Original Article

Comparison of Gene Expression Profiling in Pressure and Volume Overload–Induced Myocardial Hypertrophies in Rats

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Gene expression profiling has been conducted in rat hearts subjected to pressure overload (PO). However, pressure and volume overload produce morphologically and functionally distinct forms of cardiac hypertrophy. Surprisingly, gene expression profiling has not been reported for in an animal model of volume overload (VO). We therefore compared the gene expression profiles in the hypertrophied myocardium of rats subjected to PO and VO using DNA chip technology (Affymetrix U34A). Constriction of the abdominal aorta and abdominal aortocaval shunting were used to induce PO and VO, respectively. The gene expression profiles of the left ventricle (LV) 4 weeks after the procedure were analyzed by DNA chips. There were comparable increases in the left ventricular weight/body weight ratio in rats subjected to PO and VO. Echocardiography revealed concentric hypertrophy in the PO animals, but eccentric hypertrophy in the rats subjected to VO. The expressions of many genes were altered in VO, PO, or both. Among the genes that were upregulated in both forms of hypertrophy, greatly increased expressions of B-type natriuretic peptide, lysyl oxidase–like protein 1 and metallothionein-1 (MT) were confirmed by real-time reverse transcription–polymerase chain reaction (RT-PCR). Because free radicals are increased in the hypertrophied heart and may contribute to apoptosis, we examined the role of MT, a free radical scavenger, in apoptosis. The overexpression of MT in H9c2 cells inhibited norepinephrine-induced apoptosis, suggesting that MT may act as an anti-apoptotic molecule in cardiac hypertrophy. In conclusion, we found that many genes were regulated in VO, PO, or both. In addition, a novel role of MT in the hypertrophied myocardium was suggested. (*Hypertens Res* 2006; 29: 1029–1045)

Key Words: gene expression, hypertrophy, DNA chip, metallothionein-1, apoptosis

Introduction

Pressure and volume overload (PO and VO, respectively) produce morphologically and functionally distinct forms of cardiac hypertrophy (1–4); PO produces concentric hypertro-

phy whereas VO produces eccentric hypertrophy. At the cellular level, cardiomyocytes grow vertically in PO and longitudinally in VO. The molecular mechanisms of these differences have been unknown. Changes of some specific genes have been reported in the hearts of PO and VO, *i.e.*, the expressions of collagen isoforms (5, 6) and matrix metallo-

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Fig. 1. Echocardiographic measurements of the hypertrophied rat heart. Echocardiography was performed 28 days after hemodynamic overload as described in Methods. VO, volume overload group; PO, pressure overload group; LVDd, end-diastolic diameter of the left ventricle; IVST, interventricular septal wall thickness; %FS, percent fractional shortening; LAD, left atrial diameter. Values are the mean $\pm$ SD. ** $p < 0.01$ vs. the sham group.

proteinases (7), the expression of β myosin heavy chain (8), the transcriptional regulation of adrenomedullin (9), and the expressions of growth factors (10, 11) and β -tubulin (12). Based on these previous reports, it is anticipated that some specific genes are regulated similarly or differentially in PO and VO, but the regulation of genes other than those listed above has not been clarified. Microarray analysis is a useful method to analyze the behavior of many genes. With this method, gene profiling has been conducted in animal hearts subjected to PO (13, 14). Surprisingly, however, gene expression profiling has not been conducted in hearts subjected to VO. Accordingly, we compared the gene expression profiles in the hypertrophied myocardium of rats subjected to PO and VO using the DNA chip technology.

Methods

Animal Models

Male Wistar rats were used for the experiments. PO was produced in rats by abdominal aortic banding (15), and VO by aortocaval shunting (16). The rats were sacrificed 28 days after the procedure. All animal procedures were conducted according to the guidelines provided by the Kurume University Institutional Animal Care and Use Committee under an approved protocol.

Echocardiography

At the day before sacrifice, rats were lightly anesthetized with

pentobarbital, then examined by echocardiography with an SSD 5500 and a 7.5 MHz probe (Aloka, Tokyo, Japan).

