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Gene expression patterns of hippocampus and cerebral cortex of senescence-accelerated mouse treated with Huang-Lian-Jie-Du decoction

Zheng Yue, Cheng Xiao-Rui, Zhou Wen-Xia, Zhang Yong-Xiang*

Department of Neuroimmunopharmacology, Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Beijing 100850, China

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ABSTRACT

Alzheimer's disease (AD) is a progressive, neurodegenerative disease, which primarily affects the elderly. Clinical signs of AD are characterized by the neuron loss and cognitive impairment. At gene and protein levels, the senescence-accelerated mouse/prone 8 (SAMP8) is a suitable animal model to investigate the fundamental mechanisms of age-related learning and memory deficits. Huang-Lian-Jie-Du decoction (HL), a well-known traditional Chinese medicinal prescription, has been employed in the treatment of wide range of disease conditions. Modern pharmacological studies have showed that HL possesses many effects, which include amelioration of learning and memory function of CNS. This paper investigated the gene expression patterns of hippocampus and cerebral cortex of SAMP8, which were treated with HL employing the cDNA microarray and real time quantitative RT-PCR techniques. The results showed that HL has the significant modulating effects on age-related changes of the gene expressions in the hippocampus and cerebral cortex in SAMP8, which include genes that involved in signal transduction (Dusp12, Rps6ka1, Rab26, Penk1, Nope, Leng8, Syde1, Phb, Def8, Ihpk1, Tac2, Pik3c2a), protein metabolism (Ttc3, Amfr, Prr6, Ube2d2), cell growth and development (Ngrn, Anln, Dip3b, Acrbp), nucleic acid metabolism (Fhit, Itm2c, Cstf2t, Ddx3x, Ercc5, Pcgfr6), energy metabolism (Stub1, Uqcr, Nsf), immune response (C1qb), regulation of transcription (D1ertd161e, Gcn5l2, Ssu72), transporter (Slc17a7, mt-Co1), nervous system development (Trim3), neurogila cell differentiation (Tspan2) and 24 genes whose biological function and process were still unknown. It was suggested by the changes of the 62 genes with HL treatment that the ameliorating effect of HL on the cognitive impairments of SAMP8 might be achieved by multi-mechanism and multi-targets.

Alzheimer disease (AD) is the most common form of progressive dementia in the elderly. It is a neurodegenerative disorder characterized by the neuropathologic findings of intracellular neurofibrillary tangles (NFTs) and extracellular amyloid plaques that accumulate in vulnerable brain regions. Genes of amyloid precursor protein (APP), APOE4, presenilin-1 (PSEN1), PSEN2, cystatin-3 (CST3) [11], PAXIP1 and NOS3 [19], etc. have been identified as causes of AD, but for this multi-factorial diseases, there could be many more unknown genes which play very important roles in AD.

The current drugs only provide limited or transient benefit to many patients [27]. In the past, drug discovery merely based on a single-target-directed strategy, such as β-amyloid, AChE inhibitor, NMDA-receptor antagonists, neurotrophic factor, etc. The method seems inefficient for the treatment of complex diseases which have multiple pathogenic factors [2]. In fact, the traditional Chinese medicine and their effective components have been proven their own inimitable predominance with multi-factorial, multi-target and multi-functional action, such as modulation of cholinergic systems, anti-oxidant, anti-amyloid, and anti-inflammatory [1].

Huang-Lian-Jie-Du decoction (HL) is a very recognized traditional Chinese medicinal prescription, which consists of *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex* and *Gardeniae fructus*. The main effects of HL, described in traditional Chinese medicine, are "Purging the fire and detoxifying". It has been used for treating many diseases conditions over the century. Modern pharmacological studies demonstrate that HL possesses wide effects, including the therapies of cerebrovascular disease [13], gastritis [18], inflammation [24], etc., especially used for the therapies of many kinds of dementia in clinic in China and Japan. Studies showed HL plays a crucial protective role in ischemia-induced brain injury [12] and increases cerebral blood flow in the areas around margins of ischemia and reduces the size of the infarction [16]. It improves the microcirculation through lipid and protein metabolisms, and is useful for the treatment of cerebral vascular attack in human [21]. Moreover, investigation showed that HL can improve the disruption of spatial cognition induced by cerebral ischemia and central

* Corresponding author. Tel.: +86 10 68182998; fax: +86 10 68270676.
E-mail address: zhangyx@nic.bmi.ac.cn (Y.-X. Zhang).

