multiple systems, cell types, and pathways. In addition, recent advances in our information about the genome and development of the novel technology like microarray can provide much better chance to identify significant changes in gene expression as compared to a few years ago. For that reason, we used 10K oligonucleotide microarray analysis for the simultaneous investigation of gene expression changes in cerebral cortex, cerebellum, and hippocampus in young and old mice.

Materials and Methods

Experimental animals

Male C57BL/6 mice were purchased at 1.5 months of age from Polars International Corp. or obtained at 17 months of age from Silver Biotechnology Research Center of Hallym University (Korea).

Mice were sacrificed by rapid cervical dislocation and brains were removed. For RNA extractions, the cerebral cortex, hippocampus, and cerebellum were dissected and immediately frozen in liquid nitrogen and stored at -80°C.

RNA isolation

Total RNA was extracted from the frozen tissues using TRIZOL reagent (Life Technologies, USA) and a power homogenizer (Fisher Scientific) with the addition of chloroform for the phase separation before isopropyl alcohol precipitation oftotal RNA. RNA concentrations

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were determined spectrophotometrically and the quality of the isolated RNA samples was assessed using gel electrophoresis.

Hybridization to expression array

Total RNA (5 μg) was converted into double stranded cDNA using the cDNA synthesis Sytem (Roche) using T7-(dT)24 primer. The each cDNA was purified using the RNeasy kit (Qiagen;Valencia, USA). Each Cy3-(young mice brain), or Cy5-(old mice brain) labeled cRNA was synthesized using the Megascript T7 kit (Ambion; Austin, USA), using Cy3-CTP and Cy5-CTP (APB/ Uppsala Sweden). The cRNA was purified using the RNeasy. 15 ug of each purified cRNA was mixed and fragmented in the fragmentation buffer (40 mM Tris, pH 8.1, 100 mM KOAc, and 30 mM MgOAC) by heating to 94°C for 15 min. The fragmented cRNA was mixed with the hybridization MAGIC II-10K Oligo Chip (Macrogen;Seoul, Korea) for 16 h at 42°C. All preparations met Macrogen's recommended criteria for use on their expression arrays. The arrays were then washed and scanned with the Array scanner (APB). Acquired images were processed and analyzed statistically for interpretation of analyzed spot intensity results using Imagene v4.1 software (Roche). Nonbiological factors that may contribute to variability of data were minimized using global normalization/scaling with data from all probes sets. Each chip contains a total of 10,368 elements of which 10,108 are unique genes/clusters. The length of oligonucleotides was 50-mer.

Clustering and expression analysis

For the cluster analysis, total 1828 genes were selected by the criteria of two-fold change in at least two different samples. The software packages Cluster (version 2.11) and TreeView (version 1.60) were downloaded from the website of Eisen Lab (http://rana.lbl.gov/EisenSoftware.htm) and used for the cluster analysis and visualization. The distance metric based upon uncentered Pearson correlation was used for the hierarchical clustering. For tissue-specific expression analysis, the genes that showed more than 2-fold expression change in at least two samples from the same type of tissues were selected.

Results and Discussion

Each microarray data was sequentiallyprocessed through global normalization, intensity-dependent normalization, and print-tip normalization. Finally each of three data from the same tissue was normalized according to the scale differences of multiple slides. Among the 11,520 probes mounted on the MAGIC II-10K Oligo Chip, we selected 1,828 genes that showed more than two fold difference in at least two array data sets for the cluster analysis. Hierarchical clustering analysis revealed that each set of arrays from the same tissue was clustered together except only in one sample, cerebellum #1 (Fig. 1). We found the similar results when different methods such as Spearman Rank correlation or Kendall's Tau method were applied for the cluster analysis (data not shown). A different pattern of gene expression in cerebellum #1 sample was so obvious that we concluded to exclude this sample for further analysis

