Genomic Expression for Rat Model of Damp Obstruction in Chinese Medicine-Application of Microarray Technology

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Abstract: Damp obstruction refers to the stagnation of vital energy (qi) caused by dampness resulting in dysfunction of body and limbs movement, as well as impairment of spleen and stomach digestive function. Damp obstruction is the dampness-induced imbalance of 5 elements; thus it serves as an ideal model for genomic study using cDNA microarray. We have performed microarray analyses to major organs of damp-obstructed rats. Cluster analysis for the expression profiles of major organs indicated that spleen, stomach, and kidney respond to dampness differently from heart, liver, lung, and brain. Gene expression profile specific to each element or group of elements was also identified. Our results are consistent with the philosophy of Chinese medicine that the 5 elements, metal (lung), wood (liver), water (kidney), fire (heart), and earth (spleen and stomach) coordinate by subjugation or restriction to maintain a healthy, physiological state. This is the first time that a powerful genomic tool was applied to probe the ancient theory of Chinese medicine.

Keywords: Damp obstruction; Microarray; Elements; Gene expression.

Running title: Microarray profiling of damp-obstructed rats

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Introduction

Damp obstruction refers to the stagnation of vital energy (qi) caused by dampness resulting in dysfunction of body and limbs movement, as well as impairment of spleen and stomach digestive function (Li and Yen, 2000). Dampness (humidity) is one of the major pathogenic factors on the human body as defined by Chinese medicine. Dampness is characterized by fluidity, latency, universality, and persistence. Dampness is sticky, retards flow of qi, and consequently causes disregulation of “middle-energizer” (Huang et al., 2000). In Chinese medicine, the general term for humidity-induced syndrome is “damp obstruction” as described in *Yellow Emperor Classic of Internal Medicine (Huang Di Nei Jing)*.

Damp obstruction includes “external dampness” and “internal dampness.” External dampness is induced in many cases by environmental factors, typically during the long summer days under hot and humid weather conditions. Internal dampness refers to the imbalance of metabolism due to dysfunction of liquid and humor, usually by an over-intake of raw, cold, greasy, or sweetened food (Li, 1976; Chen, 1999).

As described above, damp obstruction occurs frequently in areas with long

periods of hot and humid weather year round, areas such as Southeast Asia. Due to the retardation of middle-energizer, the major organs involved are spleen, stomach, and kidney. Damp obstruction also presents minor symptoms such as stomach duct and abdominal fullness and distension, a feeling of oppression and discomfort, dizziness, general anxiety, insomnia, weak or fatigued cumbersome limbs, poor appetite, nausea or vomiting, a bitter taste in the mouth, and thick and slimy tongue, frequently with edema (Li, 1976; Chen, 1999).

In general, damp obstruction is defined as a moisture-caused imbalance of organs. Because the spleen and stomach belong to “tu” (earth) among the five elements, they are the first organs affected.

Like many other syndromes in Chinese medicine, there is lack of precise description and molecular mechanism that is instantly realizable to the scientific community. In addition, the symptoms associated with damp obstruction are not immediately life-threatening; thus, they attract only limited attention even to traditional doctors practicing Chinese medicine. However, damp obstruction presents a clear case for the flow of vital energy and obstruction/subjugation of the 5 elements; therefore, it is here singled out as an ideal system to approach with modern molecular biology.

Animal models of damp obstruction were established and characterized physiologically and biochemically (Huang and Zao, 2000; Kuo, 1988; Lu, 1994 and 1995). Damp-obstructed animal showed symptoms analogous to human as described in the literature. The treated animals showed fatigue, loss of appetite, change of fur color, weight loss, and reduction of Na^+-K^+-ATPase activity. Although the symptoms could be cured by traditional medicine, the molecular mechanism underlying damp obstruction was largely unexplored.

Microarray simultaneously presents the expression of tens of thousands of

genes on a genomic scale (Schena et al., 1995; Alizadeh et al., 2000). This technology enables cancer researchers to compare gene expression between normal and malignant tissues and to quickly identify genes that are differentially regulated during cancerous progression (DeRisi et al., 1996). Microarray datasets can also be used to categorize tumors on the basis of their expression profile and provide useful biological, diagnostic, and prognostic information for mechanistic studies.

The hypothesis underlying this study was to utilize microarray to obtain genomic expression profiles of the liver (mu), heart (huo), stomach (tu), spleen (tu), kidney (shui), and lung (jin) of damp-obstructed rats. With statistical approaches, we were capable of obtaining expressional relationships between organs affected by this pathogenic factor. To our best knowledge, this is the first time that modern molecular biology might provide a genomic interpretation and perhaps molecular evidence for the pathogenesis of damp obstruction and subjugation/restriction among the 5 elements.