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cholinergic dysfunction in rats [10], and showed HL prolonged the step-down latency significantly and decreased the step-down errors in the passive avoidance task, as well as shortened the latency of escaping markedly onto the platform in the training trial and increased the percentage of crossing the former platform quadrant in the probe trial in the Morris water maze test [26]. In addition, another study showed that HL significantly improved the learning behaviors in AD rats, which suggested that HL ameliorates age-related deterioration of learning and memory [8]. Although the studies showed HL against the impairment of learning memory might be associated with the prevention of a decrease in acetylcholine contents or amelioration immune function and reduction of oxidative stress, the active mechanism of HL on enhancing the cognitive ability is still unknown.

The senescence-accelerated mouse/prone 8 (SAMP8) strain, a substrain of SAM, has been proposed as a suitable animal model of age-related cognitive decline with relevance to alterations of the gene expression and protein abnormalities in AD [3,6], with the features of an increase in hyperphosphorylated forms of tau in the brain [4], age-related increases in the level of hippocampal Aβ-peptide, learning and memory deficits, and a shorter lifespan than their controls, SAM/resistant1 (SAMR1) [17].

Original SAMR1 and SAMP8 mouse were kindly provided by Dr. T. Takeda at Kyoto University (Kyoto, Japan) and maintained at Beijing Institute of Pharmacology and Toxicology under conditions of natural light–dark cycle (12-h light; 12-h dark), temperature ($25 \pm 1\,^{\circ}C$) and relative humidity ($50 \pm 5\%$). All procedures were carried out according to the Care and Use guide of laboratory animals by the NIH. Only 6-month-old male SAMP8 and SAMR1 were employed.

Traditional Chinese medicinal herbs, *C. rhizoma*, *S. radix*, *P. cortex* and *G. fructus* were purchased from Beijing Tong-Ren-Tang drug store and subjected to pharmacognosy identification before preparing the decoction. HL decoction was prepared with decocting *C. rhizoma* and *P. cortex* in the ratio of 3:2 (dry weight), keeping on 30 min in 75–80 °C water each time and three times in total. Meanwhile *S. radix* and *G. fructus* were decocted with 75–80 °C water keeping on 50 min each time and three times in total, according to the ratio of 2:3 (dry weight). These two decoctions were filtered, concentrated, sterilized and made to the final concentration of 1 g/ml d.w. This process was done according to the preparing method of Chinese traditional medicine, respectively and put together partes aequales before treatment. To ensure homogenicity of HL extract, coldspray ionisation-mass spectrometry (CSI-MS) technique was applied to detect the active components of HL extract including coptisine, berberine, palmatine, baicalin, wogonoside and as a quality control method (Fig. 1).

Eight SAMP8 were administrated by gastric intubation 5 g/kg mouse body weight of HL suspended in distilled water every day for 1 month as HL-treated group. Eight SAMR1 and 24 SAMP8 were used for comparison with same volume distilled water as above group every day for 1 month as normal animal control group and model control group, respectively.

