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EXPERIMENTAL STUDY

Effect of electro-acupuncture on gene expression in heart of rats with stress-induced pre-hypertension based on gene chip technology

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Abstract

OBJECTIVE: To explore electro-acupuncture's (EA's) effect on gene expression in heart of rats with stress-induced pre-hypertension and try to reveal its biological mechanism based on gene chip technology.

METHODS: Twenty-seven Wistar male rats were randomly divided into 3 groups. The stress-induced hypertensive rat model was prepared by electric foot-shocks combined with generated noise. Molding cycle lasted for 14 days and EA intervene was applied on rats in model + EA group during model preparation. Rat Gene 2.0 Sense Target Array technology was used for the determination of gene expression profiles and the screened key genes were verified by real-time quantitative polymerase chain reaction (RT-PCR) method.

RESULTS: Compared with blank control group, 390 genes were changed in model group; compared with model control group, 330 genes were changed in model+EA group. Significance analysis of gene function showed that the differentially expressed genes are those involved in biological process, molecular function and cellular components. RT-PCR result of the screened key genes is consistent with that of gene chip test.

CONCLUTION: EA could significantly lower blood pressure of stress-induced pre-hypertension rats and affect its gene expression profile in heart. Genes that related to the contraction of vascular smooth muscle may be involved in EA's anti-hypertensive mechanism.

Key words: Stress; Prehypertension; Electroacupuncture; Gene expression profiling; Heart

INTRODUCTION

Stress-induced hypertension refers to high blood pressure that caused by the long-term tension and stress.[1] A modern life style with fast pace can cause psychological and emotional stress, which may develop into a gradual increase in hypertension in young adults.

Present studies showed that stress-induced hyperten-

sion is of complicated pathogenesis which mainly involve the hypothalamus-pituitary-adrenal cortex (HPA) axis,[2] sympathetic nerve-adrenal medulla (SAM) system,[3] renin-angiotensin-aldosterone system (RAS).[4]

The 7th report released by the Joint National Committee report on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) pointed out that, pre-hypertension is a transition stage during which the blood pressure rises from normal to diagnostic hypertension. In this stage, the systolic blood pressure ranges between 120-139 mm Hg and (or) diastolic blood pressure between 80-89 mm Hg.[5] The study found that incidence of cardiovascular events in people with pre-hypertension in future time was as two times as people with normal blood pressure.[5] It is also proved that stress-induced hypertension is preceded by damage of target organs like blood vessels, heart, brain, and changes of the expression of relative proteins and genes.[6,7] Also, studies showed that treatment in the pre-hypertension stage can significantly reduce admission rate and mortality of hypertensive patients, and enhance the protection of target organs of the disease.[8]

Electro-acupuncture therapy was widely applied in the treatment of various kinds of hypertension-related cardiovascular diseases in clinical practices.[9] Lots of researches confirmed that acupuncture reduces blood pressure by immune system, nervous system, and vascular endothelial cell.[10,11]

Gene chip technology, characterized by high sensitivity and throughput, is a new method to analyze gene expression. It could detect multiple gene expression at the same time which provides a platform for the study of multiple genes influenced hypertension.[12] However, previous researches on treatment of hypertension were more concentrated on anti-hypertensive treatment and protection of target organs, which are applied in the middle and late stages. Fewer researches were focused on gene expression and intervention of pre-hypertension.

In this study we will explore the effect of acupuncture on stress-induced pre-hypertension, and try to systematically reveal its biological mechanisms from the aspect of gene by monitoring key genes and targets with gene chip technology, thereby, providing a new idea for the prevention and treatment of hypertension by acupuncture.

MATERIALS And METHODS

Animals preparation

Specified pathogen free (SPF) Wistar ratswere purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., License number: SCXK (Beijing) 200223. Experimental animals were raised in a controlled environment with a temperature of (20 ± 1) ℃, humidity of 50%, and 12-h light-dark cycle was maintained throughout the whole study. All procedures for animal experiments were conducted in accordance with World Health Organization's International Guiding Principles for Biomedical Research Involving Animalsand were approved by the Animal Research Ethics Board of Beijing University of Chinese Medicine.

Grouping

Twenty-seven 9-week-old Specified Pathogen Free (SPF) Wistar male rats of (220 ± 30) g were randomly divided into 3 groupsby random number table method: blank control group, model control group, and model + EA group (n = 9 per group).

Model preparation

The stress-induced hypertensive rat (SIHR) model was established by electric foot-shocks combined with generated noise. Rats in model control group and model + EA group were placed in a cage (22 cm × 22 cm × 26 cm) with a grid floor and received electric foot-shocks (30 V, 5 ms duration, 2-25 s intervals)and noises (80-100 db) produced by a buzzer (MG-2TYPE, Huai Bei Zheng Hua, Anhui, China) randomly delivered by a computer. The procedure repeated twice a day (8:00 to 10:00 in the morning and 2:00 to 4:00 in the afternoon). Rats in blank control group were put into the same cage in the same time period, with no foot-shocks or noise.

Intervention

All the rats in three groups were loosely immobilized in a specially made restrainer with four limbs exposed. Taichong (LR 3) is located in the dorsum of the foot, in the depression anterior to the junction of the first and second metatarsals. Quchi (LI 11) is located at the proximal end of radius, in the depression lateral and anterior to the elbow joint.