DNA Chip Analysis

DNA chips (GeneChip Rat Genome U34A arrays) were purchased from Affymetrix (Santa Clara, USA). Poly (A) RNA was extracted from the left ventricle (LV) by using a FAST track RNA purification kit (Invitrogen, Carlsbad, USA). We mixed poly (A) RNAs prepared from the LV of five rats and the samples were analyzed by three DNA chips in each group. DNA chip analysis was performed according to the manufacturer's recommended protocol. Each of the groups was compared with each of the other groups, resulting in 27 comparison tests. Average intensity values for each probe set were obtained from the Affymetrix MicroArray Suite 4.0. A p value less than 0.01 was arbitrarily assigned as the level of statistical significance. We considered genes that were elevated by >2 fold compared with controls as "upregulated," genes that were reduced to <0.5 as "downregulated," and genes that were altered by 0.8–1.2 fold as showing "no change." The genes that were expressed at very low levels (those with a signal intensity less than 100) or genes that were considered not to be present based on low signal intensity by the Affymetrix software under all conditions were omitted.

For clustering analysis, the CEL files were converted into DCP files using dCHIP analysis software (www.biostat.harvard.edu/compalab/cdhip/), as described previously by Li and Wong (17). Genechips were normalized, and model-based expression values were generated. We used hierarchical clus-

Fig. 2. Left ventricular weight and heart weight 28 days after hemodynamic overload. A: LVW/BW. B: HW/BW. VO, volume overload group; PO, pressure overload group; LVW/BW, left ventricular weight normalized to body weight; HW/BW, heart weight normalized to body weight. Values are the mean $\pm$ SD, * $p < 0.05$, ** $p < 0.01$ vs. the sham group.

Fig. 3. Comparisons of differentially regulated genes. Venn diagrams were produced to compare the number of genes altered in the volume overload (VO), pressure overload (PO), and sham groups. A: Genes differentially upregulated between the sham group and the VO group, PO group, or both. B: Genes differentially downregulated between the sham group and the VO group, PO group, or both. C: Genes unaltered between the sham group and the VO group, PO group, or both. D: Genes upregulated in the PO group, downregulated in the VO group, or both. E: Genes upregulated in the VO group, downregulated in the PO group, or both.

tering with the average linkage method. The functions of these altered genes were classified by clusters of orthologous groups analysis.

Real-Time Reverse Transcription–Polymerase Chain Reaction

Total RNA was prepared with an RNeasy Midi Kit (QIAGEN, Tokyo, Japan) from LV. Real-time reverse transcription–polymerase chain reaction (RT-PCR) was performed by a GeneAmp 5700 and a TaqMan One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instruction. Expression values for each gene were normalized to 18 S ribo-

somal RNA (TaqMan ribosomal RNA control reagents; Applied Biosystems). The primers and TaqMan probes used in these experiments were as follows: guanosine monophosphate (GMP) reductase TaqMan probe, CTCTGACGG AAGCTGCACATGTCCA; GMP reductase sense-primer, GGCCTCAAGGGACACATCA; GMP reductase antisense-primer, AAAGGCTTTGGCGACATCTC; metallothionein-1 (MT) TaqMan probe, TGCAAAGGTGCCTCGGACAAG TGC; MT sense-primer, GTGGGCTGCTCCAAATGTG; MT antisense-primer, GGTCCGGAATTATTTACACC TGA; B-type natriuretic peptide (BNP) TaqMan Probe, CGGCGCAGTCAGTCGCTTGG; BNP sense-primer, TGG GCAGAAGATAGACCGGA; BNP antisense-primer, ACA ACCTCAGCCCCGTACAG; lysyl oxidase-like protein 1