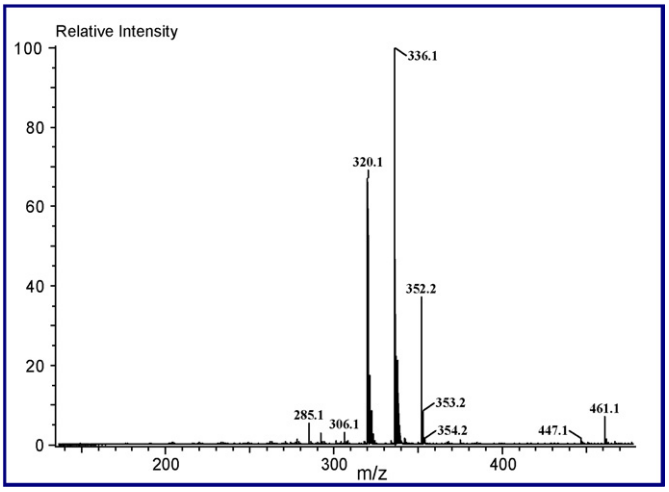

Fig. 1. The active components of HL analyzed by (+) CSI-MS spectrum. *m/z* 320, *m/z* 336, *m/z* 352, *m/z* 447, *m/z* 461 in the figure represent coptisine, berberine, palmatine, baicalin, wogonoside of the five active components in Huang-Lian-Jie-Du decoction, respectively.

Total RNA was purified from the hippocampus and cerebral cortex tissue of each group using TRIZOL Reagent (Invitrogen Cat. No. 15596-026). The integrity of total RNA was detected by agarose gel; the purity and concentration were detected by the spectrophotometer (NanoDrop, ND-1000).

Fifty micrograms of total RNA from hippocampus and cerebral cortex of each group were used, respectively for reverse transcription according to standard protocols using SUPERSCRIPT III reverse transcriptase (Invitrogen Cat. No. 18080-044) and aa-dUTP (Amersham Pharamacia Biotech). Subsequently, aa-cDNA first strand was purified using QIAquick PCR purification kit (QIAGEN Cat. No. 28104). The purified aa-dUTP of control group and treated group were labeled with cy5 or cy3 monofunctional dye (Amersham Pharamacia Biotech), respectively. The corresponding fluorescent labeled with cy3 and cy5 cDNA were combined, purified using MinEluteTM Reaction Cleanup Kit (QIAGEN Cat. No. 28204) and lyophilized in the nucleic drier (SPD1010 SpeedVac).

Amino-groups on the spotted slide [7] were blocked at room temperature through washing with $NaBH_4$/PBS/ethanol. Pre-hybridization in hybridization box was performed for 1 h at 42 °C plating with buffer containing 5× SSC, 0.1% SDS, 1% BSA under glass cover-slip in hybridization chamber (Robbins Scientific Co.). After pre-hybridization, the slide was washed and dried immediately. The above-mentioned dried fluorescent labeled cDNA was resolved with 15 μl hybridization buffer, denatured at 98 °C for 5 min, centrifuged at 12,000 rpm for 5 min and then the whole supernatant was pipetted and spread on the slide. The procedure of hybridization was same to the pre-hybridization except 18-h hybridization time. Subsequently, the slide was washed and dried immediately.

Table 1
The primer sequence of seven genes for real-time quantitative PCR

Gene name	Forward (5′→3′)	Reverse (5′→3′)	Size (bp)
Rab26	AGAAGCTGGCCAAGGAGTATG	TCGACCCTCCCTCTTAACGTA	170
Slc17a7	CCCCACCCTTAGAACGGAGT	GGAGACGAGCAGCCAGAACA	176
CaMKII	CCTACACGAAGATGTGCGACC	ATCAGGTGGATGTGAGGGTT	163
Stub1	GAGGCCAAGCACGATAAATAC	GTGATACCACTGGGTGTAATGC	152
Ube2d2	TTCTACGGTCACAGTGGTCTC	CGCATACTTCTGAGTCCATTCC	176
Ngrn	ACCATTGGCCACTTTGGAGTA	CAACAGAGAGACCACCAAGCA	151
Ttc3	GGAAGATGCTGTCTGCTCAC	TGGTGGAAGGATAAGGAAGGAG	173
β-Actin	TTGCTGACAGGATGCAGAAGGAG	GTGGACAGTGAGGCCAGGAT	127

In the hybridization, the gene expression patterns of SAMP8 were that the water-treated SAMP8 group was hybridized with the water-treated SAMR1 group, and the HL-treated SAMP8 was that the HL-treated SAMP8 group hybridized with the water-treated SAMP8.