Model + EA group: the needles (0.32 mm × 25 mm, purchased from Suzhou Acupuncture Goods Co., Ltd., Suzhou, China) were directly inserted into Taichong (LR 3) and Quchi (LI 11) bilaterally for about 1.5-2 mm and 4 mm respectively. After the insertion, Hans (LH202H, Si Sheng Da, Beijing, China) was connected with Quchi (LI 11) and Taichong (LR 3) to form a circulation of 1mA with a frequency of 2 Hz. The needles were then withdrawn after a 20 minutes' retention. The acupuncture intervention took once a day (from 5 pm) since the first day of model preparation for 14 days. All the intervention was given by the same person. Blank control group and model control group: rats were immobilized in the same restrainer for 20 min without acupuncture intervention.

Samples obtaining

After the measurement of caudal artery blood pressure at the 15th day, rats were sacrificed after anesthetized by intraperitoneal injection of 100 g/L chloral hydrate

(500 mg/kg) (Bioway biological technology Co., Ltd., Beijing, China). Heart were obtained on the ice, washed with 4 ℃ saline, dried, fixed in liquid nitrogen, and stored at - 80 ℃ refrigerator.

Measurement of blood pressure

Two hours after the stimulation of model preparation, systolic pressure of caudal artery was measured in a controlled environment at of a temperature of (22 ± 2) ℃. Sober rats were preheated in 36 ℃ for 15 min. Then the systolic pressure was measured with noninvasive blood pressure instrument when the rat is quiet and conscious. Each rat was measured for 3 times with the average blood pressured recorded. Blood pressure was recorded one day before and at the 3rd, 5th, 7th, 9th, 11th, 13th and 15th day after modeling.

Sample preparation and microarray image analysis

Total RNA was separately extracted from all the 27 individual samples using the mirVana miRNA isolation Kit (P/N AM1552, Life Technologies, Carlsbad,NM, USA). Microarray analysis was performed and repeated 3 times using a biological sample in each group with Gene Chip Rat Gene 2.0 ST Array (Affymetrix, Santa Clara, CA, USA). Total RNA of each sample was used for labeling and array hybridization. The labeling and hybridization steps were carried out according to the Affymetrix protocol (Gene Chip WT PLUS Reagent Kit, P/N 902280, Affymetrix, Santa Clara, CA, USA). Array hybridization using the Affymetrix Gene Chip 645 System and followed by washing with the Affymetrix Gene Chip 450 System. Array scanning using the Affymetrix Gene Chip 7G microarray scanner (Affymetrix, Santa Clara, CA, USA).

Data analysis

Scanned images were then imported into Affymetrix Expression console software (Affymetrix, Santa Clara, CA, USA) for grid alignment and expression data analysis. Expression data were normalized through quantile normalization in the Affymetrix Expression console software (Affymetrix, Santa Clara, CA, USA). The 27 gene level files were imported into Transcriptome Analysis console further analysis. Gene expression profiles of model control group were compared to those from blank control group or model + EA group. The changed genes were selected by the criteria: $P < 0.05$ and fold changes ≥ 1.5.

Gene functional annotation

The DAVID 6.7b of DAVID Bioinformatics Resources (http://david.abcc.ncifcrf.gov/) was used to perform gene ontology (GO) enrichment analysis. The biological process, cellular component and molecular functions were analyzed simultaneously. Differentially expressed genes of model control group (*vs* blank control group) or model + EA group (*vs* model control group) dataset were selected and were tested against the background set of all genes present in the Affymetrix GeneChip Gene 2.0 ST Array (Affymetrix, Santa Clara, CA, USA).

Clustering and tree view for identified transcripts

Hierarchical clustering was performed with help of-Cluster 3.0 (Michiel de Hoon Laboratory, Palo Alto, CA, USA). A list was prepared of the selected genes to be clustered. The normal signal data of selected genes were adjusted to log transform data. Then the data was arranged according to the requirements of Cluster 3.0 and "median" was selected for center genes and arrays.

Selected differentially expressed genes validation

Total RNA was reverse-transcribed to cDNA with a reverse transcription kit (Life Technologies, Carlsbad, NM,USA). Quantitative real-time polymerase chain reaction (qRT-PCR) method was used to detect gene expression by RT-PCR instrument (Applied Biosystems, Foster city, CA, USA) with Power SYBR Green PCR Master Mix kit (Applied Biosystems,Foster city, CA, USA). We used primer express software (Applied Biosystems, Foster city, CA, USA) to design primers (Table 1). β-actin was selected as an internal reference. Amplification conditions are as follows: 1 cycle of 10 min at 95 ℃, 40 cycles of 15 s at 95 ℃ and 1min at 60 ℃. After cycling, a melting protocol was performed with 15 at 95 ℃, 15 s at 60 ℃ and 15 at 95 ℃ in the end. After the end of the experiment RQ Manager 1.2.1 (Applied Biosystems, Foster city, CA, USA) and Data Assist V3.0 software (Applied Biosystems, Foster city, CA, USA) were used for the calculation of Ct value. △CT method was used for the relative quantification of the gene expression, target gene: = $2^{-\triangle\triangle Ct}$. All data were analyzed using SAS 9.0 statistical software (SAS Institute Inc., Cary, NC, USA). Quantitative data are expressed as the mean ± standard deviation ($\bar{x} \pm s$). The single factor analysis of variance of completely randomized design was used for parameter comparison between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Blood pressure