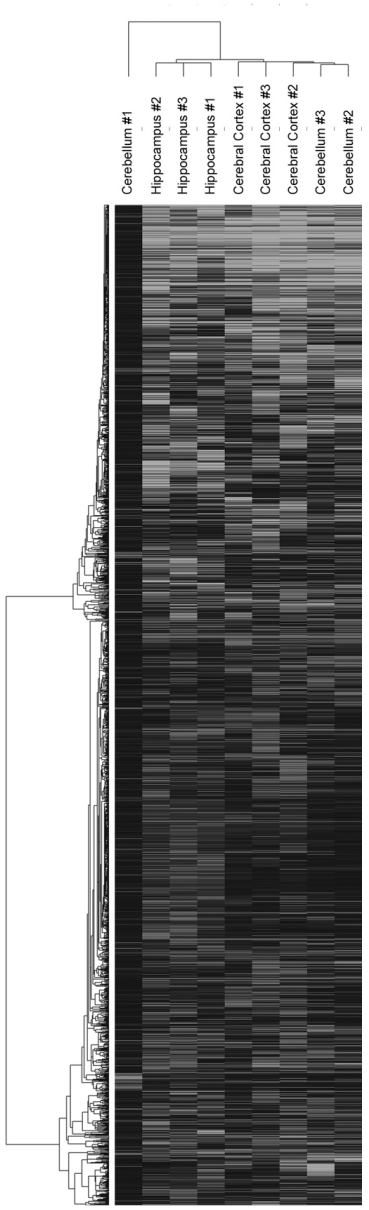

Fig. 1. Hierarchical clustering analysis of gene expression change in three different tissues of old mice

Then, we further selected the gene sets that showed the same pattern of expression in the same tissue. Total 1,098 genes showed more than two fold upregulation or downregulation in at least twoarray experiments from the same tissue. Table 1 shows the classification of 1,098 genes according to the pattern of expression changes. Interestingly, nine genes showed a different pattern of expression changes in different tissues, that is, upregulation in one tissue, but downregulation in another tissue. That is the reason why the sum of upregulated genes (656) and downregulated genes (451) is greater than 1,098. Table 1 also shows that the number of genes upregulated or downregulated in cerebellum is much less than in other tissues. This may be due to the exclusion of one sample from the cerebellum data set, which reduces the number of array experiments to only two. One interesting point from the table 1 is the number of upregulated genes in hippocampus.Upregulated genes in cerebral cortex (244 total, and 134 exclusive), downregulated genes in cerebral cortex (266 total, and 133 exclusive), and downregulated genes in hippocampus (252 total, 137 exclusive) showed a quite similar pattern of expression changes. However, the number of genes upregulated exclusively in hippocampus (337) was much larger than in other tissues. It suggests that the gene expression change (especially upregulation) occurs predominantly in hippocampus, which may be related to the characteristic cognitive changes in the aging process.

Table 2, 3 and 4 show the top-ranked twenty genes that are upregulated or downregulated in each of three tissues. One of the notable findings in these tables was that a member of protocadherin-gamma subfamily B showed the most dramatic decrease in the expression in all three tissues. Cadherins are calcium-dependent cell-cell adhesion molecules that mediate neural cell-cell interactions, and protocadherins (pcdh) constitute a subfamily of nonclassic cadherins. The pcdh gene clusters are known to have very similar genomic architecture with the immunoglobulin and T cell receptor gene clusters, and can potentially provide a significant molecular diversity like them (Wang *et al*., 2002). Pcdh genes are present in most neurons, and are the primary candidates for synapse formation and cell survival during development (Weiner *et al*., 2005; Junghans *et al*., 2005). The dramatic change in the expression level of one member of the pcdh-gamma families suggests the possible role of this gene product in the age-related changes in the synapse and neuronal architectures.