Hybridized array was scanned and analyzed using GenePix 4100A microarray scanner (Axon Instruments Inc.) and GenePix Pro 5.1 software (Axon Instruments Inc.) which was combined with Microsoft Excel software. Spots were analyzed by adaptive quantification, and the local background was subsequently subtracted. Spots with background-corrected signal intensity (median) in both channels less than twofold of background intensity (median) were rejected from further analysis. After normalization with data representing G3PDH and β-actin comparing expression in treated and control groups, statistic analysis of each remaining gene on six replicate microarray slides for experimental repetatur was implemented using SAM software (significance analysis microarray, Stanford University) [23] with one class mode ($\delta = 0.96938$).

Table 2
Differential expression genes in the hippocampus of SAMP8 treated with Huang-Lian-Jie-Du decoction

Gene symbol/access number	Gene name	Expression level (microarray ratio)	
		SAMP8	SAMP8 treated with HL
Nucleic acid metabolism			
Ddx3x	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked	+1.055	−1.665
Fhit	Fragile histidine triad	−1.010	+3.013
Ercc5	Excision repair cross-complementing rodent repair deficiency, complementation group 5	+1.030	−1.901
Pcgfr6	Polycomb group ring finger 6	+1.416	−1.868
Cstf2t	Cleavage stimulation factor, 3'-pre-RNA subunit 2, tau	−1.032	−1.623
Itm2c	Integral membrane protein 2C	−1.543	+1.626
Transport			
Slc17a7	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	−1.281	+3.758
Signal transduction			
Rps6ka1	Ribosomal protein S6 kinase polypeptide 1	−1.202	+2.991
Leng8	Leukocyte receptor cluster (LRC) member 8	−1.031	+1.825
Nope	Neighbor of Punc E11	+1.542	−2.199
Dusp12	Dual specificity phosphatase 12	+1.453	−1.616
Penk1	Preproenkephalin 1	+2.696	+1.809
Cell growth			
Ngrn	Neugrin, neurite outgrowth associated	−1.436	−1.747
Anln	Anillin, actin binding protein (scraps homolog, Drosophila)	+1.063	−2.100
Regulation of transcription			
D1Ertd161e	DNA segment, Chr 1, ERATO Doi 161, expressed	+1.086	−1.657
Gcn5l2	GCN5 general control of amino acid synthesis-like 2 (yeast)	−1.127	−3.054
Ssu72	Ssu72 RNA polymerase II CTD phosphatase homolog (yeast)	+1.820	−1.785
Protein metabolism			
Ttc3	Tetratricopeptide repeat domain 3	+1.021	−1.669
Prr6	Proline-rich polypeptide 6	+1.788	−1.658
Amfr	Autocrine motility factor receptor	−1.186	−1.663
Ube2d2	Ubiquitin-conjugating enzyme E2D 2	−1.530	+1.747
Energy metabolism			
Stub1	STIP1 homology and U-box containing protein 1	−1.044	−1.663
Immune response			
C1qb	Complement component 1, q subcomponent, beta polypeptide	+1.047	−1.647
Unknown			
GI:70909346	Ribosomal protein S19 binding protein 1	−1.051	+2.562
AE008685.1	T-cell receptor alpha/delta locus section 3 of 4 of the complete region	/	−2.569
AC131191.4	Mus musculus BAC clone RP24-464B16 from chromosome 18, complete sequence	+1.129	+2.693
AC087166.3	Mus musculus strain C57BL/6J chromosome 12 clone RP23-354D10, complete sequence	/	−3.985
AC044864.6	Mus musculus chromosome 3, clone RP23-168E14, complete sequence	/	−2.738
AC165962.4	Mus musculus BAC clone RP23-362B7 from chromosome 17, complete sequence	+1.203	−2.035
BC027563.1	RIKEN cDNA 2410030A14 gene	−1.133	−1.735
AK019336.1	RIKEN clone: 2900045A19	+1.201	−1.625
AC122053.3	Mus musculus BAC clone RP24-463C3 from 3, complete sequence	−1.212	−1.655
AK078087.1	RIKEN clone: 6330439K17	−1.058	−1.637
AL593843.9	Mouse DNA sequence from clone RP23-136D4 on chromosome 11, complete sequence	+1.180	−3.250
AC110028.8	Mus musculus chromosome 6, clone RP23-44E17, complete sequence	+1.390	−2.161