Materials and Methods

Animal model

Damp-obstructed rats were established based on previous reports with minor modification (Huang and Zao, 2000; Kuo, 1988; Lu 1994 and 1995). Male Sprague-Dawley rats, weighing 150-200 g, were obtained from the National Science Council Animal Center. All procedures were performed according to the Institutional Guidelines for the Care and Use of Laboratory Animals. The rats were randomly housed in groups of two per wire-mesh cage (39 × 26 × 21 cm) for at least 1 week. Animals were divided into two groups including damp-obstructed group and control group. Each group contained 6 rats. For normal control, rats

were placed in a controlled environment of 23±1 ºC and 50% relative humidity with free access to standard food in pellets (supplied and designed by Fusow Industry Co. Ltd., Taiwan) and tap water, on a 12 h light/dark cycle. For damp obstructive group, rats were placed in a controlled environment of 31±1 °C and 94±5% relative humidity with free access to standard food in pellets and tap water, on a 12 h light/dark cycle. In addition to normal chow, damp obstructive rats were given 18 ml 20% bee honey and 5 g lard daily. The control group was handled with the identical procedure but received the same volume of normal saline instead.

Serum biochemical assays

Animals were fasted for eight hours prior to blood sampling. Anesthetization was performed by intra-peritoneal injection of pentobarbital (45 mg/kg, ip). Five hundred micro-liters of blood was drawn from the tail vein from each animal. Serum in plain vial was separated at 4 °C in a cooling centrifuge for 15 min at 2,000 × *g*. Serum glutamate pyruvate transaminase activity (GPT), glutamate oxaloacetate transaminase activity (GOT), low-density lipoproteins (LDL), high-density lipoproteins (HDL), total cholesterol, triglyceride (TG), alkaline phosphatase activity (ALP), lactate dehydrogenase activity (LDH), serum creatine (CREA), serum creatine phosphokinase activity (CPK), and blood urea nitrogen (BUN) were determined using Roche COBAS MIRA automatic assay machine using standard reagents purchased from Roche Co.

Microarray

All microarrary procedures including PCR amplification, spotting, post-spotting processing, RNA extraction, probe preparation, hybridization, and

post-hybridization experiments were performed in a dust/climate control laboratory at China Medical University. Microarray design, experimental procedures, data processing, and data presentation were carefully performed according to guidelines of Minimum Information About a Microarray Experiment (MIAME) (Brazma et al., 2001).

A sequence-verified human cDNA library containing 32,064 human cDNA clones was a kind gift from the National Health Research Institute of Taiwan. The clones were originally obtained from the IMAGE consortium libraries through its distributor (Research Genetics, Huntsville, AL). We selected 9,600 clones containing mostly known genes for homemade microarray. All clones were carefully selected and manipulated to avoid systematic error usually accompanied by library rearrangement. The cDNA was PCR amplified using 96-well polycarbonate microtiter plate. Primer sequences for amplification of cDNA insert were provided by the supplier. The PCR amplifications were performed using 10 ng purified plasmid in 100 μL total volume containing 10 mM Tris-HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 μM dNTP, 0.2 μM each primer, and 0.5 units of Taq polymerase (Viogen Co., Taiwan). Thermal cycles were as follows: 94 ºC for 10 min, the first 25 cycles; 94 ºC for 30 sec; 57 ºC for 30 s; then 72 ºC for 4 min; and a final extension for 10 min at 72 ºC. Manufacturer-specified length of PCR products was verified by 1% agarose gel electrophoresis. Unreacted small molecules were purified with Millipore MultiScreen-HV plate-filtration (Millipore Co., USA). Final DNA concentration was adjusted to 0.3 μg/μL in 30% DMSO. The DNA solution was transferred to 384-well plates for array spotting. To verify library integrity after reorganization, sequencing was performed on 5 randomly selected 96-well plates. An approximate 5% error rate, including incorrect identity and missing clones, was

detected. The error rate was within the supplier's specification upon arrival of the original library.

The spotting was carried out in DNA Arrayer 6 (Wittech Co., Taiwan) using 24-pin format onto CMT-GAPS II coated slides (Corning Co., USA) under ~80% relative humidity with a density of 9,600 spots per 6 square centimeters. The printed slide was baked 2 hrs at 80 ºC and stored in desiccators at room temperature no longer than 1 week. Spotting quality was examined by hybridization to Cy3-labeled vector sequence. Point-to-point fluctuation between slides was under 5% judged by the fluorescence intensity.

RNA extraction and labeling

Total RNA was extracted using protocol supplied with TRI-reagent (Molecular Research Center, Inc.). Quality of RNA was examined by agarose gel electrophoresis and by OD 260/280 ratio (grater than 2.0).

