

Basic Investigation

Effects of Acupuncture on Expressions of the Transcription Factors NF-E2, YB-1, LRG47 in the SAMP10 Mice

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Objective: To explore the mechanism of acupuncture for delaying aging. **Methods:** Using the senescence accelerated mouse pattern SAMP10 and the normal aging mice SAMR1 as models and applying RT-PCR and digoxin (DIG)-labeled Northern blot technique to observe expressions of NF-E2, YB-1, LRG47 genes in the forebrain, cortex and hippocampus in a 8-month old SAMR1 control group, a 8-month old SAMP10 control group, a 8-month old SAMP10 acupuncture group and a 8-month old SAMP10 non-point stimulation group. **Results:** In the SAMP10 control group, the expressions of NF-E2, YB-1 and LRG47 were down-regulated in the forebrain, cortex and hippocampus, and after acupuncture they were up-regulated and tended to normal. **Conclusion:** The brain aging of the SAMP10 mice is related with abnormal expressions of NF-E2, YB-1 and LRG47 genes; and acupuncture can regulate the expressions of NF-E2, YB-1 and LRG47 genes, strengthening the functions of erythrocyte series, increasing the proliferation of cells and enhancing the cellular immune function in anti-bacteria, hence delaying aging.

Key words: aging/acup-mox eff; transcription factors, general/acup-mox eff; genes, regulator/acup-mox; blotting, northern; mice

The aging of brain is one of the glaring manifestations of human aging, and SAMP10 is a internationally recognized natural senescence accelerated mice pattern introduced from Japan, characterized biologically by rapid aging after an overgrowth stage, exfoliation of neurons in the cortex, encephalatrophy, and accompanied by learning disorder and dysmnnesia, with a mean life span of 333 days. The abnormal changes in biochemical indexes of SAMP10 indicate that the occurrences of aging and aging-related diseases are related with the abnormal expressions of some genes. It has been reported that a great number of transcription factors can regulate the transcription rate of special genes through directly influencing the regulative sequence of genetic promoter, playing a key role in anti-aging; and the transcription factors can regulate the decline of physiological functions and the apparent increase of aging genes along with aging of the organism.¹ Therefore, in the present study, the 3 transcription factors NF-E2, YB-1 and LRG47 were selected, the normal aging mice of same strain (SAMR1) were

used as the controls, and the DIG-labeled Northern blot method was adopted to observe the changes of expressions of NF-E2, YB-1, LRG47 gene mRNAs in the forebrain, cortex, hippocampus of the SAMP10 mice so as to probe into the effects of acupuncture, reveal the mechanisms of brain aging in the SAMP10 mice, and partially elucidate the molecular biological mechanisms of acupuncture in prevention and treatment of brain aging.

MATERIALS AND METHODS

Selection and grouping of animals

All of the mice were supplied by the Center for Experimental Animals, the First Affiliated Hospital of Tianjin College of TCM. Thirty SAMP10 mice aged 8 months, 15 male and 15 female, were randomly divided into 3 groups, the SAMP10 control group, the SAMP10 acupuncture group and the SAMP10 non-point stimulation group; and 10 SAMR1 mice aged 8 months were selected as the SAMR1 control group.

Method of treatment

For the SAMP10 acupuncture group, “Tanzhong” (CV 17), “Zhongwan” (CV 12), “Qihai” (CV 6), “Xuehai” (SP 10) and “Zusanli” (ST 36) were selected and located according to “*The Atlas of Acupoints of Animals*” drawn up by Experimental Acupuncture and Moxibustion Research Society, Chinese Association of Acupuncture and Moxibustion. Acupuncture methods: “Tanzhong” (CV 17), “Zhongwan” (CV 12), “Qihai” (CV 6) and “Zusanli” (ST 36) were punctured by the reinforcing method with twirling manipulation respectively for 30 s, and “Xuehai” (SP 10) by the reducing method with twirling manipulation for 30 s.

For the SAMP10 non-point stimulation group, the fixed non-points on bilateral costal regions were punctured and manipulated by the uniform reinforcing-reducing method respectively for 30 s. The mice in the SAMP10 control group and the SAMR1 control group were grasped in the same degree as that in the acupuncture group. All the groups of mice were treated once a day, for consecutive two weeks with a one-day interval in the 7th day, and then they were killed by dislocation of the cervical vertebrae. The whole brain was taken rapidly and put into liquid nitrogen for quick-freeze, and kept at -80°C .