Unknown gene name is the access number; (+) up-regulated; (−) down-regulated; (/) invalid data; that the ratio of gene expression is more than 1.6 was been defined the significant change.

Table 3
Differential expression genes in the cerebral cortex of SAMP8 treated with Huang-Lian-Jie-Du decoction

Gene symbol/access number	Gene name	Expression level (microarray ratio)	
		SAMP8	SAMP8 treated with HL
Nucleic acid metabolism			
Cstf2t	Cleavage stimulation factor, 3'-pre-RNA subunit 2, tau	+1.122	+1.882
Itm2c	Integral membrane protein 2C	+1.002	−1.667
Fhit	Fragile histidine triad	+1.518	−1.646
Signal transduction			
Syde1	Synapse defective 1, Rho GTPase, homolog 1 (*C. elegans*)	+1.199	−2.356
Rps6ka1	Ribosomal protein S6 kinase polypeptide 1	+1.052	−1.760
Phb	Prohibitin	+1.265	−2.015
Def8	Differentially expressed in FDCP 8	+1.274	−2.074
Rab26	Ribosomal protein S6 kinase polypeptide 1	+1.708	−2.006
Dusp12	Dual specificity phosphatase 12	+1.269	−2.381
Ihpk1	Inositol hexaphosphate kinase 1	+1.654	−1.704
Pik3c2a	Phosphatidylinositol 3-kinase, C2 domain containing, alpha polypeptide	/	+1.913
Tac2	Tachykinin 2	/	−2.020
Transport			
Slc17a7	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	+1.008	−1.920
Cell growth and development			
Ngrn	Neugrin, neurite outgrowth associated	/	−2.018
Dip3b	Dip3 beta	+1.195	−1.870
Acrbp	Proacrosin binding protein	+1.398	−1.820
Regulation of transcription			
D1Ertd161e	DNA segment, Chr 1, ERATO Doi 161, expressed	+1.347	−2.139
Protein metabolism			
Ttc3	Tetratricopeptide repeat domain 3	+1.250	−2.197
Prr6	Proline-rich polypeptide 6	+1.339	−2.347
Amfr	Autocrine motility factor receptor	+1.178	−1.708
Energy metabolism			
Stub1	STIP1 homology and U-Box containing protein 1	+1.509	−1.608
Uqcr	Ubiquinol-cytochrome *c* reductase (6.4 kDa) subunit	+1.564	−1.642
Nsf	*N*-Ethylmaleimide sensitive fusion protein	+1.243	−2.003
Immune response			
C1qb	Complement C1q subcomponent, B chain precursor	+1.145	−1.692
Nervous system development			
Trim3	Tripartite motif protein 3	+1.236	−2.286
Electron transport			
mt-Co1	Cytochrome *c* oxidase I, mitochondrial	+1.205	−2.137
Neurogila cell differentiation			
Tspan2	Tetraspanin 2	/	+1.932
Unknown			
AC131191.4	Mus musculus BAC clone RP24-464B16 from chromosome 18, complete sequence	+1.430	−1.622
AC127342.3	Mus musculus chromosome 18 clone RP23-191B18, complete sequence	+1.152	−1.752
AL845475.12	Mouse DNA sequence from clone RP23-403E20 on chromosome 11, complete sequence	+1.097	−1.723
AK078087.1	RIKEN clone: 6330439K17	+1.271	−2.013
NM_177049.4	RIKEN cDNA 9330157P13 gene	+1.138	−2.040
AL596331.15	Mouse DNA sequence from clone RP23-81G14 on chromosome 11, complete sequence	+1.245	−2.461
AK086633.1	RIKEN clone: D930023J12	+1.216	−1.654
AL808119.6	Mouse DNA sequence from clone RP23-70J9 on chromosome 2	+1.212	−1.657
AC116950.5	Mus musculus chromosome 15 clone RP23-56K4, complete sequence	+1.056	−2.148
BC055934.1	Mus musculus RIKEN cDNA 6720401G13 gene, mRNA (cDNA clone IMAGE: 3965282), partial cds	+1.130	−2.143
AL672100.7	Mouse DNA sequence from clone RP23-125A1 on chromosome 2, complete sequence	+1.187	−2.189
AB175685.1	Mus musculus mpp-GalNAc-T18(O18) mRNA for UDP-*N*-acetyl-alpha-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase, complete cds	/	+1.770
AK081295.1	RIKEN clone: C030048J15	+1.328	−2.452
NM_130885.1	RIKEN cDNA 1110064A23	+1.314	−2.383
AK013859.1	RIKEN clone: 3000002F03	+1.298	−2.212