Among the upregulated genes, grb2-related adaptor protein 2 (grap2), lipocalin 2, cystatin 7 (cystatin F), sushi-repeat-containing protein (srpx, or drs), and matrix metallopeptidase 1b were prominent. There are some reports about the roles of these gene products in hematopoietic cells (Ludwig *et al*., 2003; Flo *et al*., 2004; Nathason *et al*., 2002), carcinogenesis (Hanai *et al*., 2005; Yamashita *et al*., 1999), or other disorders (Meindl *et al*., 1995). However, the distinct role of these genes in brain or senescence is largely unknown. One exception is the report from MacManus *et al*. (2005) that showed the upregulation of lipocalin 2 in mouse brain after focal ischemia. Itsuggests that these genes have very restricted roles in normal brain, but may be related to aging process itself or aging-related phenotypes in some part of the nervous system.

Table 5 and 6 show the list of genes up- or down-regulated in tissue specific manners. We believe that this type of analysis would be more efficient in estimating the role of possible target genes in aging process. For example, laminin alpha 1 was downregulated significantly in all three tissues although it could not be easily foundin the list of top-ranked downregulated genes. Laminin is a basement membrane protein, and a variety of different laminins can be found in the vascular basement membrane of the adult and aged brain (Jucker *et al*., 1996). Laminin alpha 1 has been found to be overexpressed in Alzheimer's disease frontal cortex, and localized to reactive astrocytes of the gray and white matter, and as punctate deposits in the senile plaques of the Alzheimer's brain tissue (Palu *et al*., 2002). On the contrary, Morita *et al*. (2005) reported a decrease in laminin immunolabeling in the capillary basement membranes of old dogs. The investigation of correlation between the heterogeneity of laminin expression and the structural and functional

Table 1. Age-related changes of gene expression in different brain tissues of old mice

Number of upregulated genes	
only cerebral cortex	134
only cerebellum	60
only hippocampus	337
cerebral cortex and cerebellum, not hippocampus	19
cerebellum and hippocampus, not cerebral cortex	15
cerebral cortex and hippocampus, not cerebellum	68
All three tissue	23
Sum	656
Number of downregulated genes	
only cerebral cortex	133
only cerebellum	29
only hippocampus	137
cerebral cortex and cerebellum, not hippocampus	27
cerebellum and hippocampus, not cerebral cortex	19
cerebral cortex and hippocampus, not cerebellum	44
All three tissues	62
Sum	451

diversity of the vascular basement membranes in aging process would be an interesting topic in this field.

The genes up- or down-regulated in only one tissue, especially in hippocampus, would be attractive targets for research about cognitive changes in the aging process. Transthyretin (also known as prealbumin), the major transporter of thyroid hormones was one of the most highly upregulated genes in hippocampus (Table

Table 2. The most highly upregulated or downregulated genes in aged cerebral cortex