Reverse transcription polymerase chain reaction (RT-PCR):

1) Designing synthesis of the primers: All the 4 pairs of primers were quoted from uniSTS of PUBMAD. NF-E2: upstream, AACGACCCTGTTCTTAGCGA; the downstream, GCTCGGCAGATTTGACGATG. YB-1: the upstream, CATCAACAGGAATGACAC; the downstream, the GCGTCTATAATGGTTACG. LRG47: the upstream, GAACACCAACTGCTTTTCCG; the downstream, CAGAAGGAAAATTGATTTGGACA. β -action: the upstream, GTATGCC-TCTGGTCGTACCAC; the downstream, CTAGAC-TTCGAGCAGGAGATGG. For the length of amplification, NF-E2: 376bp; YB-1: 220bp; LRG47: 238bp; and β -action: 256bp.

2) Extraction of total RNA of the mouse forebrain: The total RNA of the mice forebrain was extracted by the method (spun-column type) introduced by the kit for total RNA extraction supplied by Beijing Tianwei

Shidai Biological Company. Electrophoresis was used for examination of the integrity, the OD260/280 ratio was determined by an ultraviolet spectrometer, and the RNA content was calculated and regulated to a same concentration.

3) RT-PCR: RT-PCR was carried out according to directions of RT-PCT kit made by Shanghai Shenggong Company. Reaction condition of PCR: Pre-denaturation for 2 min at 94°C , followed by amplification under the following circulation condition: denaturation 45 s at 94°C → annealing for 45 s at 60°C → extension for 90 s at 72°C , circulation 35 times; and finally, extension for 10 min at 72°C .

Analysis of the DIG labeled northern blot

The products of PCR were extracted and purified by phenol-chloroform, and labeled with digoxin (DIG) RNA probe PCR method of Lou's DIG northern starter Kit, and the labeling efficacy was detected. After the total RNA of the mouse was isolated and denatured by 1.5% MOPS formaldehyde denatured gel, electrotransfer membrane was carried out by a transfer electrophoresis apparatus made by Beijing Liuyi Factory, and dried for 0.5 h at 200°C . The RNA was fixed at a nylon membrane with positive charge, and Northern hybridization was made according to directions of Lou's DIG northern starter Kit, under X-ray-film squash was conducted for 4 h in a dark box. The staining degrees of the hybrid zones on the X-ray film were analyzed by genetools of gel imager made by Cyngene Company. The ratio of the staining degrees of the 3 gene hybrid zones to that of the β -actin hybrid zone, i.e., relative levels of the mRNAs were used to analyze expressions of the 3 gene mRNAs.

Statistical analysis

One-way ANOVA of SPSS11.0 was used for statistical analysis.

RESULTS

Detection of RNA

The OD260/280 ratio, detected by ultraviolet spectroscopy, was 1.7–2.0, indicating that the purity of the extracted RNA was higher with lower contamination. Two electrophoretic zones, 28 s and 18 s with brightness ratio of about 2:1, could be clearly seen. This indicates that RNA is intact with no degradation (Fig.1).

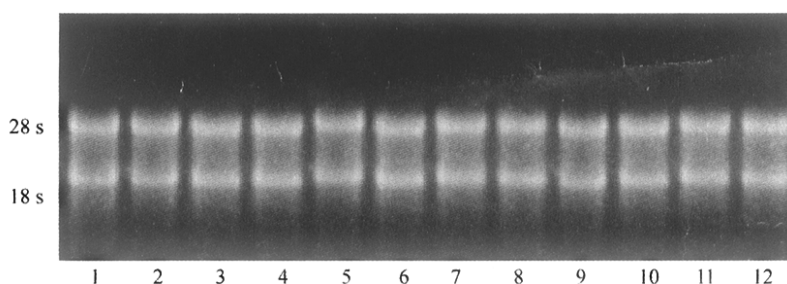


Fig.1. The photo of agarose gel electrophoresis of the total RNA. 1: Forebrain of the R1 control group; 2: Forebrain of the P10 control group; 3: Forebrain of the P10 acupuncture group; 4: Forebrain of the P10 non-point stimulation group; 5: Cortex of the R1 control group; 6: Cortex of the P10 control group; 7: Cortex of the P10 acupuncture group; 8: Cortex of the non-point stimulation group; 9: Hippocampus of the R1 control group; 10: Hippocampus of the P10 control group; 11: Hippocampus of the P10 acupuncture group; 12: Hippocampus of the P10 non-point stimulation group (The same in the following photos).

Results of transfer membrane after electrophoresis of the formaldehyde denatured RNA

As shown in the Fig. 2, 28 s and 18 s of RNAs in all the groups were clear and intact, indicating that

RNAs in all of the groups had been completely transferred to the nylon membrane with positive charges.