Systolic blood pressure of Wistar rats in blank control group stayed normal through the whole process of the experiment. Systolic blood pressure in model control group reached higher than 120 mm Hg ($P < 0.01$) at the 3rd day, and kept increasing to a significantly higher level when measured at the 3rd, 5th, 7th, 11th, 13th and 15th days during model preparation ($P < 0.01$), compared with that of the blank control group. In the modeling process, model control group were of elevated blood pressure, irritation, squealing, gnawing, hard stool, yellow urination, rough hair and bloodshot eyes. With the value kept in the range between 120-139 mm Hg, the model was successfully established.

Compare with model control group, systolic blood pressure in model + EA group remarkably decreased at the 5th, 7th, 9th day (all $P < 0.01$) and 11th, 13th and 15th days during EA intervention(all $P < 0.05$), indicating that EA at Taichong (LR 3) and Quchi (LI 11) can significantly lower the blood pressure of stress-induced pre-hypertension rats with better short-term effects (Figure 1).

Gene expression changes

Gene microarray analysis showed that there were 390 gene expressions changed in model control group compared to blank control group. Of these, 182 were down-regulated and 208 were up-regulated. EA intervention changed 330 gene expressions compared with model control group. Among these genes, 169 were down-regulated and 161 were up-regulated ($P < 0.05$ and fold change ≥ 1.5 were identified). Gene microarray analysis showed that there were 71 genes whose expression up-regulated in the model control group (*vs* blank control group) but down-regulated in model + EA group (*vs* model control group) (Table 2); and 37 genes whose expression down-regulated in the model control group (*vs* blank control group) but up-regulated in model + EA group (*vs* model control group) (Table 3).

Go analysis

To identify the biological processes associated with gene expression changes with EA treatment, we performed GO enrichment analysis. Enriched GO terms are displayed in Figure 2 and arranged according to biological processes, molecular functions and cellular components.The categories that were significantly enriched

Table 1 Sequences of primers employed for RT-PCR and their anticipated PCR product size

Gene	Primer	Oligonucleotide sequences 5'-3'	Length (bp)
HSPB1	Front	5'- CGGTGCTTCACCCGGAAATA-3'	152
	Rear	5'- TCGAAAGTGACCGGAATGGT-3'	
OLR1	Front	5'- GGCCATCCTTTGCCTAGTGT-3'	131
	Rear	5'-ACATCTGCCCCTCCAGGATA-3'	
GADPH	Front	5'-CAGGGCTGCCTTCTCTTGTG-3'	217
	Rear	5'-ACCAGCATCACCCCATTTGA-3'	
PLA2G4A	Front	5'-AGTACCAGAGAACACCTGGGA -3'	232
	Rear	5'-TTGTTCGCTTCCTGCTGTCA -3'	
CD 38	Front	5'-CAGCACCTTTGGAAGTGTGG-3'	186
	Rear	5'-GGTCGGTAGTTATCCTGGCA-3'	
PTGS2	Front	5'-CTCAGCCATGCAGCAAATCC-3'	172
	Rear	5'-GGGTGGGCTTCAGCAGTAAT-3'	
HSPA1A	Front	5'-GGCCTTGAGGACTTTGGGTT-3'	131
	Rear	5'-CTGGGAATGCAAAGCACACG-3'	

Notes: RT-PCR: real-time polymerase chain reaction; HSPB1: heat shock protein beta-1; OLR1: oxidized low-density lipoprotein receptor 1; GADPH: glyceraldehyde-3-phosphate dehydrogenas; PLA2G4A: phospholipase A2; PTGS2: prostaglandin-endoperoxide synthase 2; HSPA1A: heat shock 70 kDa protein 1.

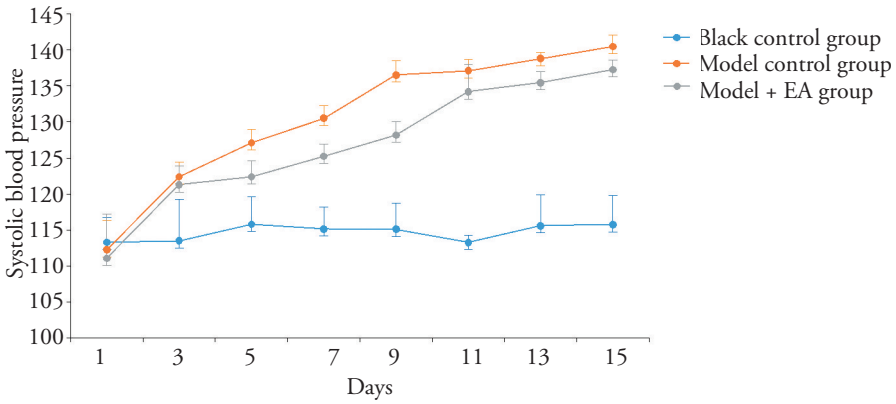

Figure 1 Effects of EA on systolic blood pressure of stress-induced pre-hypertension rats

Blank control group: wistar rats without operated; model control group: SIHR without treatment; EA + model group: SIHR with treatment by electro-acupuncture. SIHR: the stress-induced hypertensive rat model was established by electric foot-shocks combined with generated noise. $^{a}P < 0.01$, *vs* blank control group; $^{b}P < 0.01$, $^{c}P < 0.05$ *vs* model control group.