Unknown gene name is the access number; (+) up-regulated; (−) down-regulated; (/) invalid data; that the ratio of gene expression is more than 1.6 was been defined the significant change.

In this study, the absolute value of gene change fold exceeding 1.6 represented significant expression change.

Seven genes were chosen to conduct quantitative RT-PCR (q-RT-PCR) in order to validate the altered gene expression identified by cDNA microarray experiments. The genes represented different expression character, including up-regulated, down-regulated and quiet regulated and possessed different biological functions. The synthesis of first cDNA strand from total RNA was abided by above reverse transcription procedure using oligo dT. Q-PCR was implemented on the LightCycler (ROCHE, LightCycler Operator) by monitoring the amount of fluorescence increase due to the binding of SYBR green to double-stranded DNA which used specific designed primer (Table 1). Dissolve curve analysis was performed after the end of every PCR reaction to ensure the product was specific. The amplification was performed according to the following scheme: 94 °C denature, 60 °C anneal and 72 °C elongation. Data was calculated with respective standard curve of each gene template, normalized with β-actin, calculated the mean ± S.D. ($n = 3$) in the Excel software.

To identify the changes of gene expression of HL effecting on the cognitive deterioration of SAMP8 and study the mechanism of the amelioration of HL on learning and memory in SAMP8, two gene expression patterns in hippocampus and cerebral cortex were compared and assessed, respectively. The results showed there were 35 differentially expressed genes in the hippocampus (Table 2) and 42 differentially expressed genes in the cerebral cortex of SAMP8 after administrated with HL (Table 3).

Gene expression change of Ube2d2 turned from low expression in the hippocampus of SAMP8 to up-regulation after administrated with HL, namely some gene expression in animal model of accelerated senescence were adversely affected by the HL administration. The nucleic acid metabolism gene Itm2c also showed the same regulation as gene Ube2d2 after HL administrated. In addition, gene Fhit, Slc17a7, Rps6ka1, Rps19bp1, Leng8, and AC131191.4 expression turned from the mild down-regulation in the hippocampus of SAMP8 to the significant up-regulation after treatment with HL.

Gene Dusp12, Ssu72, Prr6, Nope, Pcgfr6, Penk and AC110028.8 showed down-regulation in the hippocampus of SAMP8 after treatment with HL, while up-regulation in different level in SAMP8. Analogously, gene Ddx3x, Ercc5, Cstf2t, Anln, D1ertd161e, Gcn5l2, Amfr, Stub1, Ngrn, C1qb, AC087166.3, AC044864.6, AK019336.1, AC122053.3, AK078087.1, BC027563.1, AE008685.1, AC165962.4 and AL593843.9 showed down-regulation in the hippocampus of SAMP8 after treatment with HL, except for significant change of expression in SAMP8.