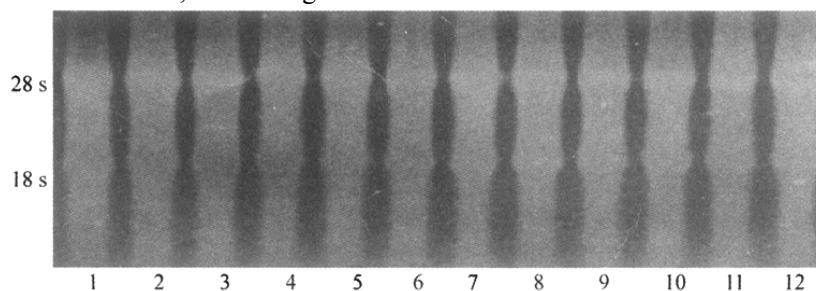


Fig.2. The photo of transfer membrane after electrophoresis of the formaldehyde denatured RNA.

Effects of acupuncture on expressions of aging-related gene mRNAs

The results and analysis of Northern blot (Fig. 3a and Fig. 3b, Table 1):

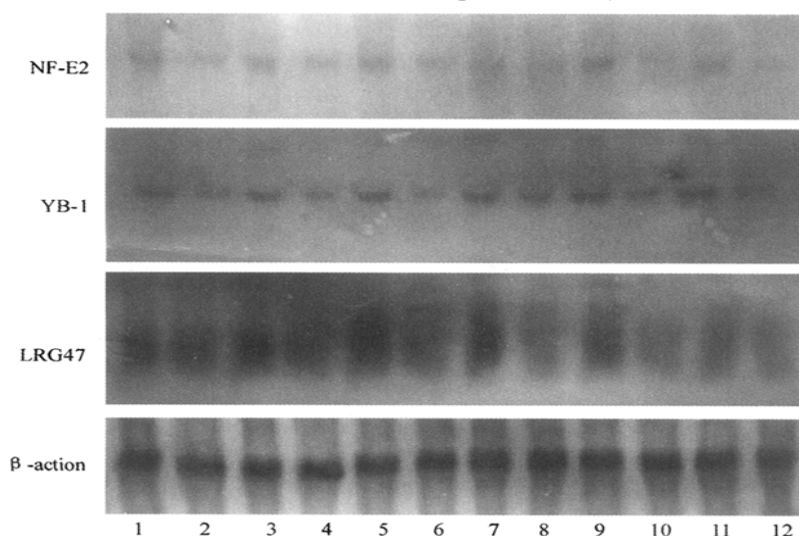


Fig. 3a. The photos of Northern hybridization of NF-E2, YB-1, LRG47 in the senescence accelerated mice of all the groups.

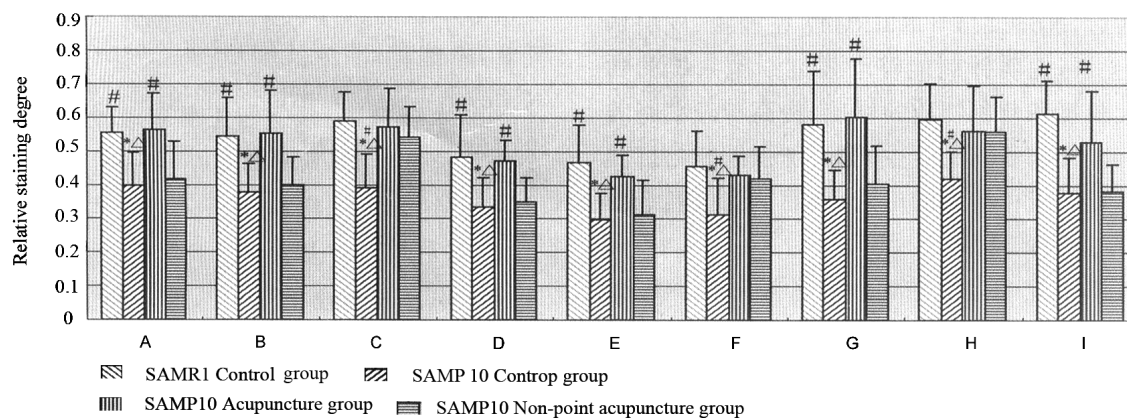


Fig.3b. Histogram of Northern hybridization of NF-E2, YB-1, LRG47 in the forebrain, cerebral cortex and hippocampus. Compared with the SAMR1 control group, $*P<0.05$; compared with the SAMP10 acupuncture group, $^{\Delta}P<0.05$; and compared with the non-point stimulation group, $^{\#}P<0.05$. A: NF-E2 in forebrain; B: NF-E2 in cortex; C: NF-E2 in hippocampus; D: LRG47 in forebrain; E: LRG47 in cortex; F: LRG47 in hippocampus; G: YB-1 in forebrain; H: YB-1 in cortex; I: YB-1 in hippocampus.