Gene	Fold change	GenBank Accession No.
upregulated		
eosinophil-associated ribonuclease 2 precursor: ear2	↑6.94	AF238402
matrix metallopeptidase 1b (interstitial collagenase)	↑5.60	NM_032007
sushi-repeat-containing protein, x-chromosome: srpx	↑4.93	NM_016911
NK2 transcription factor related, locus 6 (Drosophila)	↑4.81	AF045150
lipocalin 2: 24p3 protein	↑4.68	X14607
cDNA sequence BC020077: mKIAA1799	↑4.36	BC020077
killer cell lectin-like receptor subfamily a member 1d: klrb1d	↑4.24	NM_030599
myeloid/lymphoid or mixed lineage-leukemia translocation to 4 homolog (Drosophila): afadin	↑4.19	AF172447
bruton agammaglobulinemia tyrosine kinase: btk	↑4.15	NM_013482
sodium channel, voltage-gated, type iv, alpha polypeptide: scn4a	↑4.11	NM_133199
interleukin 1 family, member 10	↑4.08	AY071844
cystatin F (leukocystatin): cystatin 7	↑3.90	NM_009977
GRB2-related adaptor protein 2: monocytic adaptor: mona	↑3.86	NM_010815
glycine receptor alpha 3 subunit	↑3.59	AF214575
serine/threonine kinase 22c (spermiogenesis associated): stk22c	↑3.57	NM_021479
a-1 alpha-amylase	↑3.36	M11895
cdna clone immunoglobulin lambda chain, variable 1	↑3.36	AK008145
similar to programmed cell death 6 interacting protein	↑3.32	BC002261
gamma-aminobutyric acid (gaba-a) receptor, subunit rho 1: gabrr1	↑3.29	NM_008075
fibroblast growth factor 21: fgf21	↑3.26	NM_020013
downregulated		
protocadherin gamma subfamily b, 2: pcdhgb2	↓8.97	NM_033575
zinc finger protein 114: zfp110	↓7.18	AF167317
killer cell lectin-like receptor, subfamily a, member 4: klra4	↓7.14	NM_010649
RE1-silencing transcription factor: neural-restrictive silencer factor nrsf/rest	↓6.28	AB024496
phosphatidylinositol 4-kinase type 2 beta	↓5.99	NM_028744
caspase 3, apoptosis related cysteine protease: casp3	↓5.86	NM_009810
solute carrier family 8 (sodium/calcium exchanger), member 1	↓5.76	AF409089
immunoglobulin mu-chain v-region	↓5.57	M12369
cdna clone chromodomain helicase dna binding protein 1	↓5.19	AK021188
endothelin 1: preproendothelin	↓5.15	U07982
formin: fmn	↓5.07	NM_010230
dnaj (hsp40) homolog, subfamily c, member 2: dnajc2	↓5.06	NM_009583
4a11 antigen	↓4.84	S74697
mutS homolog 3 (E. coli): msh3	↓4.65	NM_010829
RIKEN cDNA 9430034D17 gene: cdna clone homolog to transcription factor nrf (fragment)	↓4.60	AK020455
ets homologous factor	↓4.39	BC005520
carbonic anhydrase 6: car6	↓4.38	NM_009802
paraoxonase 2	↓4.32	BC021887
calcium channel, voltage-dependent, L type, alpha 1C subunit	↓4.14	L06233
glomulin, FKBP associated protein: aw227515	↓4.07	NM_133248

Table 3. The most highly upregulated or downregulated genes in aged cerebellum

Gene	Fold change	GenBank Accession No.
upregulated		
GRB2-related adaptor protein 2: monocytic adaptor: mona	↑5.01	NM_010815
sushi-repeat-containing protein, x-chromosome: srpx	↑4.96	NM_016911
ankyrin-like protein: 1700012m14rik	↑4.34	NM_023816
bcl2-antagonist/killer 1: bak1	↑4.21	NM_007523
matrix metallopeptidase 1b (interstitial collagenase)	↑3.79	NM_032007
sulfotransferase, hydroxysteroid preferring 2: sth2	↑3.74	NM_009286
peptidylprolyl isomerase F, opposite strand transcription unit	↑3.66	AK019686
interleukin 6: il6	↑3.66	NM_031168
testis-specific histone h2b: mth2b	↑3.63	X90778
similar to hypothetical protein flj12644	↑3.47	BC007165
vanin 3: vnn3	↑3.43	NM_011979
formyl peptide receptor, related sequence 2: fpr-rs2	↑3.42	NM_008039
lipocalin 2: 24p3 protein	↑3.38	X14607
arylacetamide deacetylase (esterase): aadac	↑3.34	NM_023383
eosinophil-associated ribonuclease 2 precursor: ear2	↑3.33	AF238402
RIKEN cDNA 4933424C13 gene: src homology domain 2 containing protein	↑3.32	AK016886
gamma-aminobutyric acid (GABA-A) receptor, subunit gamma 1	↑3.24	AF156490
cystatin F (leukocystatin): cystatin 7	↑3.19	NM_009977
ikappab related protein: nfkbil2	↑3.18	AJ294539
toll-like receptor 5: tlr5	↑3.14	NM_016928
downregulated		
protocadherin gamma subfamily b, 2: pcdhgb2	↓8.53	NM_033575
formin: fmn	↓6.74	NM_010230
zinc finger protein 114: zfp110	↓6.64	AF167317
4a11 antigen	↓6.10	S74697
solute carrier family 8 (sodium/calcium exchanger), member 1	↓6.02	AF409089
g protein-coupled receptor: mrga5	↓5.58	AY042195
paraoxonase 2	↓5.40	BC021887
mutS homolog 3 (E. coli): msh3	↓5.14	NM_010829
m65 odorant receptor	↓4.73	AF283814
loss of heterozygosity, 11, chromosomal region 2, gene A homolog (human)	↓4.66	AK018055
RIKEN cDNA 4931431F19 gene: uba domain containing protein	↓4.66	AK016497
ATP-binding cassette, sub-family F (GCN20), member 1	↓4.59	AF213383
phosphatidylinositol 4-kinase type 2 beta	↓4.56	NM_028744
tyrosinase: tyr	↓4.55	NM_011661
troponin i, skeletal, fast 2: tnni2	↓4.37	NM_009405
similar to 2'-5' oligoadenylate synthetase 1b	↓4.24	BC012877
glucose-6-phosphatase, catalytic: g6pc	↓4.23	NM_008061
RF-amide G protein-coupled receptor: mrga1	↓4.05	AY042191
t-cell receptor gamma, variable 4: tcrg-v4	↓4.02	NM_011558
calcium channel, voltage-dependent, L type, alpha 1C subunit	↓3.88	L06233