For each standard labeling, 0.5 microgram of total RNA was annealed with 0.5 microgram polydT(18) in a total volume of 20 μL. The cDNA synthesis was performed in a 50 μL mixture containing 1 mM each dATP, dCTP, dGTP; 40 μM dTTP, 40 μM Cy3-dUTP (or Cy-5-dUTP) (Boehringer Mannheim), 10 mM DTT; 0.5 units/μL Human Placental Ribonuclease Inhibitor (HT Biotechnology Ltd., UK); and 50 units of Superscript RT II (Life Science). The mixture was incubated for 90 min at 42 ºC and terminated by heating at 95 ºC for 5 min. The RNA was degraded by addition of 5.5 μL of 3N NaOH and incubated at 50 ºC for 30 min. The mixture was neutralized by addition of 5.5 μL of 3M acetic acid and filter-purified by Microcon YM-100 (Amicon Co., USA). Final volume was 30 μL.

Hybridization of microarray

The microarray was pre-hybridized in 30 ml prehybridization buffer containing 25% formamide, 5X SSC, 0.1% SDS, and 0.1 mg/mL BSA in 50 mL conical tube at 42 ºC for 1 h. The labeled probe was mixed with 20 μg polydA(10) and 20 μg human Cot-1 DNA (Gibco BRL) and denatured at 95 ºC for 5 min. The denatured probe was dried and suspended in 20 μL prehybridization buffer. Hybridization was performed in Corning hybridization chamber and incubated at 42 ºC for 12-16 h. The slide was washed twice with 30 ml of 2X SSC and 0.1% SDS for 5 min at room temperature, followed by three washes for 20 min each with 30 ml of 0.1X SSC and 0.1% SDS at 42 ºC. All washing procedures were performed in 50-mL conical tubes with gentle shaking. Fluorescence scanning was performed using Axon Genepix 4000B. The fluorescent image was processed by GenePix Pro 3.0 to obtain raw expression dataset. Mean intensity and mean background intensity were utilized for data processing.

Normalization

After background subtraction, raw datasets of normal organs were averaged. The mean datasets were used as the bases of normalization. We employed global array intensity for normalization controls. Non-linear normalization using locally weighted linear regression (lowess) was performed (Cleveland, 1979). Logarithmic ratios based on 2 were calculated accordingly. All datasets are posted on our websites (http://www.cc.nctu.edu.tw/~shuang/download/5-elements/; or http://140.113.133.134/download/5-elements/).

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

The total RNA was extracted as described above. Superscript II RT

(Invitrogen Co., USA) was employed to perform reverse transcription. Two micrograms of denatured total RNA was annealed with 0.5 µg poly $dT_{(15)}$ in a total 20 µl volume containing 1 mM dNTP, 25 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM DTT, 5 mM $MgCl_2$, as well as 40 unit RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen Co., USA). Subsequently, twenty units of Super RT were added and then incubated at 42 °C for 1 hour. In a typical polymerase chain reaction (PCR), two micro-liters of reaction mixture was applied. Thermal-cycle was programmed to 94 °C, 60 °C, and 72 °C for 35 cycles of 1 minute each. The PCR product underwent electrophoresis using 1 % agarose gel in 1x TBE buffer, which contained 89mM of Tris-HCl, 89 mM of boric acid, and 2 mM of EDTA, followed by a standard staining of ethidium bromide. PCR primers were designed and synthesized according to the published sequence of rat genes from GenBank.

Results

Damp-obstructed rat model

Damp obstructed rat model was established following previous descriptions (Huang and Zao, 2000; Kuo, 1988; Lu, 1994 and 1995). There was a dramatic difference in the fur color of damp-obstructed rats compared to normal group, typically brownish. The skin underneath has rashes, bruising, and hemorrhaging (Fig. 1C, 1D).

Serum biochemical assays indicated significant organ damage

To investigate organ damage induced by damp-obstruction, serum biochemical analyses including GOT, GPT, LDL, HDL, total cholesterol, TG, ALP, LDH, CPK, CREA, BUN, and animal weight were performed for animals on day 0, 1, 3, 5,

and 7 (Fig. 2). The significant increase in LDL, HDL, TG, and total cholesterol was likely due to the high-cholesterol diet meant for internal dampness. Increase of GTP for damp-obstructed group indicated minor damage to heart or liver. The decrease of BUN might indicate mild malfunction of kidney. In general, biochemical analyses were consistent with symptoms of damp obstruction.

RT-PCR of heat shock proteins excluded similarity to heat stroke

It is possible that heat treatment might induce molecular consequences similar to heat stroke. Heat shock proteins were shown at elevated levels and might have played a protective role during heat stroke of animals (Welch, 2001; Yang, 1999). To further distinguish the damp-obstructed rat model from heat stroke, semi-quantitative RT-PCR was performed using a primer pair specific to selected heat shock proteins. The damp-obstructed groups showed no significant differences in expression levels for all heat shock proteins (Fig. 3), indicating the distinct molecular difference between the two seemingly similar models.

Microarray profiling indicated marginal similarity among all organs

Microarray experiments were performed on heart, liver, kidney, lung, stomach, spleen, and brain of damp-obstructed rats and normal rats. We performed damp obstructive experiments on 5 livers, 3 lungs, 2 spleens, 2 stomachs, 2 kidneys, 3 brains, 3 hearts, and on the same number of corresponding normal organs. Scattered plots were drawn which show the high-quality data acquisition (Fig. 4). Student's *t*-test was performed to search for genes generally differentially expressed in all organs. Only sixty genes with *P*-values less than 0.05 were identified and exhibited only marginal differences (Table 1). The result indicates that the differential expression of seven organs induced by dampness share only

very limited homology in gene expression profile.