Table 1. Comparison of the relative ratio of expressions of NF-E2, YB-1, LRG47 genes in the forebrain, cortex and hippocampus ($\bar{x} \pm s$)

Group	Region	n	LRG47	NF-E2	YB-1
SAMR1 control	Forebrain	10	0.48±0.12 [#]	0.55±0.07 [#]	0.58±0.15 [#]
	Cortex	10	0.46±0.11 [#]	0.54±0.11 [#]	0.59±0.10
	Hippocampus	10	0.45±0.10	0.58±0.08	0.61±0.09 [#]
SAMP10 control	Forebrain	10	0.33±0.08 ^{*Δ}	0.39±0.10 ^{*Δ}	0.35±0.08 ^{*Δ}
	Cortex	10	0.29±0.08 ^{*Δ}	0.37±0.08 ^{*Δ}	0.41±0.08 ^{*Δ#}
	Hippocampus	10	0.31±0.11 ^{*Δ#}	0.39±0.10 ^{*Δ#}	0.37±0.10 ^{*Δ}
SAMP10 acupuncture	Forebrain	10	0.47±0.06 [#]	0.56±0.11 [#]	0.60±0.17 [#]
	Cortex	10	0.42±0.06 [#]	0.55±0.12 [#]	0.56±0.13
	Hippocampus	10	0.43±0.05	0.57±0.11	0.52±0.15 [#]
SAMP10 non-point Stimulation	Forebrain	10	0.35±0.07	0.41±0.11	0.40±0.09
	Cortex	10	0.31±0.10	0.40±0.08	0.55±0.11
	Hippocampus	10	0.42±0.07	0.54±0.09	0.38±0.10

Notes: Compared with the SAMR1 control group, $*P<0.05$; compared with the SAMP10 acupuncture group, $^{\Delta}P<0.05$; and compared with the SAMP10 non-point stimulation group, $^{\#}P<0.05$.

It can be seen from Table 1 that the expressions of NF-E2, YB-1, LRG47 mRNAs in the SAMP10 control group were down-regulated as compared with those of the SAMR1 control group (all $P<0.05$); No significant differences were found between the SAMP10 acupuncture group and the SAMR1 control group ($P>0.05$); the expressions of NF-E2, YB-1, LRG47 were up-regulated in the SAMP10

acupuncture group as compared with those of the SAMP10 control group ($P<0.05$); no significant differences were found between the SAMP10 non-point stimulation group and the SAMP10 control group in the expressions of LRG47 and NF-E2 mRNAs in the forebrain and cortex and in the expressions of YB-1 mRNA in the forebrain and hippocampus ($P>0.05$), while the expressions of

LRG47, NF-E2 mRNAs in the hippocampus and the expressions of YB-1 mRNA in the cortex were up-regulated ($P < 0.05$). It is suggested that the 3 genes in the forebrain, cortex and hippocampus in the aging mice are in the low expression state; and that acupuncture up-regulates the expressions of the 3 genes, tending to those in the normal group; and stimulation of the non-points only influence the expressions of NF-E2, LRG47 mRNAs in the hippocampus and the expressions of YB-1 mRNA in the cortex in the aging.

DISCUSSION

A great number of medical literature have reported that acupuncture, a traditional therapy of Chinese medicine, may show obvious therapeutic effects in delaying brain aging, improving the brain function for the old people, and relieving the symptoms of dementia.^{2,3} In the present study, Prof. HAN Jing-xian's acupuncture method for "supplementing *qi*, regulating blood, strengthening the kidney and promoting the mental activities" was adopted, with the point prescription including Tanzhong (CV 17), Zhongwan (CV 12), Qihai (CV 6), Xuehai (SP 10) and Zusanli (ST 36). Tangzhong (CV 17) is used for the upper-*jiao* to supplement the lung-*qi*, regulate and reinforce the pectoral *qi*, and promote the circulation of *qi* and blood. Zhongwan (CV 12) and Zusanli (ST 36) are used for the middle-*jiao* to supplement *qi* of the spleen and stomach, strengthen acquired constitution, produce *qi* and blood, and remove the phlegm. Qihai (CV 6) is used for the lower-*jiao* to nourish the liver and kidney, reinforce the congenital constitution. And Qihai (CV 6) may help to promote flow of *qi* and nourish the blood. All the above acupoints were used in an attempt to delay aging of the brain.