Table 1. Genes That Respond to Pressure or Volume Overload

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
VO↑ and PO↑						
AA799773	re_AA799773_at	Filamin C, γ	2.42	2.03	actin binding	19
AA892378	re_AA892378_at	Similar to tetratricopeptide repeat domain 11 (LOC288584)	2.27	2.05	apoptosis	
S69383	S69383_at	12-Lipoxygenase	2.61	2.02	arachidonate 12-lipoxygenase activity, iron ion binding	20
L18948	L18948_at	S100 calcium-binding protein A9 (calgranulin B)	3.25	3.22	calcium ion binding	19
U17919	U17919_s_at	Allograft inflammatory factor-1	2.05	2.00	calcium ion binding	
AI102562	re_AI102562_at	Metallothionein-1 (MT-1)	4.05	3.82	copper ion binding, metal ion binding, zinc ion binding	14, 19, 21
L16532	L16532_at	2',3'-cyclic nucleotide 3'-phosphodiesterase (CnplI)	3.28	2.94	cyclic nucleotide catabolism	
U17254	U17254_g_at	Immediate early gene transcription factor NGFI-B	2.14	2.06	DNA binding, ligand-dependent nuclear receptor activity	
U75397	U75397UTR#1_s_at	Early growth response 1 (Egr 1)	2.37	2.03	DNA binding, nucleic acid binding, transcription factor activity	21
L22761	L22761_at	GATA binding protein 4 (Gata4)	2.42	3.06	DNA binding, transcription factor activity	13
AA859805	re_AA859805_at	Lysyl oxidase like protein (LOXL)	3.95	2.48	elastogenesis	19
AA800517	re_AA800517_s_at	Vesicle associated protein (VAP1)	2.07	2.02	endosome protein	
AI231472	re_AI231472_s_at	Collagen α 1 type I	3.67	3.90	extracellular matrix protein	13, 14
M17526	M17526_at	GTP (guanine nucleotide protein)-binding protein (G α o)	2.09	2.49	GDP binding	19
AA800784	re_AA800784_at	Cysteine-rich protein 61 (CYR61)	2.18	2.18	growth factor binding, heparin binding	14
AA799498	re_AA799498_at	Natriuretic peptide precursor type B	4.45	2.62	hormone activity	
E00775	E00775cds_s_at	Natriuretic peptide precursor type A	4.12	2.56	hormone activity	13, 19, 22
M32062	M32062_at	Fe- γ receptor (IgG)	2.88	2.31	IgG binding, IgG receptor activity, receptor activity	13, 14, 19, 20, 22
AI231213	re_AI231213_at	Metastasis suppressor homolog (KAI1)	2.03	2.01	integral to membrane	
X04979	X04979_at	Apolipoprotein E	2.16	2.00	lipid transporter activity, lipid binding, heparin binding	
J00692	J00692_at	Skeletal muscle α -actin gene	2.47	2.02	motor activity, structural constituent of cytoskeleton	14
AA799678	re_AA799678_s_at	EGL nine homolog 3	2.33	2.00	oxidoreductase activity	19
X71127	X71127_at	Complement protein C1q β -chain	2.23	2.00	phosphate transport, immune response, complement activation	
AI069982	re_AI069982_s_at	Myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK)	2.09	2.02	protein serine/threonine kinase activity	
AA875405	re_AA875405_at	Forkhead-like 18	2.21	2.07	transcription, regulation of transcription, development	
AI639185	re_AI639185_s_at	Potassium channel, subfamily K, member 3	3.20	3.26	transport, ion transport, potassium ion transport	
M91235	M91235_f_at	VL30 element	2.60	2.15	—	
AA892847	re_AA892847_at	Similar to α -N-acetyl-galactosaminidase (LOC315165)	2.95	2.71	—	
AA891828	re_AA891828_g_at	Similar to UV excision repair protein RAD23 homolog A	2.03	2.07	—	
AA799992	re_AA799992_at	Similar to C11orf17 protein (LOC361624)	2.57	2.18	—	
AA800678	re_AA800678_at	Similar to AW046014 protein (LOC363328)	2.03	2.00	—	
AA875523	re_AA875523_s_at	Similar to 17 kDa myosin light chain (LOC362816)	2.04	2.00	—	
AA800803	re_AA800803_g_at	EST190300 (Lung derived cDNA)	2.05	2.11	—	
VO↑ and PO→						
AI105348	re_AI105348_i_at	Cofilin 1 (CFL1)	2.20	1.13	actin binding	
M58404	M58404_at	Thymosin β -10 (testis-specific) gene	2.44	1.04	actin binding, actin monomer binding	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