5). Transthyretin has been well known to bind the Alzheimer beta-peptide and to be a possible protector against neurodegeneration. Recent findings suggest new roles of this protein related with depression-like behavior or other psychiatric disorders (Sousa *et al*., 2004).

cAMP responsive element binding protein 1 (CREB1) is included in the list of genes exclusively downregulated in hippocampus (Table 6). The role of CREB1 in long-term memory formation in hippocampus is already well known, and a number of reports showed that the change of this protein is closely related to the memory

Table 4. The most highly upregulated or downregulated genes in aged hippocampus

Gene	Fold change	GenBank Accession No.
upregulated		
GRB2-related adaptor protein 2: monocytic adaptor: mona	↑6.06	NM_010815
dachshund (drosophila): dach1	↑5.04	NM_007826
sulfotransferase family, cytosolic, 1c, member 1: sult1c1	↑4.63	NM_026935
transthyretin: ttr	↑4.35	NM_013697
peptidylprolyl isomerase F, opposite strand transcription unit	↑4.25	AK019686
homeobox transcription factor nkx2-3: nkx2-3	↑3.96	AF202036
T-cell receptor alpha, variable 22.1	↑3.93	M33590
chordin-like 1: neuralin 1	↑3.93	NM_031258
gamma-aminobutyric acid (gaba-a) receptor, subunit rho 1: gabrr1	↑3.82	NM_008075
sushi-repeat-containing protein, x-chromosome: srpx	↑3.78	NM_016911
similar to angiopoietin-like factor	↑3.68	BC023373
procollagen, type v, alpha 3: col5a3	↑3.60	NM_016919
dna methyltransferase 3b: dnmt3b	↑3.58	NM_010068
caspase 14: casp14	↑3.31	NM_009809
inhibitor of growth family, member 5	↑3.29	AK006421
matrix metallopeptidase 1b (interstitial collagenase)	↑3.25	NM_032007
hypothetical gene supported by AK048951: cdna clone rab7, member ras oncogene family	↑3.22	AK020089
RIKEN cDNA 4930431F10 gene, cdna clone similar to rnp particle component (fragment)	↑3.21	AK015266
cystatin 7: cst7	↑3.15	NM_009977
meprin 1 alpha: mep1a	↑3.15	NM_008585
downregulated		
protocadherin gamma subfamily b, 2: pcdhgb2	↓7.64	NM_033575
histocompatibility 2, class II antigen E beta	↓6.76	M28408
mutS homolog 3 (E. coli): msh3	↓6.02	NM_010829
Immunoglobulin heavy chain (gamma polypeptide)	↓5.84	X67210
formin: fmn	↓5.62	NM_010230
proteosome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2)	↓5.48	S59862
expressed sequence AI324046, protein for MGC:68300	↓5.24	L36938
endothelin 1: preproendothelin	↓4.91	U07982
alkaline phosphatase 2, liver: akp2	↓4.88	NM_007431
zinc finger protein 114: zfp110	↓4.88	AF167317
formyl peptide receptor-like 1: fprl1	↓4.43	NM_008042
RF-amide G protein-coupled receptor: mrga1	↓4.36	AY042196
glucagon receptor: gcgr	↓4.32	NM_008101
phosphatidylinositol 4-kinase type 2 beta	↓4.24	NM_028744
m65 odorant receptor	↓4.19	AF283814
membrane-spanning 4-domains, subfamily A, member 4B	↓4.18	NM_021718
adenosine a3 receptor: adora3	↓4.13	AY011209
immunoglobulin alpha heavy chain constant region: igh-2	↓3.89	AY045743
phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase, type III	↓3.79	AK017186
calcium channel, voltage-dependent, L type, alpha 1C subunit	↓3.79	L06233