Table 2 Selected genes up-regulated in model control group (*vs* blank control group) but down-regulated in model + EA group (*vs* model control group) based on gene microarray analysis

Gene	FC_1	*P* value	FC_2	*P* value
UAP1	1.58211	2.43E-11	- 3.84041	6.63E-11
COP10B	2.33618	6.29E-09	- 2.13814	1.83E-08
LOC688318	2.81178	9.63E-08	- 3.35472	2.12E-08
RGS2	2.74147	3.17E-07	- 3.43924	4.63E-08
TINAGl1	3.19025	7.13E-08	- 2.65601	3.63E-07
P4HA1	2.68679	1.61E-07	- 2.62653	2.00E-07
GFPT2	2.25611	2.89E-06	- 1.98377	1.34E-05
HSPH1	2.14991	1.43E-05	- 2.34283	5.58E-06
TNFRSF12A	1.96883	9.46E-06	- 1.93423	1.19E-05
CCRN4l	2.11015	6.47E-06	- 2.08010	7.68E-06
CD38	1.53121	1.90E-05	- 1.66954	3.71E-06
PTGS2	2.24993	1.11E-05	- 1.85358	0.000111
PDK4	4.17347	7.04E-06	- 2.12498	0.0011898
HSPB1	1.88297	0.000290583	- 2.52067	1.28E-05
ZBTB16	3.42483	1.96E-05	- 2.52959	0.0002016
HSPA1A	1.70622	0.00037365	- 1.92875	7.31E-05
REG3G	9.00765	3.05E-05	- 4.11327	0.0009653
OLR1	2.11512	0.000413181	- 2.29848	0.000184
PLA2G4A	1.70934	0.00133431	- 1.54617	0.0050377
ETNK1	1.66363	1.99E-07	- 1.93096	1.71E-08

Notes: FC1: gene expression level in model control group /gene expression level in blank control group. FC2: gene expression level in model + EA group /gene expression level in model control group. Positive values indicate higher expression in model samples, while negative values indicate lower expression in model + EA samples. UAP1: UDP-N-acetylglucosamine pyrophosphorylase 1; COP10B: coenzyme Q10 homolog B; LOC688318: leucine rich repeat containing 45; RGS2: regulator of G-protein signaling 2; TINAGl1: tubulointerstitial nephritis antigen-like 1; P4HA1: prolyl 4-hydroxylase; GFPT2: glutamine-fructose-6-phosphate transaminase 2; HSPH1: heat shock 105 kDa/110 kDa protein 1; TNFRSF12A: tumor necrosis factor receptor superfamily, member 12A; CCRN4l: CCR4 carbon catabolite repression 4-like; CD38: CD38 molecule; PTGS2: prostaglandin-endoperoxide synthase 2; PDK4: pyruvate dehydrogenase kinase, isozyme 4; HSPB1: heat shock 27 kDa protein 1; ZBTB16: zinc finger and BTB domain containing 16 ; HSPA1A: heat shock 70 kDa protein 1A; REG3G: regenerating islet-derived 3 gamma; OLR1: oxidized low density lipoprotein (lectin-like) receptor 1; PLA2G4A: phospholipase A2, group IVA; ETNK1: ethanolamine kinase 1.

in our gene set were response to stimulus, biological adhesion, cellular process, immune system process, membrane part, receptor activity and molecular transducer activity and so on.

Validation of genes expression

To validate the microarray results, we selected several transcripts to cluster (Figure 3A) and validated the clustering result using qRT-PCR (Figure 3B). These genes were selected based on fold change differences, previous association with blood pressure regulation, and/or involvement in processes that may influence blood pressure.The expression of heat shock 70 kDa protein (HSPA1A) ($P < 0.01$), heat shock protein beta-1 (HSPB1) ($P < 0.01$), oxidized low-density lipoprotein receptor 1 (OLR1) ($P = 0.035$), phospholipase A2 (PLA2G4A) ($P < 0.01$) and prostaglandin-endoperoxide synthase 2 (PTGS2) ($P = 0.041$) was significantly higher in model control group compared to blank control group. HSPA1A ($P < 0.01$), HSPB1 ($P < 0.01$), OLR1 ($P < 0.01$), PLA2G4A ($P < 0.01$) and PTGS2 ($P = 0.015$) expression was significantly lower in model + EA group when compared to model control group. To some extent, the identified results confirmed the reliability of the array analysis.