Cluster analysis identified organ-specific expression profiles

To investigate the differences of expression profiles between organs ANOVA was performed to select genes significantly expressed from organ to organ. Four hundred seventy-seven genes with *P*-values less than 0.001 were selected for unsupervised cluster analysis. The results showed that the same organs from different individuals clustered to the same group (Fig. 5). Moreover, their spleen, stomach, and kidney were clustered in the same sub-group, while brain, heart, liver, and lung showed a similar sub-grouping. Genes exhibiting organ-specific expressions were isolated in groups. For instance, Figure 5A shows genes overly expressed in kidney, stomach, and spleen; these might be defined as shui-tu-specific, overly-expressed genes. Genes in Figure 5B and 5C show down-regulation of kidney (shui), stomach (tu), and spleen (tu); these could thus be defined as shui-tu-specific, down-regulated genes. The complete representation of cluster analysis can be found on our websites.

Discussion

We performed biochemical and molecular analysis on damp-obstructed rats. Microarray analysis for organs of the damp-obstructed animals versus normal group indicated that dampness behaves like a pathogenic factor and unequally affects the genomic expression of all organs. Cluster analysis for the expression patterns indicates that spleen, stomach, and kidney respond to dampness differently from heart, liver, lung, and brain. Gene expression specific to each element or group of elements was identified.

DNA microarray is a powerful tool to explore the gene expression on a

genomic scale. The prospective contribution of this technique, however, depends on the complexity and identity of the cDNA library from which microarray is made. The human cDNA collection contains the largest number of known genes while the progress of other mammalians still stumbles. Due to the high degree of homology between human and mammalian genes, interspecies hybridization using human cDNA microarray under lower stringency of hybridizing conditions has been performed (Huang and Yang, 2000; Hsueh el al., 2003). Many important genes associated with pathogenic factors have been identified. Consequently, the interspecies hybridization technique is capable of bringing invaluable genomic information to damp obstruction in rats.

Edema is one of the many symptoms induced by damp obstruction. Microarray study of buffered saline-induced rabbit lung edema was performed and validated by semi-quantitative RT-PCR (Sabbadini, 2003). Genes associated with inflammatory response are consistently up-regulated from both microarray and RT-PCR evidence. Genes of other functional categories are less conclusive due to the inconsistency between microarray expression and RT-PCR validation. In damp-obstructed rats, the down regulation of TNF-alpha and FOS indicated the lesser degree of inflammatory response than that in the lung edema model. Due to the limited information revealed in the lung edema report, further comparison of expression profiling is not available at the present time. Judging from the expression of inflammatory responding genes, edema induced by damp obstruction is mechanistically different from lung edema induced by saline injection.

In the heat stroke animal model, elevated expression of heat shock proteins protects organs from heat damage (Welch, 2001; Yang, 1999). In damp obstructed rats, we observed no significant difference in expression levels for heat shock

proteins, indicating that damp obstruction is different from heat stroke.

According to the philosophy of Chinese medicine, the 5 elements, metal (lung, jin), wood (liver, mu), water (kidney, shui), fire (heart, huo), and earth (spleen and stomach, tu) coordinate by subjugation or restriction to maintain a healthy, physiological state. Normal organ function promotes circulation of vital energy (qi). Introduction of dampness, overwhelming humidity in a hot environment, or abnormal intake of fats and sweets retards the flow of vital energy at middle-energizer, thus inducing dysfunction of spleen and stomach. However, this description is subjective, non-specific, and non-medical. Microarray is capable of observing expression of thousands of genes in a parallel way, thus generating results potentially describing the whole picture of biological events instantly. We have observed the imbalanced gene expression on a genomic scale spreading through all organs including spleen, stomach, kidney, liver, heart, lung, and brain. Expression profiles of spleen, stomach, and kidney clustered in the same group, consistent with the description in the literature for damp obstruction.

We suspected that differential expression of liver and perhaps heart was caused by a massive intake of greasy food. This was indicated by the significant elevation of blood cholesterol and TG. However, the subtle differences between external and internal dampness are intriguing and worth further pursuit.

This is the first time a genomic tool has been used to probe a fundamental theory of Chinese medicine. Our results indicate that it is possible to translate the ambiguous language of ancient medicine into genetic terms that are well known to the scientific community.

Chinese medicine is based on the harmonic coordination of several philosophical systems; qi, elements, and environment. Although differentially

expressed genes are readily available by microarray experiment, we did not intend to identify a specific group of genes representing damp obstruction nor to identify the specific organ that might be a target of a therapeutic approach. Specific genes might lead to a focused investigation for damp obstruction; however, such an approach might be subjective from the point of view of Chinese medicine. Our observations might provide evidence for the ancient description of damp obstruction. Alternative genomic tools such as bioinformatics calculation and proteomics should be incorporated to reveal molecular description for all possible aspects behind reams of ancient literature describing damp obstruction.