Of these 42 differentially expressed genes in the cerebral cortex of SAMP8, the expression change of gene Fhit, Rab26, Ihpk1, Stub1, Uqcr and AC131191.4 showed down-regulated significantly after treatment with HL corresponding to the up-regulation in SAMP8 comparing with SAMR1.

Furthermore, the expression of gene Itm2c, Syde1, Rps6ka1, Phb, Def8, Dusp12, Slc17a7, Dip3b, Acrbp, D1ertd161e, Ttc3, Prr6, Amfr, Nsf, C1qb, Trim3, mt-Co1, AC127342.3, AL845475.12, AK078087.1, NM_177049.4, AL596331.15, AK086633.1, AL808119.6, AC116950.5, BC055934.1, AL672100.7, AK081295.1, NM_130885.1, AK013859.1 showed down-regulated significantly in the cerebral cortex of SAMP8 with HL treatment, but no obviously expression change in SAMP8. Corresponding to these genes, gene Cstf2t, Pik3c2a, Tspan2 and AB175685.1 showed significant up-regulation in the cerebral cortex of SAMP8 with HL treatment.

By comparing the above differentially expressed genes in the hippocampus and cerebral cortex, we procured the common differential expression gene responding to the HL, except for some specific gene in hippocampus or cerebral cortex. There were 14 genes responding to the HL at the same time, but the genes showed different change patterns, which were Dusp12, Ngrn, D1ertd161e, Ttc3, Prr6, Amfr, Stub1, C1qb, Itm2c, Fhit, Slc17a7, Rps6ka1, AC131191.4 and AK078087.1. Of these 14 genes, Dusp12, Ngrn, D1ertd161e, Ttc3, Prr6, Amfr, Stub1, C1qb in hippocampus and cerebral cortex presented the similar directional response to the HL, but gene Itm2c, Fhit, Slc17a7, Rps6ka1, AC131191.4, AK078087.1 did not.

In order to validate the gene differential expression identified by the cDNA microarray screening and analysis, the expression of some gene was detected using the q-RT-PCR technique. These genes which represented different biological function were Stub1, Slc17a7, Ube2d2, Ngrn, Ttc3, CaMKII, Rab26, and the housekeeping gene β-actin as control. Results showed that the relative expression ratios of these seven genes in the hippocampus and cerebral cortex of SAMP8 after HL administration comparing with SAMP8 detected by the q-RT-PCR did not significantly differ from the results of microarray screening and analysis (Fig. 2). Although the detailed