A1180288	rc_A1180288_s_at	Caldesmon 1 (Cald1)	3.79	1.14	actin binding, calmodulin binding, myosin binding	
AA875132	rc_AA875132_at	Tropomyosin 1, α	2.36	0.95	actin binding, structural constituent of cytoskeleton	
AA893280	rc_AA893280_at	Adipose differentiation-related protein	2.01	0.82	adipocyte differentiation	
M23601	M23601_at	Monoamine oxidase B (Maobf3)	3.71	1.13	amine oxidase activity, oxidoreductase activity	
AA859829	rc_AA859829_g_at	Macrophage erythroblast attacher	2.08	0.96	apoptosis, cell adhesion, development	
S54008	S54008_i_at	Fibroblast growth factor receptor 1 β -isoform	2.22	1.12	ATP binding, fibroblast growth factor receptor activity	
AA875002	rc_AA875002_at	Leucine-rich repeat-containing 8 (LOC311846)	2.52	0.84	B cell development	
M83143	M83143_g_at	β -Galactoside- α 2,6-sialyltransferase	2.06	0.81	β -galactoside α -2,6-sialyltransferase activity	
U90121	U90121_at	Thrombomodulin	3.22	1.11	blood coagulation	
AF027984	AF027984_at	Low voltage-activated, T-type calcium channel α subunit	2.13	1.11	calcium channel activity, calcium ion binding	
AA891204	rc_AA891204_s_at	Secreted acidic cysteine rich glycoprotein (Sparc) or osteonectin	2.46	1.13	calcium ion binding	22
X06916	X06916_at	Protein p9Ka homologous to calcium-binding protein	2.14	0.85	calcium ion binding	
AA849769	rc_AA849769_at	Follistatin-related protein precursor or Follistatin-like (Fstl)	3.22	1.19	calcium ion binding, heparin binding	21
X51529	X51529_at	Platelet phospholipase A	3.79	0.85	calcium ion binding, phospholipase A2 activity	
X89963	X89963_at	Thrombospondin 4 (Thbs4)	4.41	0.93	calcium ion binding, structural molecule activity	
A1145680	rc_A1145680_s_at	Monocarboxylate transporter or Solute carrier family 16, member 1	2.17	1.14	carrier activity, monocarboxylate porter activity	
AB000778	AB000778_s_at	Phospholipase D	2.46	0.85	catalytic activity, hydrolase activity, phospholipase D activity	
X82396	X82396_at	Cathepsin B	2.12	1.12	cathepsin B activity, cysteine-type endopeptidase activity	
L03201	L03201_at	Cathepsin S	2.38	0.90	cathepsin S activity, cysteine-type endopeptidase activity	
AF020618	AF020618_g_at	Myeloid differentiation primary response gene 116	2.24	0.86	cell differentiation	
AA874848	rc_AA874848_s_at	Thy-1 gene for cell-surface glycoprotein	2.42	1.20	cell-cell interaction	
AA799803	rc_AA799803_at	Complement component 1, r subcomponent	2.36	0.96	chymotrypsin activity, trypsin activity	
M14656	M14656_at	Osteopontin	3.43	1.03	cytokine activity, growth factor activity, integrin binding	
X59864	X59864mRNA_g_at	ASM15 gene	2.98	1.15	cytoplasmic protein	
U16025	U16025_at	Class Ib RT1 or M3 protein (M3 gene)	2.82	1.18	defense response, immune response	
A1232374	rc_A1232374_g_at	H1 histone family, member 0 (H1f0)	2.07	1.19	DNA binding	
M31322	M31322_at	Sperm membrane protein (YWK-II)	2.20	1.19	DNA binding, endopeptidase inhibitor activity	
AA800613	rc_AA800613_at	Gene for TIS11 or Zinc finger protein 36 (Zfp36)	2.83	0.94	DNA binding, transcriptional activator activity	
M65149	M65149_at	CCAAT/enhancerbinding, protein (C/EBP) δ (Cebpd)	2.30	0.83	DNA binding, protein binding	
X60769	X60769mRNA_at	Interleukin-6-dependent binding protein	2.57	1.05	DNA binding, protein binding, transcriptional activator activity	
AA891041	rc_AA891041_at	Jun-B oncogene	3.21	0.93	DNA binding, transcription factor activity	
AB012231	AB012231_s_at	NF1-B2	2.11	1.11	DNA binding, transcription factor activity	
AA891717	rc_AA891717_at	Upstream transcription factor 1	2.32	1.19	DNA binding, transcription factor activity	