In contrast to pathology-based Western medicine, Chinese medicine is based on the balancing of physiological conditions. Although the philosophies are different, it is possible to interpret this ancient language into a scientifically acceptable concept. Our ambition is to apply genomic language as a physiological description to better describe the wisdom of ancient Chinese doctors. Presenting Chinese medicine in a vivid and specific genetic scenario is certainly one of the best ways to resurrect the medicine that has served millions of Chinese for thousands of years. We sincerely hope this is the beginning of a novel application of the most contemporary genomic tools.

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Table 1. Generally differentially expressed genes of damp-obstructed rats

Symbol	Brain	Heart	Kidney	Liver	Lung	Spleen	Stomach	Mean	SD
CSPG2	0.71	0.70	1.97	1.07	-0.10	0.46	2.23	1.01	0.83
MEF2D	0.70	1.09	0.53	0.83	0.30	1.27	1.28	0.85	0.38
GPCR150	0.33	0.65	0.88	0.03	-0.97	0.19	1.53	0.38	0.78
CRP2BP	0.51	0.43	1.40	0.48	0.67	0.24	0.94	0.67	0.39
WRN	-0.09	0.79	0.11	0.44	-0.53	1.20	1.04	0.42	0.63
ACO1	0.18	-0.10	-0.24	-0.15	-0.57	1.59	1.06	0.25	0.78
SLC2A1	0.12	1.04	-0.47	0.74	0.09	1.34	1.09	0.56	0.66
TNFSF7	0.16	0.63	0.18	-0.39	-0.19	1.59	-0.05	0.27	0.66
CHRM3	-0.73	0.71	0.53	0.03	0.52	0.88	0.17	0.30	0.54
CFL2	0.61	-0.20	0.68	1.00	-0.16	0.94	-0.05	0.40	0.52
GLRB	-0.89	-0.88	1.38	0.08	-0.77	-0.96	0.73	-0.19	0.94
PSG11	0.19	-0.25	0.46	-0.42	0.93	0.22	0.72	0.26	0.49
LOC51303	0.60	0.73	0.42	1.80	-0.26	0.65	0.30	0.60	0.62
D2S448	0.05	0.61	-0.47	1.32	-0.39	1.54	0.47	0.45	0.78
EGF	-0.42	0.99	0.31	0.04	0.77	0.28	0.60	0.37	0.47
TCEB1L	0.20	0.42	-0.41	0.03	0.50	1.53	0.19	0.35	0.60
T54	0.44	-0.29	0.16	0.94	0.14	0.44	0.45	0.32	0.38
PIK3C2G	0.15	0.96	-0.27	0.50	0.03	1.06	0.14	0.37	0.50
FRG1	0.31	0.02	0.19	-0.16	0.13	0.85	-0.20	0.16	0.35
SLC35A2	1.10	1.46	0.24	0.47	0.70	1.18	-0.46	0.67	0.65
CHRNA7	1.31	1.41	0.15	0.85	1.07	0.36	0.18	0.76	0.53
KIAA1172	0.46	-0.36	0.07	0.71	-0.14	1.05	-0.53	0.18	0.58
ATF1	0.59	0.56	0.51	-0.27	0.16	0.44	-0.50	0.21	0.44
PPP2R5A	0.45	0.69	-0.08	0.03	1.10	0.49	-0.04	0.38	0.44
DYSF	0.14	0.72	-0.25	1.09	0.05	0.21	0.31	0.32	0.45
ZFP161	0.22	1.30	0.00	0.39	0.75	0.62	-0.24	0.44	0.51
ATDC	0.37	1.05	0.26	-0.38	1.05	0.46	-0.51	0.33	0.61
TOP2A	0.77	-1.39	-0.11	2.00	1.18	0.43	-0.11	0.40	1.08
BK65A6.2	1.47	1.26	-0.20	0.90	1.04	1.21	-0.74	0.71	0.84
ARIH2	-0.30	0.89	-0.17	0.32	-0.51	0.61	-0.64	0.03	0.58
CEACAM5	-1.19	-0.68	-0.10	-1.41	-0.31	-0.53	-0.03	-0.61	0.53
DSG3	-0.18	0.51	-0.28	0.98	0.88	0.12	-0.44	0.23	0.57
FAF1	-2.28	-1.15	0.11	-0.97	0.16	0.01	-0.78	-0.70	0.88
BAZ2B	1.26	1.19	-0.67	1.22	1.11	-0.48	0.18	0.54	0.85
LCN2	0.05	1.66	-0.27	1.28	0.99	0.94	-1.31	0.48	1.04
HMG1	-1.35	-1.62	0.08	-1.14	-2.14	-0.36	-0.67	-1.03	0.76
TMEPAI	1.11	1.21	-0.42	0.98	0.37	-0.38	-0.51	0.34	0.77
TNF	-1.13	-0.77	-0.44	-1.17	-1.32	-0.47	-0.58	-0.84	0.36
TEAD3	-0.68	-0.89	-0.01	-0.39	-0.93	-1.12	-0.91	-0.70	0.38
SORT1	-1.37	-1.04	0.13	-1.12	-0.10	-0.82	-1.31	-0.80	0.59
DXS9879E	-0.29	-0.98	-0.52	-0.15	-0.09	-1.44	-0.85	-0.62	0.50