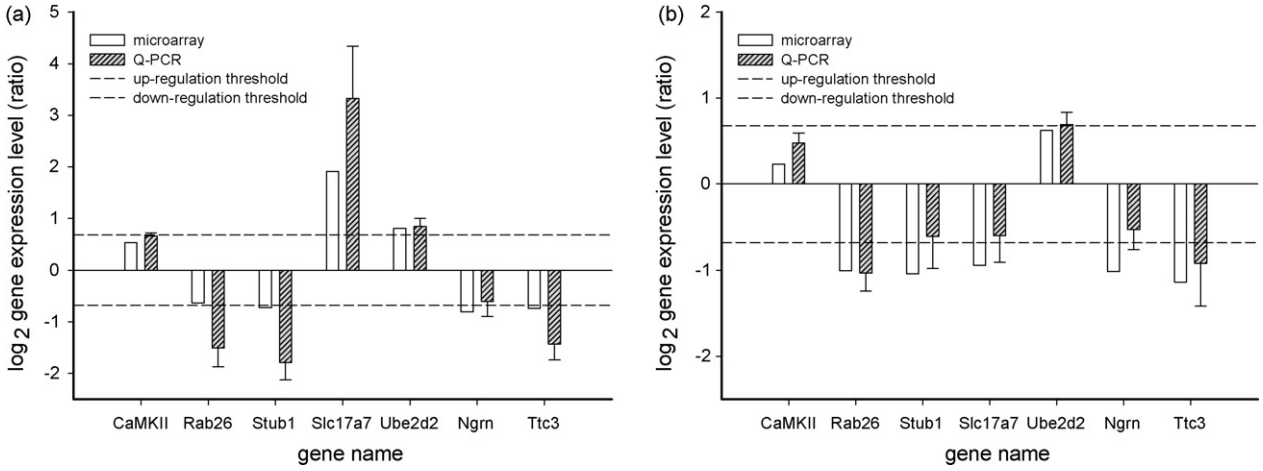

Fig. 2. The gene expression in the hippocampus (a) and cerebral cortex (b) of SAMP8 treated with Huang-Lian-Jie-Du decoction comparing with SAMP8 by the cDNA microarray and real-time quantitative RT-PCR. In order to validate the gene differential expression identified by the cDNA microarray analysis, the gene expression in the same sample used by the cDNA microarray analysis was detected by the quantitative RT-PCR. Seven genes were Stub1, Slc17a7, Ube2d2, Ngrn, Ttc3, CaMKII and Rab26, respectively. Statistical analysis of microarray was performed by the significance analysis microarray software. Results of quantitative RT-PCR were expressed as mean ± S.D. from three individual experiments. The dashed lines indicated the interval −1.6- to 1.6-fold regulation (corresponding to $\log_2 = 0.678$) in which changes in expression were considered not significant.

data was not completely equal, the tendency of expression change was same or consistent. Therefore, the genes of differential expression identified by the cDNA microarray analysis were creditable.

Of the 62 differential expression genes, gene ubiquitin-conjugating enzyme E2D 2 (Ube2d2) is a highly conserved 76-amino acid polypeptide which is found covalently bound to target proteins as monomers or chains. Endoplasmic reticulum-associated degradation (ERAD) serves to degrade misfolded proteins or otherwise functionally denatured proteins. This ubiquitylation has important implications for many diseases, which include cystic fibrosis, neurodegenerative disorders [5,9]. As all known, in the pathway of protein degradation in eukaryotic cells, the substrate protein is catalyzed by ubiquitin-conjugating enzyme and ubiquitin-protein ligases, namely the ubiquitin is transferred from the former to the substrate protein through the latter. Interestingly, Ube2d2 and its interaction protein including Amfr and Stub1 were all in the list of differential expression genes in the hippocampus and cerebral cortex of SAMP8 after HL administration in this study. In fact, Stub1 and Amfr are ubiquitin-protein ligases [14,25], this result hinted that protein degradation and quality control were one of HL's regulatory functions. In addition, tau binds to Hsc70, and its phosphorylation is a recognition requirement for the addition of ubiquitin by the ubiquitin-protein ligases Stub1. The study showed Hsp70/Stub1 played an important role in the pathogenesis of tauopathies [22]. A report [15] demonstrated that hippocampal expression of NLK and its receptor Amfr were associated with maze learning in rats. Another report [20] demonstrated that NLK is up-regulated in the brain during Huntington's disease (HD), a neurodegenerative disorder. This might be a balanced regulation process which is still obscure. This study showed that HL regulated the expression of gene Ube2d2, Stub1 and Afmr at the same time in the hippocampus and cerebral cortex of SAMP8 characterizing cognitive deterioration with aging.

Meanwhile there are 25 novel genes without any functional clues response to HL to further study. These genes could be very important to identify the mechanisms of HL improving the cognitive impairment of SAMP8.

In conclusion, the effects of HL in treating SAMP8's cognitive deterioration with aging might be associated with cellular proliferation and differentiation, protecting the normal synaptic transmission, regulating signaling transduction, and improving energy and protein metabolism. The differential expression genes caused by HL involvement in multi-pathway indicated that HL produced marked effect with multi-factorial, multi-target, and multi-functional action. These above-described genes including genes with functional clue and uncertain function might be the potential gene targets for HL effects on the cognitive impairments. Further experiments are being conducted to advance more into an in-depth analysis of the gene targets and mechanism of Huang-Lian-Jie-Du decoction effects on the cognitive impairments and AD.

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