DISCUSSION

Hypertension affects approximately 1 billion individuals worldwide.[13] It is believed that high-pressure environment is crucial for the prevalence of hypertension and coronary diseases.[2] Stress-induced hypertension usually results from endocrine disorders related to increased levels of vasopressin, aldosterone, cortisol, endorphins and catecholamines.[14] Previous researches on

Table 3 Selected genes Down-regulated in the model control group (*vs* blank control group) but up-regulated in model + EA group (*vs* model control group) based on gene microarray analysis

Gene	FC_1	*P* value	FC_2	*P* value
LOC306096	-2.14136	8.55E-08	1.52132	2.00E-05
SYNJ2	-1.80596	6.89E-07	1.58761	6.47E-06
NREP	2.5273	4.60E-07	2.06676	4.34E-06
NAV3	-2.67667	2.00E-05	1.96893	1.25E-05
KCNN3	-1.99797	1.04E-06	1.54168	6.50E-05
FREM2	-1.72394	1.07E-05	1.67734	1.68E-05
LSMEM1	-2.14671	0.0003924	2.31810	0.0001877
PRC1	-2.37436	6.01E-05	1.57840	0.0058411
PDCD1LG2	-2.71689	6.88E-05	1.91174	0.0017846
OLR1531	-1.51335	0.0116087	1.50782	0.0121603
KCNA2	-1.66961	0.036979	1.69514	0.0327385
OLR1729	-1.88576	0.0141577	2.05270	0.007219
FANCB	-1.66599	0.0045538	1.72657	0.0029982
RBP7	-1.78662	0.0015391	1.55916	0.0080404
PIRT	-1.76689	0.0044303	2.39980	0.0002215
SERPINB9	-1.56302	0.0005949	1.66035	0.0002283
LRRC3B	-1.85978	0.0014212	1.86193	0.0014032
RGD1305184	-1.79733	0.0156537	1.61668	0.038513

Notes: FC_1: gene expression level in model control group/gene expression level in blank control group. FC_2: gene expression level in model + EA group / gene expression level in model control group. Positive values indicate higher expression in model samples, while negative values indicate lower expression in model + EA samples. LOC306096: dachshund family transcription factor 1; SYNJ2: synaptojanin 2; NREP: neuronal regeneration related protein; NAV3: neuron navigator 3; KCNN3: potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 3; FREM2: Fras1 related extracellular matrix protein 2; LSMEM1: leucine-rich single-pass membrane protein 1; PRC1: protein regulator of cytokinesis 1; PDCD1LG2: programmed cell death 1 ligand 2; OLR1531: olfactory receptor 1531; KCNA2: potassium channel, voltage gated shaker related subfamily A, member 2; OLR1729: olfactory receptor 1729; FANCB: fanconi anemia, complementation group B; RBP7: retinol binding protein 7, cellular; PIRT: phosphoinositide-interacting regulator of transient receptor potential channels; SERPINB9: serpin peptidase inhibitor, clade B (ovalbumin), member 9; LRRC3B: leucine rich repeat containing 3B; RGD1305184: similar to CDNA sequence BC023105.

treatment of hypertension were more concentrated on anti-hypertensive treatment and protection of target organs, fewer researches were focused on intervention of pre-hypertension. However, it has been proved that early stage treatment could effectively reduce the incidence of high blood pressure, and protect target organs.[15] Therefore, in this study, we focused on the early stage of pre-hypertension to observe the EA's effect on anti-hypertensive.

In the present study, the stress-induced hypertensive model is well established by electric foot-shocks combined with generated noise,[16] it may simulate the present environment in which humans live: high work pressures and fast paced.[16]

Our results show that, compared with the blank control group, rats in the model control group got significantly increased blood pressure from the third day (higher than 120 mm Hg), suggesting a successfully prepared pre-hypertension model. The blood pressure continuously rose and reached higher than 140 mm Hg at the 15th day, supporting the theory that repeated chronic physical or emotional stress could lead to the elevation of blood pressure.[9]

The traditional Chinese medicinal theory of preventive treatment of disease shows its unique advantages in preventing and treating pre-hypertension and high blood pressure. Especially the acupuncture, it could reduce blood pressure by unblocking the meridians and pacifying the liver to subdue *Yang*.[17]

Syndromeis the basic unit and a key concept in TCM theory, all diagnostic and therapeutic methods in TCM are based on the differentiation of TCM syndrome. Previous studies have shown that Syndromes of hypertension are always divided into liver *Yang* hyperactivity syndrome and liver-kidney *Yin* deficiency syndrome.[18] In the modeling process of our study, stress-induced hypertension rats were of elevated blood pressure, irritation, squealing, gnawing, hard stool, yellow urination, rough hair and bloodshot eyes, which were similar to performance of liver *Yang* hyperactivity syn-