AA892520	rc_AA892520_g_at	Vesicle amine transport protein 1 homolog	2.27	0.99	DNA binding, zinc ion binding, oxidoreductase activity	
AF041066	AF041066_at	Ribonuclease 4	2.09	1.17	endonuclease activity, hydrolase activity, nuclease activity	
U42719	U42719_at	Complement component 4a	2.00	0.82	endopeptidase inhibitor activity	
J04035	J04035_at	Tropoelastin	2.54	1.17	extracellular matrix constituent conferring elasticity	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
U17834	U17834_at	Biglycan	2.66	1.16	extracellular matrix protein	22
X84039	X84039_at	Lumican	2.10	1.03	extracellular matrix protein	
X17053	X17053mRNA_s_at	Immediate-early serum-responsive JE gene	2.15	0.81	G-protein-coupled receptor binding, chemokine activity	
X05834	X05834_at	Fibronectin gene 3' end	2.46	0.82	heparin binding, mercury ion binding, oxidoreductase activity	
M36317	M36317_s_at	Thyrotropin-releasing hormone (TRH) precursor	3.34	0.86	hormone activity, thyrotropin-releasing hormone activity	
H31839	rc_H31839_at	BCL2-antagonist/killer 1 (Bak1)	2.17	0.94	induction of apoptosis	
A123219	rc_A123219_at	Endothelial cell-specific molecule 1	4.78	1.19	insulin-like growth factor binding	
AA894029	rc_AA894029_at	Endothelial type gp91-phox gene	3.03	1.09	ion channel activity, voltage-gated ion channel activity	
X16554	X16554_at	Phosphoribosylpyrophosphate synthetase subunit I	2.56	1.19	kinase activity, lipoate-protein ligase B activity	
U02553	U02553cds_s_at	Protein tyrosine phosphatase	2.74	1.19	MAP kinase phosphatase activity	
S74351	S74351_s_at	Protein tyrosine phosphatase	2.30	1.11	MAP kinase phosphatase activity	
S81478	S81478_s_at	Oxidative stress-inducible protein tyrosine phosphatase	2.20	0.84	MAP kinase phosphatase activity	
A1169327	rc_A1169327_at	Tissue inhibitor of metalloproteinase-1 (TIMP1)	2.32	1.19	metalloendopeptidase inhibitor activity	
X06801	X06801cds_i_at	Vascular α -actin	3.09	1.15	motor activity, structural constituent of cytoskeleton	
L00088	L00088expanded_cds#2	Fast myosin alkali light chains	2.90	0.81	motor activity, structural constituent of muscle	
AA866276	rc_AA866276_at	Myeloid-associated differentiation marker	2.43	0.94	myeloid blood cell differentiation	
AA945737	rc_AA945737_at	Chemokine receptor LCR1	2.35	0.95	neuronal cell recognition	
U90610	U90610_at	CXC chemokine receptor (CXCR4)	2.18	1.11	neuronal cell recognition	
AA874999	rc_AA874999_at	Sec61 β subunit	2.11	1.14	protein targeting, protein transport	
U42627	U42627_at	Dual-specificity protein tyrosine phosphatase (rVH6)	2.58	1.18	protein tyrosine/serine/threonine phosphatase activity	
AA874889	rc_AA874889_g_at	Unc-5 homolog B	2.29	0.95	receptor activity, netrin receptor activity, protein binding	
X07636	X07636_at	Hepatic lectin	2.35	1.14	receptor activity, sugar binding	
M91590	M91590_at	β -Arrestin2	2.65	1.08	regulation of G-protein coupled receptor protein signaling	
A1008423	rc_A1008423_at	Unc-50 related protein (UNC5)	2.01	1.19	RNA binding	
AA860043	rc_AA860043_at	Guanine nucleotide binding protein γ subunit 11 (Gng11)	2.26	1.03	signal transducer activity	20
AA894345	rc_AA894345_at	Phosphoprotein enriched in astrocytes 15	2.17	0.85	sugar porter activity, protein binding	
AA893235	rc_AA893235_at	Similar to G0S2-like protein	2.60	0.96	—	
X52815	X52815cds_f_at	Similar to γ actin-like protein	2.01	1.17	—	
AA892391	rc_AA892391_at	Similar to hypothetical protein FLJ20531 (LOC303164)	3.85	1.16	—	
AA893267	rc_AA893267_at	Similar to proline-serine-threonine phosphatase-interacting protein 1	2.06	0.84	—	
AA799571	rc_AA799571_at	Similar to RIKEN cDNA 1110001M20 (LOC298308)	2.01	1.19	—	