Figure legends

Figure 1. Pictures of damp-obstructed rats. (A) normal SD rat, (B) damp-obstructed rat, (C) dorsal view of damp-obstructed rat with hair removed, (D) back view of damp-obstructed rat with hair removed.

Figure 2. Serum biochemical analyses for damp-obstructed rats. (A) serum biochemical analyses for damp-obstructed rat (gray bars) and normal rat, (B) stereo view. Symbols are: D, damp-obstructed rats; N, control rats. Numbers under x or y axis indicate days of damp treatment. Significance is shown as * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$).

Figure 3. Semi-quantitative RT-PCR of heat-shock proteins and other proteins for organs in damp-obstructed rats. Primers for RT-PCR of specific genes were designed based on the published rat sequences at GenBank. The PCR products were verified by 1% agarose gel electrophoresis. Three individuals were subjected to RT-PCR for each gene. RT-PCR of ribosomal proteins L18 and L21 were also performed as controls.

Figure 4. Scatter plots for livers, hearts, lungs, kidneys, stomachs, spleens, and brains. Y-axis is damp-obstructed organs and X-axis is normal organs. Shown in the figure are heart (A), kidney (B), liver (C), lung (D), spleen (E), and stomach (F). All plots were drawn at linear scale to show the quality of microarray datasets.

Figure 5. TreeView for the cluster analysis of expression ratios from seven organs of damp-obstructed rats versus normal rats. Cluster analysis for microarray datasets from all organs were performed and visualized by TreeView. The grouping of cluster analyses is shown.

Fig. 1

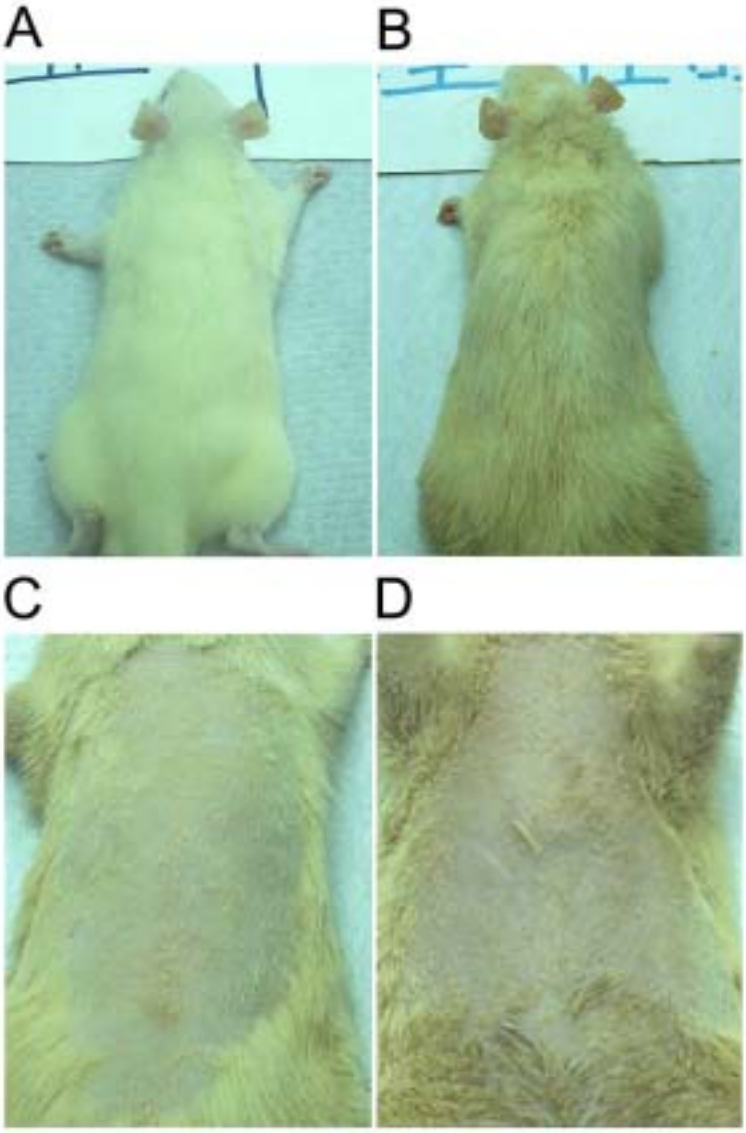
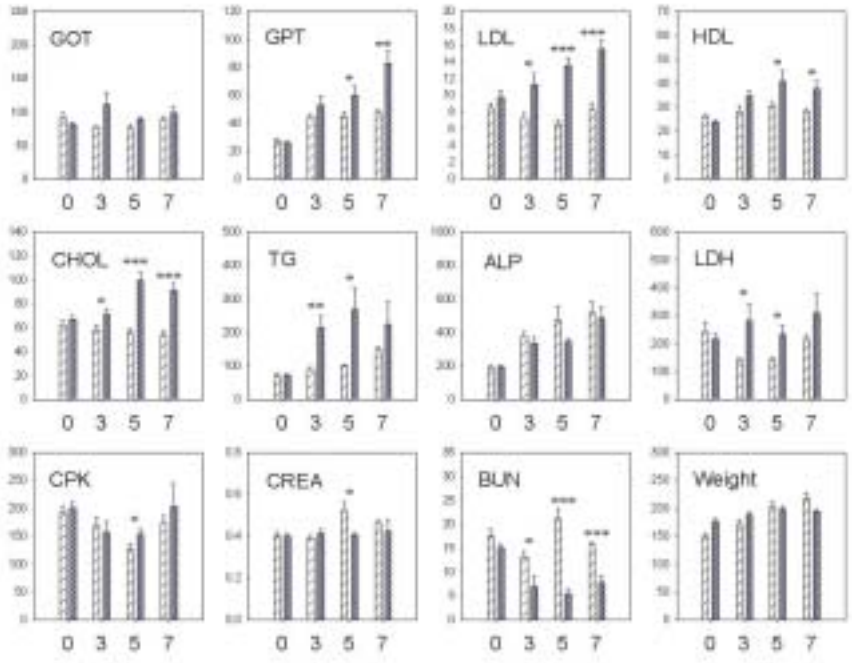