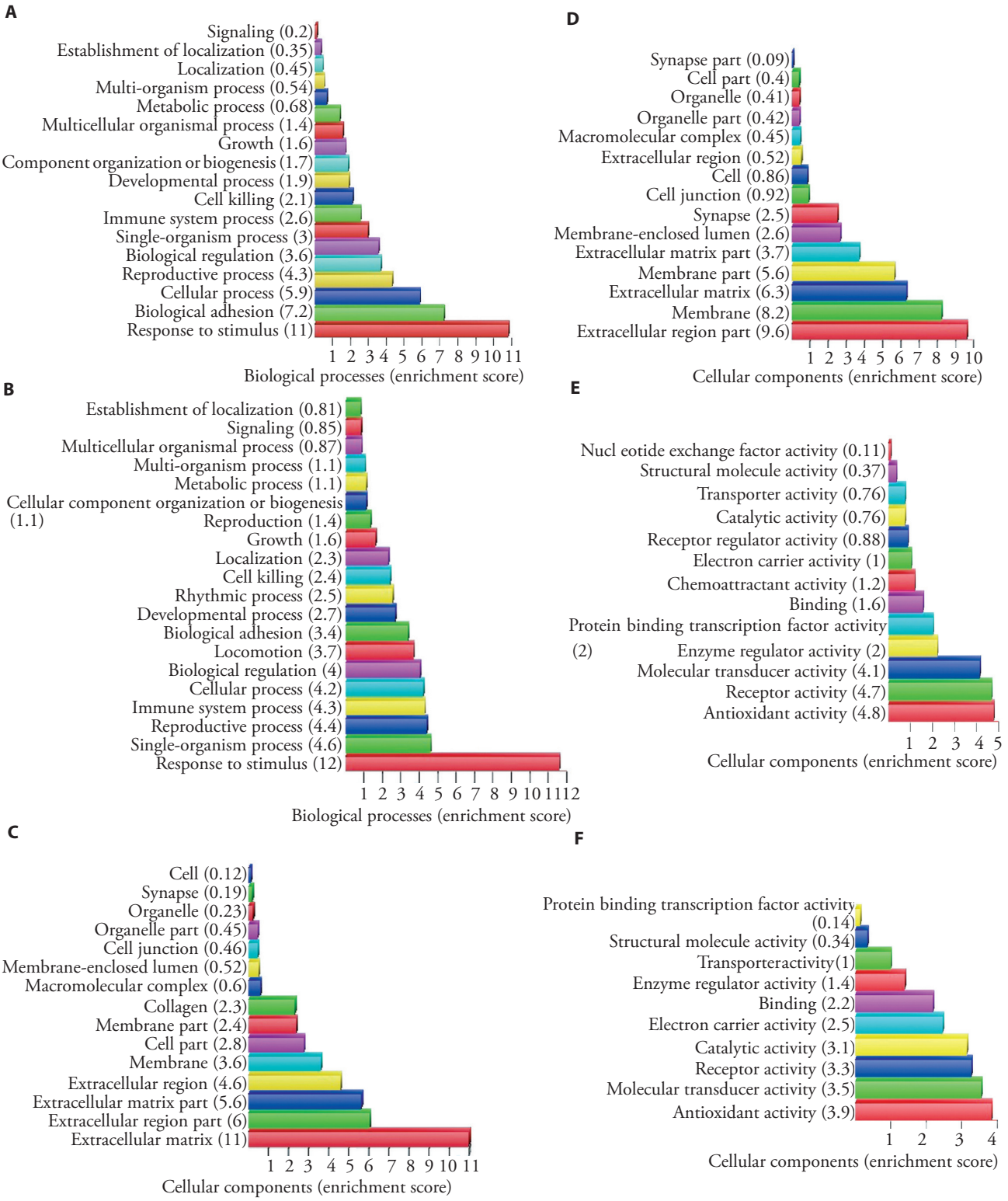

Figure 2 Enriched GO terms according to biological processes, molecular functions and cellular components
A, B: biological processes; C, D: molecular functions; E, F: cellular components. Blank control group: wistar rats without operated; model control group: SIHR without treatment; EA + model group: SIHR with treatment by electro-acupuncture. GO terms are ordered by enrichment score with highest enriched term at the bottom of the list. GO: gene ontology; SIHR: stress-induced hypertensive rat; EA: electro-acupuncture. Differentially expressed transcripts involved in the term (count) with $P < 0.05$ and fold-change > 1.5 were included. A, C, E: blank control group *vs* model control group. B, D, F: model control group *vs* model+EA group.

drome of human hypertension.

According to TCM theory, Quchi (LI 11) and Taichong (LR 3) can treat hypertension with liver *Yang* hyperactivity syndrome by pacifying the liver to subdue *Yang* and promoting *Qi* circulation to resolve depression.[19] Our study showed that after EA treatment, compared with model control group, the blood pressure of model + EA group decreased significantly from the fifth day, suggesting EA can reduce blood pressure of stress-induced pre-hypertension rats. This