The three genes, NF-E2, YB-1 and LRG47, are the transcription factors. NF-E2 of the erythrocytic series, originating from precursor of the red cells, plays an important role in remodeling of the chromatin and

transcription activation of the globin gene;^{1,4,5} it is a key regulative molecule for controlling hemoglobin and globin, making an even production of hemoglobin; and it also plays a key role in production and development of hemoglobin, platelet, globin and megacaryocyte. YB-1 gene is one of the cold-shock structural domain protein families. It has wide cellular actions, including transcription regulation, translation regulation, DNA repair, drug resistance and stress reaction to the cellular signals, and it is closely related with cellular proliferation.⁶⁻⁸ The interferon-induced protein LRG47 (Ifil) is a necessary gene for congenital immunity. Re-staying on the vacuolus of pathogen, LRG47 can prevent the confluence of phagolysosome and the killing of bacteria.⁹ In addition, LRG47 can also resist invasion of the intercellular pathogens by controlling proliferation and existence of the peripheral T-cells, playing an important role in immunity of anti-bacteria.¹⁰

It was found in the present study that the expressions of NF-E2, YB-1, LRG47 genes were down-regulated in the forebrain, cerebral cortex and hippocampus of the senescence accelerated mice SAMP10 as compared with the normal mice SAMR1 of the same strain, indicating that the NF-E2, YB-1, LRG47 contents in the SAMP10 mice decrease along with the aging. We have known that NF-E2 plays a key role in the production and development of hemoglobin, platelet, globin and megacaryocytes, YB-1 plays a key role in the transcription regulation, translation regulation, DNA repair, drug resistance and stress reaction to the cellular signals, and LRG47 plays an important role in resisting invasion of the intercellular pathogens and in the immunity of anti-bacteria. After acupuncture treatment with Prof. HAN Jing-xian's method for "supplementing *qi*, regulating blood, strengthening the kidney and promoting the mental activities", the expressions of all the NF-E2, YB-1 and LRG47 genes were up-regulated in the forebrain brain, cerebral cortex

and hippocampus, indicating that acupuncture can strengthen the functions of the erythrocyte system, improve supply of oxygen and nutrient substance to the brain, increase proliferation ability of the nervous cells, reduce the occurrence and development of encephalatrophy, strengthen the anti-bacterial and immune functions, delay the occurrence of the aging symptoms, thus yielding the anti-aging effects.

The results of the present study show that the non-point stimulation can up-regulate the expressions of Ifil and NF-E2 mRNAs in the hippocampus and the expression of YB-1 mRNA in the cortex, which may be respectively related with the short-term memory the stress reaction. Although both acupuncture at the acupoints and the non-point stimulation can cause changes in the expressions of Ifil, NF-E2 and YB-1, yet their effects are essentially different.

REFERENCES

1. Roy AK, Oh T, Rivera O, et al. Impacts of transcriptional regulation on aging and senescence. *Ageing Res Rev* 2002; 1: 367-380.
2. Tian WF, Li Q, Liu Y. Advances of the study on the mechanisms of acupuncture for anti-aging. *Journal of Shangdong TCM University* 2004; 28: 320.
3. Han JX, Yang ZR, Zheng HY, et al. Effect of acupuncture positive cell counts of apoptosis in the brain tissue of the senescence accelerated mouse (SAMP10). *Acupuncture Research* 1998; 23: 266.
4. Andrews NC. The NF-E2 transcription factor. *Int J Cell Biol* 1998; 30: 429-432.
5. Toki T, Arai K, Terui K, et al. Functional characterization of the two alternative promoters of human p45 NF-E2 gene. *Exp Hematol* 2000; 28: 1113-1119.
6. Kohno K, Izumi H, Uchiumi T, et al. The pleiotropic functions of the Y-box-binding protein, YB-1. *Bioessays* 2003; 25: 691-698.
7. Holm PS, Bergmann S, Jurchott K, et al. YB-1 relocates to the nucleus in adenovirus-infected cells and facilitates viral replication by inducing E2 gene expression through the E2 late promoter. *J Biol Chem* 2002; 277: 10427-10434.
8. Ashizuka M, Fukuda T, Nakamura T, et al. Novel translational control through an iron-responsive element by interaction of multifunctional protein YB-1 and IRP2. *Mol Cell Biol* 2002; 22: 6375-6383.
9. MacMicking JD. IFN-inducible GTPases and immunity to intracellular pathogens. *Trends Immunol* 2004; 25: 601-609.
10. Feng CG, Collazo-Custodio CM, Eckhaus M, et al. Mice deficient in LRG-47 display increased susceptibility to mycobacterial infection associated with the induction of lymphopenia. *J Immunol* 2004; 172: 1163-1168.

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