Fig. 2A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TG
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LDH
CPK
CREA
BUN
Weight
0 3 5 7

Fig. 2B

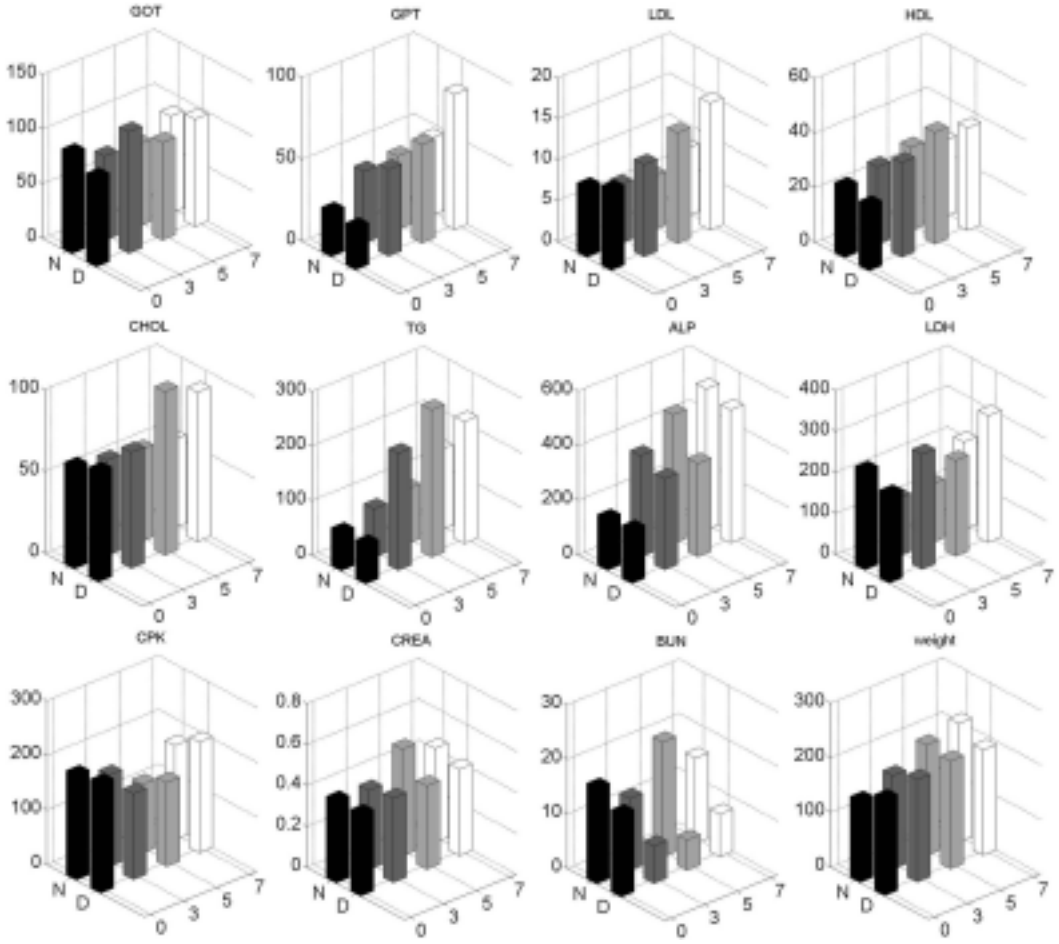
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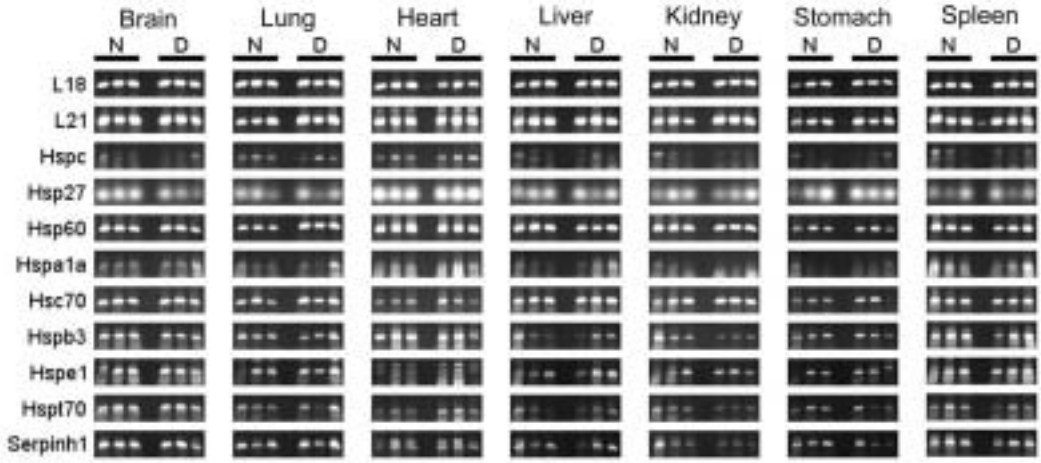
Fig. 3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Lung
Heart
Liver
Kidney
Stomach
Spleen
N
D
L18
L21
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Hsc70
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Hspe1
Hspt70
Serpinh1