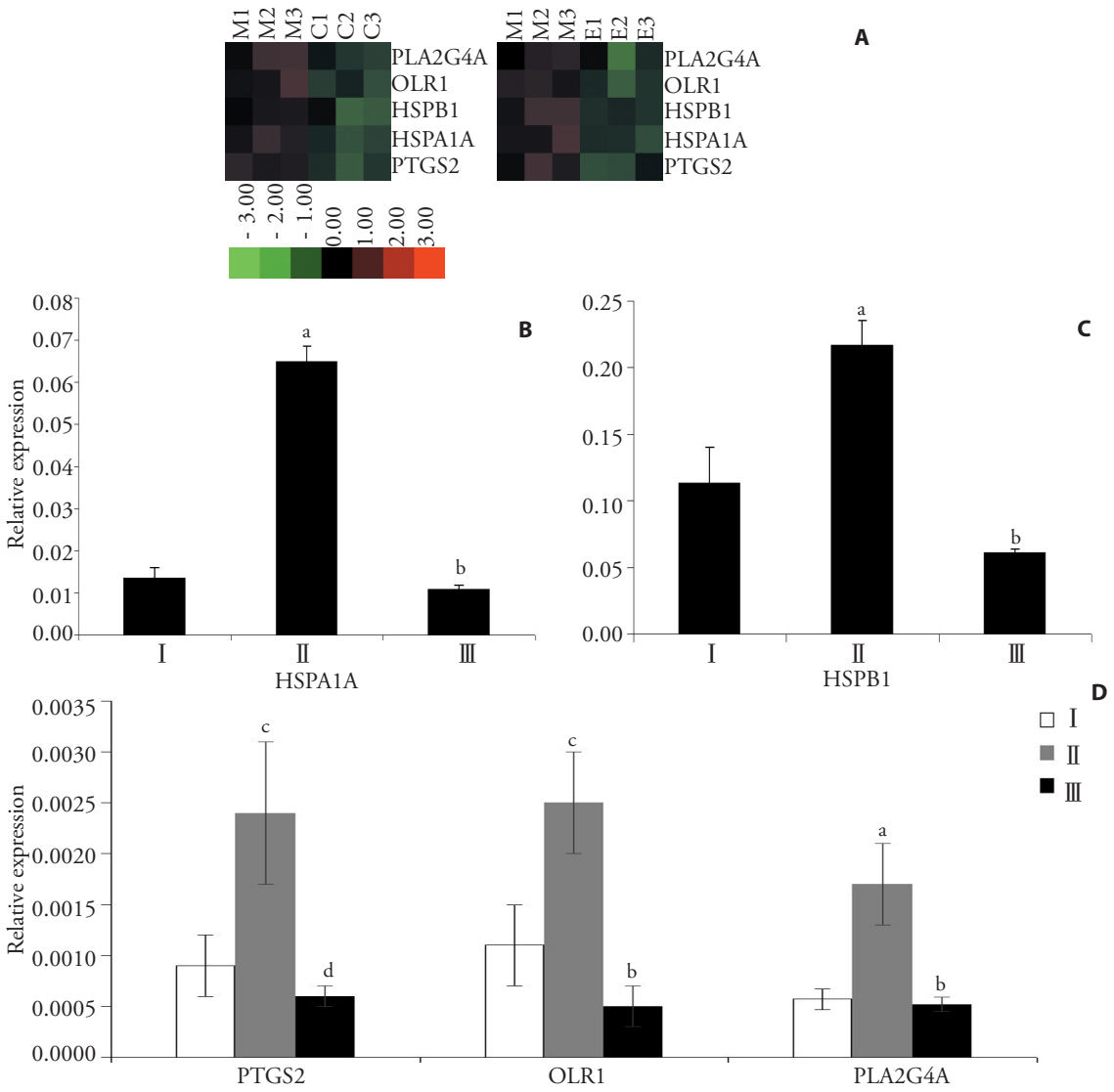

Figure 3 Selected transcripts clustered and validated with real-time PCR. First, selected genes were clustered using Cluster 3.0 according to the LOG value

A: red is relatively up-regulated and green is relatively down-regulated in different samples. The three "Cs" are the three blank control samples. The three "Ms" are the model control samples. The three "Es" are the EA + model samples. Then, to verify the reliability of the microarray analysis. B-D: verified these selected genes from the clustering diagram using qRT-PCR. HSPA1A, HSPB1, OLR1, PLA2G4A and PTGS2 gene expression in blank control group, model control group and EA + model group were presented on Figure 3B-D. Blank control group: wistar rats without operated; model control group: SIHR without treatment; EA + model group: SIHR with treatment by electro-acupuncture. SIHR: the stress-induced hypertensive rat model was established by electric foot-shocks combined with generated noise. These transcripts analyzed here showed coherent profiles with cluster A. qRT-PCR: quantitative real-time polymerase chain reaction; HSPA1A: heat shock 70 kDa protein 1; HSPB1: heat shock protein beta-1; OLR1: oxidized low-density lipoprotein receptor 1; PLA2G4A: phospholipase A2; PTGS2: prostaglandin-endoperoxide synthase 2. SIHR: stress-induced hypertensive rat; EA: electro-acupuncture. Ⅰ: Black control group; Ⅱ: model control group; Ⅲ: model + EA group. $^{a}P < 0.01$, $^{c}P < 0.05$, *vs* blank control group; $^{b}P < 0.01$, $^{d}P < 0.05$, *vs* model control group.

result was consistent with previous studies.[19]

Hypertension is a polygenic disease that related to the interaction of genetic and environmental factors. Therefore, the scanning of all the related gene expression and the analysis of the differences between groups could study the regulatory mechanism of hypertension in a more systematic way. Gene chip technology, characterized by high sensitivity and throughput, provides a platform for the study of multiple genes influenced hypertension.[20] However, fewer researches are focused on the gene expression in pre-hypertension and the relation between pre-hypertension and gene expression has not been confirmed. In this study, gene chip results showed that significant differences of gene expression existed among blank control group, model control group and model + EA group. Some of them have already been related to hypertension.