dysfunction in the aging process (Kudo *et al*., 2005; Monti *et al*., 2005; Brightwell *et al*., 2004). This would be another example providing the reliability of our study results.

Finally we tried to analyze the distribution of up- or down-regulated genes according to the gene ontology classification that was provided by the microarray company, Macrogen. Although most of the gene ontology classes had similar distribution of up- and down-regulated genes, some of them showed interesting patterns (Table 7). For example, the genes involved in G-protein coupled receptor protein signaling pathways

Table 5. Examples of the genes that showed tissue-specific upregulation In old mice

Gene	GenBank Accession No	Fold Change		
		Cortex	Cerebell.	Hippocamp.
significantly upregulated in all three tissues				
GRB2-related adaptor protein 2: monocytic adaptor: mona	NM_010815	3.86	5.01	6.06
sushi-repeat-containing protein, x-chromosome: srpx	NM_016911	4.93	4.96	3.78
matrix metallopeptidase 1b (interstitial collagenase)	NM_032007	5.60	3.79	3.25
lipocalin 2: 24p3 protein	X14607	4.68	3.38	2.94
cystatin 7: cst7	NM_009977	3.90	3.19	3.15
significantly upregulated in cerebral cortex and cerebellum, but not in hippocampus				
RIKEN cDNA 4933424C13 gene: src homology domain 2 containing protein	AK016886	2.24	3.32	0.25
interferon regulatory factor 1: irf1	NM_008390	2.13	2.15	-1.53
interleukin 18 receptor accessory protein: il18rap	NM_010553	1.86	2.21	0.52
significantly upregulated in cerebellum and hippocampus, but not in cerebral cortex				
3-hydroxyanthranilate 3,4-dioxygenase: haao	NM_025325	0.07	1.88	2.80
serine protease inhibitor 13: spi13	NM_011455	-0.19	1.52	2.52
significantly upregulated in cerebral cortex and hippocampus, but not in cerebellum				
homeo box A3	K02591	3.12	0.08	2.59
basic leucine zipper transcription factor, ATF-like	NM_016767	2.61	1.18	2.71
chloride intracellular channel 5	AK017800	2.30	1.19	2.69
chemokine (C-C motif) receptor 7	NM_007719	2.53	0.13	2.24
significantly upregulated in only cerebral cortex				
fibroblast growth factor 21: fgf21	NM_020013	3.26	0.91	1.28
dickkopf homolog 4 (Xenopus laevis)	BC018400	3.22	0.23	0.47
tripartite motif protein trim6: trim6	AF220031	2.66	1.09	-0.45
cholinergic receptor, nicotinic, beta polypeptide 3	AK017571	2.63	0.98	0.99
similar to mannose-p-dolichol utilization defect 1 protein homolog	BC025220	2.44	-0.56	0.13
kinesin family member 23	AB054027	2.40	0.25	-0.30
significantly upregulated in only cerebellum				
bcl2-antagonist/killer 1: bak1	NM_007523	1.25	4.21	0.67
arylacetamide deacetylase (esterase): aadac	NM_023383	0.94	3.34	0.12
RIKEN cDNA 2610205E22 gene	BC027220	0.65	3.03	0.55
crystallin, beta b1: crybb1	NM_023695	0.48	2.78	0.38
flavin containing monooxygenase 1: fmo1	NM_010231	0.39	2.59	-0.60
gro1 oncogene: gro1	NM_008176	1.11	2.59	1.13
udp-n-acetyl-alpha-d-galactosamine:polypeptide n-acetylgalactosaminyltransferase 3: galnt3	NM_015736	1.13	2.51	0.00
s100 calcium-binding protein a9 (calgranulin b): s100a9	NM_009114	0.76	2.41	0.83
significantly upregulated in only hippocampus				
transthyretin: ttr	NM_013697	-0.02	-0.11	4.35
meprin 1 alpha: mep1a	NM_008585	0.65	-0.18	3.15
t-cell receptor beta chain	AF126466	0.00	-0.58	3.05
plectin 1	AK017743	-0.78	-0.04	3.03
purinergic receptor p2x-like 1, orphan receptor: p2rxl1	NM_011028	1.31	0.98	2.99
carbonic anhydrase 8	AK004923	-1.14	-0.13	2.93
corticotropin releasing hormone receptor 2: crhr2	NM_009953	-0.25	-0.04	2.87
histocompatibility 2, Q region locus 2	M14830	0.72	-0.77	2.75
calcium channel, voltage-dependent, R type, alpha 1E subunit	NM_009782	-1.32	1.10	2.73
transmembrane protein 27	NM_020626	0.85	0.84	2.67
triadin	AK009816	-0.35	0.38	2.55
RIKEN cDNA 1700013H16 gene: similar to synaptonemal complex protein 3	AK005953	1.32	0.44	2.50