Fig. 4

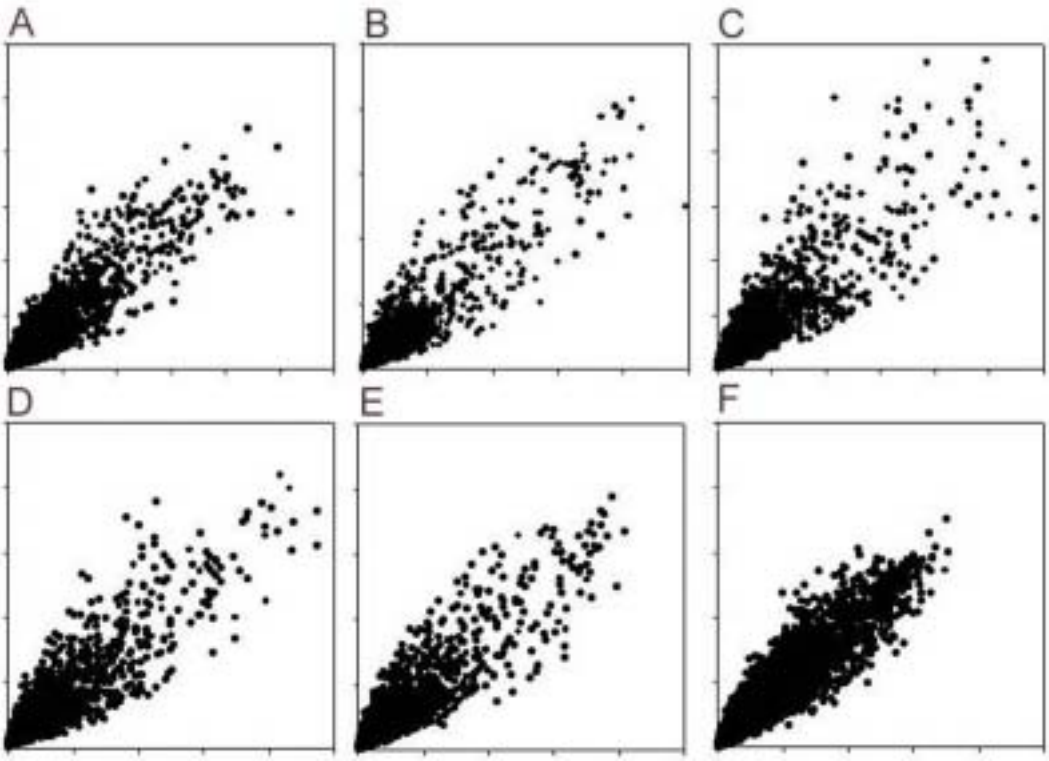
A
B
C
D
E
F

Fig. 5