It is proved that hypertension is preceded by damage of target organs like blood vessels, heart, brain and kidney.[6,7] Besides, the abnormal vascular function, which may be the reason for the damage of target organs like heart, brain and kidney, is an important initial link in the development of cardiovascular and cerebrovascular diseases. It is also demonstrated that impaired vascular

function is closely associated with inflammation and the level of oxidative stress.[6] Altered genes including PLA2G4A (FC = 1.70934), HSPB1 (FC = 1.88297), HSPA1A (FC = 1.70622), PTGS2 (FC = 2.24993), OLR1 (FC = 2.11512), which are related to the cytoskeleton and the contraction of vascular smooth muscle, up-regulated in model control group when compared with blank control group, but down-regulated in model + EA group, PLA2G4A (FC = - 1.54617), HSPB1 (FC = - 2.52067), HSPA1A (FC = - 1.92875), PTGS2 (FC = - 1.85358), OLR1 (FC = - 2.298 48), when compared with model control. PCR results of these genes are consistent with the gene chip test.

PLA2G4A also known as phospholipase A2 (cytosolic phospholipaseA2, cPLA2), plays an important role in inflammation reactions. It is closely related to the cell proliferation and apoptosis after being activated through phosphorylation.[21] It is believed that in the hypertension condition, cPLA2 could accelerate cell proliferation, leading to the proliferation and aggregation of interstitial collagen fiber, the remodeling of large artery, and the decrease of its elasticity and compliance.[22] Our result indicated that PLA2G4A gene may be one of the target genes of stress-induced pre-hypertension. Electro-acupuncture intervention can reduce expression of PLA2G4A, indicating a relation between anti-hypertensive effect of EA and regulation of PLA2G4A gene. The specific mechanism needs to be further studied.

HSPB1, also known as heat shock protein 25 (HSP25). HSPA1A, also called HSP70. HSPB1 and HSPA1A proteins belong to the HSP family. HSP genes were activated when the cell was damaged by stressors of various kinds, and therefore act as biomarker of stress response of the cell. Activated HSP genes can improve cell tolerance to stressors, take part in the endogenous protective mechanisms of cardiovascular system and play an important role in the pathological process of cardiovascular diseases.[23] In stress conditions like ischemia, HSPB1 can enhance myocardial cells' resistance to ischemic injury,[24] indicating that HSPB1 is an important kind of heat shock protein with protective function in myocardial tissues. The mechanism may concerns the stabilization and repair of the structure of damaged cytoskeletal proteins or nuclear proteins of myocardial tissue. In recent years many researches also focus on the HSPA1A. Reports showed that the increased amount of serum HSPA1A antibody is positively correlated to the elevation of blood pressure, indicating that HSPA1A gene is closely related to hypertension.[25] The underlying mechanism may concern HSPA1A's influence on vascular endothelial cell function by taking part in antioxidation, modulation of immune system and vascular stress.

In our study, the elevated expression of HSPB1 and HSPA1A gene indicates that endogenous protection of the cardiovascular system has been activated by stress, HSPB1 and HSPA1A are involved in the development of hypertension, which is consistent with previous researches. After EA intervention, we speculate that EA could improve the stress state of the rat and slow down the cellular stress responses to avoid the excessive stress damage while lowering the blood pressure. Its specific mechanism needs to be further studied.

The expression of PTGS2, also known as cyclooxygenase-2. In many pathological conditions, various internal and external environmental stimuli could induce its over-expression in vascular endothelial cells, vascular smooth muscle cells, and macrophages that are closely related to inflammation. Research showed that PTGS2 expression up-regulated in atherosclerosis.[26] It may aggravate the condition through increasing vascular permeability and promoting the migration of macrophage, the generation of pro-inflammatory cytokines (such as IL-6 and IL-8), the migration and proliferation of smooth muscle cells, and the synthesis of extracellular matrix.[27] It is also reported that PTGS2 can induce the synthesis of vascular growth factor and the angiogenesis aggravate the form and expansion of atherosclerotic plaque.[28] The gene chip results showed that PTGS2 gene may be one of the target genes in stress-induced pre-hypertension and plays an important role in the development of stress-induced hypertension. EA may be an effective way to constrain PTGS2 level to treat atherosclerosis caused by hypertension.

OLR1 is the specific receptor of oxiding low density lipoprotein (Ox-LDL).[29] Ox-LDL plays an important role in the pathological process of atherosclerosis because it can activate endothelial cells to release adhesion molecules and chemokines.[30] In recent years, studies have shown that Ox-LDL can initiate CD40/CD40L endothelial cell signaling pathway through OLR1 and participate in the expression of inflammatory molecules and activation, dysfunction and injury of endothelial cells.[31,32] Our study showed that OLR1 gene may be one of the target genes in stress-induced pre-hypertension and closely related to development of stress-induced hypertension. EA's antihypertensive effect may be related with the regulation of OLR1 gene, with the underlying mechanism to be further studied.

In conclusion, our study proved that EA at Taichong (LR 3) and Quchi (LI 11) can significantly lower the blood pressure of stress-induced pre-hypertension rats and affect its gene expression profile in heart. It may perform anti-hypertensive regulation on stress-induced pre-hypertension *via* various ways. We speculate that genes that related to the contraction of vascular smooth muscle may be involved in the mechanism.

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