AA892578	rc_AA892578_at	Similar to RIKEN cDNA 6330406115 (LOC360757)	2.83	1.00	—	
AA891940	rc_AA891940_at	Similar to transforming protein RhoC (H9)	2.36	0.91	—	
AA892333	rc_AA892333_at	Similar to tubulin α -6	2.13	0.81	—	
AA891690	rc_AA891690_g_at	Similar to tumor necrosis factor ligand superfamily member 13	2.37	1.07	—	
AA894292	rc_AA894292_at	EST198095 (Spleen derived cDNA)	2.31	0.87	—	
AA894092	rc_AA894092_at	EST197895 (Spleen derived cDNA)	4.98	1.14	—	
AA891677	rc_AA891677_at	EST195480 (kidney derived cDNA)	2.07	1.14	—	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
VO↑ and PO↓						
J02780	J02780_at	Tropomyosin 4 (Tpm4)	2.70	0.48	actin binding	19
M34043	M34043_at	Thymosin β -4 (pTB4G)	2.32	0.49	actin binding	19
M83107	M83107_at	Transgelin (Smooth muscle 22 protein) (Tagln)	2.28	0.44	actin filament binding, protein binding, bridging	
L12380	L12380_at	ADP-ribosylation factor 1 (Arf1)	2.95	0.40	GTP binding, GTPase activity, protein transporter activity	
X74402	X74402_at	Guanosine diphosphate dissociation inhibitor 1 (Gdi1)	2.45	0.47	GTPase activator activity, RAB GDP-dissociation inhibitor activity	
D30740	D30740_at	14-3-3 protein mRNA for mitochondrial import stimulation factor S1	2.36	0.44	monoxygenase activity, protein domain specific binding	
AA799520	rc_AA799520_at	Integral membrane protein 2B	2.00	0.43	neurogenesis	21
X76489	X76489cds_g_at	CD9 mRNA for cell surface glycoprotein	2.37	0.33	protein binding	
AA875033	rc_AA875033_at	Fibulin 5 (Fbln5)	2.16	0.49	regulation of cell growth	19
AA850734	rc_AA850734_at	Vascular endothelial growth factor A (Vegfa)	2.12	0.49	vascular endothelial growth factor receptor binding	
AA875537	rc_AA875537_at	Similar to splicing factor arginine/serine rich 2 (SC-38)	2.55	0.32	—	
VO→ and PO↑						
M60655	M60655_at	α -1B adrenergic receptor	1.03	2.05	α 1-adrenergic receptor activity	
AI639058	rc_AI639058_s_at	Transmembrane, prostate androgen induced RNA	1.17	2.19	androgen receptor signaling pathway	
AF014009	AF014009_at	Acidic calcium-independent phospholipase A2	0.80	2.02	antioxidant activity, catalytic activity, hydrolase activity	21
M22631	M22631_at	α -Propionyl-CoA carboxylase	1.14	2.02	ATP binding, biotin binding, propionyl-CoA carboxylase activity	
U10357	U10357_at	Pyruvate dehydrogenase kinase 2 subunit p45	1.19	2.30	ATP binding, pyruvate dehydrogenase kinase activity	
X75253	X75253_at	Phosphatidylethanolamine binding protein (Pbp)	0.83	2.00	ATP binding, serine-type endopeptidase inhibitor activity	
D86039	D86039_at	Potassium inwardly rectifying channel, subfamily J, member 11	0.98	2.18	ATP-activated inward rectifier potassium channel activity	
AI169417	rc_AI169417_s_at	Phosphoglycerate mutase type B subunit	1.12	3.61	bisphosphoglycerate phosphatase activity, catalytic activity	
M86621	M86621_at	Dihydropyridine-sensitive L-type calcium channel α -2 subunit	1.02	2.18	calcium channel activity, ion channel activity	
AA892511	rc_AA892511_at	Tetcalcin	0.92	2.07	calcium ion binding	
AA892146	rc_AA892146_f_at	Similar to carboxypeptidase B gene	1.13	2.02	carboxypeptidase activity, metalloproteinase activity	
X66494	X66494_at	Choline transporter (CHOT1)	0.91	2.00	choline transporter activity, creatine transporter activity	
AB012234	AB012234_g_at	Nuclear factor I/X (Nfix)	1.11	2.01	DNA binding, transcription factor activity	19
U17837	U17837UTR#1_g_at	Zinc finger protein RIZ	0.86	2.06	DNA binding transcription factor	
AA899106	rc_AA899106_at	G1/S-specific cyclin D2 (VIN-1 proto-oncogene) (LOC297611)	1.14	2.19	G1/S transition of mitotic cell cycle, cell growth	