Table 6. Examples of the genes that showed tissue-specific downregulation In old mice

Gene	GenBank Accession No	Fold Change		
		Cortex	Cerebell.	Hippocamp.
significantly downregulated in all three tissues				
protocadherin gamma subfamily b, 2: pcdhgb2	NM_033575	-8.97	-8.53	-7.64
formin: fmn	NM_010230	-5.07	-6.74	-5.62
solute carrier family 8 (sodium/calcium exchanger), member 1	AF409089	-5.76	-6.02	-3.23
RF-amide G protein-coupled receptor: mrga1	AY042191	-4.00	-4.05	-3.53
glomulin, FKBP associated protein: aw227515	NM_133248	-4.07	-3.57	-3.63
protein O-fucosyltransferase 2	BC018194	-3.81	-3.05	-3.62
laminin, alpha 1: lama1	NM_008480	-3.77	-3.27	-3.31
significantly downregulated in cerebral cortex and cerebellum, but not in hippocampus				
calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	AB025352	-3.41	-2.51	-0.23
loop tail associated protein: ltap	NM_033509	-2.34	-3.28	-1.18
sperm associated antigen 5	X81633	-3.42	-1.95	-1.22
soluble guanylyl cyclase beta2 subunit: gc-sb2	AF038500	-1.78	-2.75	0.99
flt3 ligand isoform 5h	S76459	-1.94	-2.34	-0.68
significantly downregulated in cerebellum and hippocampus, but not in cerebral cortex				
tyrosinase: tyr	NM_011661	-0.93	-4.55	-1.94
granuloma associated factor	L17305	-0.86	-2.10	-2.02
significantly downregulated in cerebral cortex and hippocampus, but not in cerebellum				
dna primase, p49 subunit: prim1	NM_008921	-3.81	-0.73	-2.55
fat cell-specific low molecular weight protein beta: falpbeta	AY079154	-2.27	-1.26	-2.44
granzyme a: gzma	NM_010370	-2.00	-0.89	-2.14
significantly downregulated in only cerebral cortex				
carbonic anhydrase 6: car6	NM_009802	-4.38	-1.79	-0.35
angiopoietin: agpt	NM_009640	-2.45	-0.33	-1.26
teratocarcinoma-derived growth factor: tdgf1	NM_011562	-2.42	-0.26	-1.00
cytochrome b-245, beta polypeptide: cybb	NM_007807	-2.35	0.29	0.40
bcr	X52831	-2.33	-0.75	0.29
significantly downregulated in only cerebellum				
cd36 antigen: cd36	NM_007643	-0.42	-3.40	0.21
endothelial differentiation, sphingolipid g-protein-coupled receptor, 3: edg3	NM_010101	-0.78	-3.29	-1.53
pancreatic lipase-related protein 2: pnliprp2	NM_011128	-0.55	-2.46	-0.77
phospholipase a2, group iif: pla2g2f	NM_012045	-0.71	-2.45	-0.18
acyloxyacyl hydrolase: aoah	NM_012054	-0.04	-2.34	0.49
significantly downregulated in only hippocampus				
expressed sequence AI324046, protein for MGC:68300	L36938	-0.59	0.66	-5.24
immunoglobulin alpha heavy chain constant region: igh-2	AY045743	-0.40	-0.29	-3.89
c-amp-responsive-element binding protein psi: creb	X67719	-0.99	-1.19	-3.31
musashi homolog 2 (drosophila): msi2h	NM_054043	-1.30	-0.58	-3.13
immunoglobulin lambda-2 chain	J00592	-0.64	-0.67	-3.00
epithelial protein lost in neoplasm: eplin-pending	NM_023063	0.56	-0.68	-2.86
CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	AK002232	-0.65	0.46	-2.78
paraoxonase 1: pon1	NM_011134	-0.98	0.51	-2.73
su(var)3-9 homolog suv39h2: suv39h2	AF149204	-0.48	1.78	-2.68
immunoglobulin joining chain	M12557	-0.23	0.19	-2.52
solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2: slc13a2	NM_022411	-0.75	-0.08	-2.51