AA799788	rc_AA799788_s_at	Cell division cycle 34	0.99	2.10	G2 checkpoint	
AA799570	rc_AA799570_at	DnaJ (Hsp40) homolog, subfamily A, member 4	1.11	2.02	heat shock protein binding, unfolded protein binding	19
M33962	M33962_g_at	Protein-tyrosine-phosphatase	0.93	2.02	hydrolase activity, phosphoprotein phosphatase activity	
AJ006971	AJ006971_g_at	Death-associated like kinase (Dapk)	0.80	2.08	induction of apoptosis	
AI043968	rc_AI043968_at	Caveolin-3	1.03	2.00	integral to membrane	
AA859981	rc_AA859981_at	Similar to myo-inositol monophosphatase 2 (Impa2)	0.82	2.02	magnesium ion binding, inositol-1(or 4)-monophosphatase activity	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
X53054	X53054_at	RT1.D β chain	0.99	2.16	MHC class II protein	
A1227608	rc_A1227608_s_at	Microtubule-associated protein tau (Mapt)	1.16	2.02	microtubule-based process	
U59126	U59126_at	TR4-NS orphan receptor (TR4) gene	1.13	5.34	nuclear hormone receptor	
AF077354	AF077354_at	Ischemia responsive 94 kDa protein (irp94)	1.00	2.20	response to heat	
M29293	M29293_at	Small nuclear ribonucleoprotein-associated protein (snRNP)	1.12	2.03	RNA binding, pre-mRNA splicing factor activity	
U82224	U82224_at	Cardiac titin N2B isoform (Ttn)	1.15	3.79	sarcomere organization	
X78985	X78985cds_s_at	CD5 or Hocyte antigen CD5	0.81	4.28	scavenger receptor activity	
AA892843	rc_AA892843_at	Mitochondrial ribosomal protein L24	1.06	2.04	structural constituent of ribosome	
X56228	X56228_g_at	Rhodanese or thiosulfate sulfurtransferase (Tst)	0.80	2.31	thiosulfate sulfurtransferase activity, transferase activity	
AA858586	rc_AA858586_at	Suppressor of Ty 5 homolog (<i>S. cerevisiae</i>) (Supt5h)	1.14	2.16	transcription elongation factor activity	
M62752	M62752_at	Statin-related protein (S1) gene	0.87	2.10	translation elongation factor activity, GTP binding	
AA892507	rc_AA892507_at	Immature colon carcinoma transcript 1	1.12	2.00	translation release factor activity	
AA799691	rc_AA799691_at	K-Cl cotransporter KCC4	1.01	2.00	transport, ion transport, potassium ion transport	
AA799475	rc_AA799475_at	Ankyrin repeat domain 24	0.88	2.05	—	
AA875261	rc_AA875261_at	CSX-associated LIM	1.17	2.08	—	
AA893821	rc_AA893821_at	P34 protein	1.19	2.12	—	
AA892005	rc_AA892005_at	SCIRP10-related protein	1.02	2.08	—	
AA891802	rc_AA891802_at	Similar to cysteine and histidine rich 1	1.16	2.04	—	
AA800039	rc_AA800039_s_at	Similar to hypothetical protein MGC6696 (LOC293719)	1.02	2.12	—	
AA799766	rc_AA799766_at	Similar to Jtv1-pending protein	0.92	2.00	—	
AA892768	rc_AA892768_at	Similar to putative breast adenocarcinoma marker (32 kDa)	1.19	2.30	—	
AA799654	rc_AA799654_g_at	Similar to WD repeat-containing F-box protein FBW5	1.02	2.12	—	
AA866454	rc_AA866454_at	Similar to α -2(f) promoter	1.16	2.00	—	
AA892773	rc_AA892773_at	EST196576 (kidney derived cDNA)	1.02	2.02	—	
AA891631	rc_AA891631_at	EST195434 (kidney derived cDNA)	0.93	2.02	—	
AA799932	rc_AA799932_at	EST189429 (heart derived cDNA)	1.09	2.09	—	
VO→ and PO↓						
U93306	U93306_at	VEGF receptor-2/FLK-1	1.01	0.49	ATP binding, kinase activity, protein kinase activity	
X53363	X53363cds_s_at	Calreticulin (Calr)	0.82	0.36	calcium ion binding, calcium ion storage activity	
AF047707	AF047707_at	UDP-glucose:ceramide glycosyltransferase	1.13	0.45	ceramide glucosyltransferase activity	
U95727	U95727_at	DnaJ (Hsp40) homolog, subfamily A, member 2 (DnaJ2)	0.89	0.45	chaperone activity	19
X16043	X16043cds_at	Phosphatase 2A catalytic subunit isotype α	0.92	0.47	CTD phosphatase activity, calcium-dependent phosphatase activity	