Table 7. Gene ontology based analysis of gene expression change in the brain of old mice

Gene Ontology		Total	Upregulated genes			Downregulated genes		
			Cort	Cerebel	Hippoc	Cort	Cerebel	Hippoc
GO:0007389	pattern specification	63	6	0	3	0	0	0
GO:0006508	proteolysis and peptidolysis	321	14	3	14	9	2	10
GO:0006952	defense response	160	7	2	10	2	1	13
GO:0006955	immune response	203	8	11	13	9	3	14
GO:0006810	transport	667	12	5	26	15	10	17
GO:0007186	G-protein coupled receptor protein signaling pathway	351	9	3	17	14	10	8
GO:0006811	ion transport	248	8	5	12	8	6	5
GO:0006118	electron transport	172	5	4	9	2	1	3
GO:0007010	cytoskeleton organization and biogenesis	91	2	0	6	1	0	0
GO:0007242	intracellular signaling cascade	290	3	4	13	2	2	8
GO:0008152	metabolism	238	3	2	9	2	1	4
GO:0006812	cation transport	112	3	2	7	4	1	2
GO:0005975	carbohydrate metabolism	96	3	0	7	0	1	2
GO:0007264	small GTPase mediated signal transduction	106	1	3	6	1	0	1
GO:0007275	development	391	9	1	7	11	7	11
GO:0006915	apoptosis	185	4	3	4	2	2	8
GO:0007049	cell cycle	149	2	1	1	0	0	5

have a dominance of upregulation in hippocampus and downregulation in cerebellum. Similarly, the genes classified as small GTPase mediated signal transduction were more upregulated than downregulated in hippocampus. The meaning of these findings can not be easily explained. However, with the findingthat more genes are upregulated in hippocampus than in other tissues (Table 1) and several classes related with signaling or transport have tendency of upregulation in hippocampus (Table 7), we can suggest that the senescence in hippocampus may be linked to gain of function related with active signal transduction rather than just passive changes of dysfunction. We believe that a lot of investigations are still required in this area, and our result may provide some useful tools for studying the molecular aspect of aging in the brain.

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