D17711	D17711cds_s_at	Heterogeneous nuclear ribonucleoprotein K (Hnrpk)	1.13	0.32	DNA binding, RNA binding, nucleic acid binding	
AA799582	rc_AA799582_at	Heterogeneous nuclear ribonucleoprotein K (Hnrpk)	1.17	0.48	DNA binding, RNA binding, nucleic acid binding	
U53922	U53922_at	DnaJ-like protein (RDJ1)	0.82	0.47	DNA damage response, perception of DNA damage	
D21800	D21800_at	Proteasome subunit RC10-II	0.83	0.43	endopeptidase activity, hydrolase activity, peptidase activity	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
AF072411	AF072411_g_at	Fatty acid translocase/CD36	1.13	0.35	fatty acid binding, receptor activity	
S81353	S81353_s_at	Sulfated glycoprotein-1 or Prosaposin (Psap)	0.89	0.25	glycolipid transport, sphingolipid metabolism	
M83676	M83676_at	Similar to RAB12, member RAS oncogene family (Rab12)	0.92	0.49	GTP binding, protein transporter activity	
D84477	D84477_at	Pls1a ras-related homolog A2 (Arha2)	1.19	0.25	GTPase activity, GTP binding	
X68101	X68101_at	Dedicator of cytokinesis 9	1.16	0.46	guanyl-nucleotide exchange factor activity, GTP binding	
AF000942	AF000942_at	Inhibitor of DNA binding 3	1.11	0.43	inhibitor of transcription	
M19533	M19533mRNA_i_at	Cyclophilin or peptidylprolyl isomerase A (Ppia)	0.88	0.46	isomerase activity, peptidyl-prolyl <i>cis-trans</i> isomerase activity	
A1102620	rc_A1102620_at	Mitogen activated protein kinase kinase 1	0.95	0.47	magnesium ion binding, protein kinase activity	
A1011706	rc_A1011706_at	Splicing factor, arginine/serine-rich 3 (SRp20)	0.99	0.49	nucleic acid binding, RNA binding	
D84346	D84346_s_at	NCK-associated protein 1 (Nap1) protein or Hem-2	0.88	0.21	protein binding	
D78303	D78303_at	YT521 for RNA splicing-related protein	1.09	0.49	protein binding	
AA891035	rc_AA891035_at	Beclin 1 (coiled-coil, myosin-like BCL2-interacting)	1.11	0.45	protein binding	
S59892	S59892_f_at	La=autoantigen SS-B/La (3' region, clone K4)	0.89	0.43	RNA binding, nucleic acid binding	
AF030091	AF030091UTR#1_g_at	Cyclin ania-6a	1.10	0.49	RNA processing	
AB022209	AB022209_s_at	Ribonucleoprotein F	1.18	0.46	TATA-binding protein binding, pre-mRNA splicing factor activity	
AF048687	AF048687_s_at	UDP-Gal:glucosylceramide β -1,4-galactosyltransferase	1.18	0.45	UDP-galactose-glucosylceramide galactosyltransferase activity	
AF034237	AF034237_s_at	DD6A4-1	1.13	0.49	—	
U64705	U64705cds_i_at	Eukaryotic translation initiation factor 4A2	0.89	0.49	—	
AA891535	rc_AA891535_at	Hippocampus abundant gene transcript 1	1.18	0.49	—	
AA892376	rc_AA892376_at	Protein associated with PRK1	1.19	0.44	—	
AA892918	rc_AA892918_at	Similar to tight junction protein 1 (Tjp1)	1.19	0.47	—	
VO↓ and PO↑						
L22294	L22294_at	Pyruvate dehydrogenase kinase-1 (Pdk1)	0.44	2.02	ATP binding, pyruvate dehydrogenase kinase activity	19
D10754	D10754_at	Proteasome subunit R-DELTA	0.48	2.19	endopeptidase activity, hydrolase activity, peptidase activity	
X62660	X62660mRNA_at	Glutathione transferase subunit 8	0.46	2.06	—	
AA891037	rc_AA891037_g_at	Similar to 60S ribosomal protein L3-like	0.47	2.05	—	
AA799888	rc_AA799888_at	Similar to mitochondrial ribosomal protein L40	0.49	2.00	—	
VO↓ and PO→						
D16478	D16478_at	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase α subunit	0.49	0.92	3-hydroxyacyl-CoA dehydrogenase activity	
D00512	D00512_at	Mitochondrial acetoacetyl-CoA thiolase	0.43	0.98	acetyl-CoA C-acetyltransferase activity, acyltransferase activity	
J02752	J02752_at	Acyl-coA oxidase	0.48	0.83	acyl-CoA oxidase activity	
M60322	M60322_g_at	Aldehyde reductase 1 (low Km aldose reductase) (Aldr1)	0.49	1.08	aldehyde reductase activity, oxidoreductase activity	
M21060	M21060_s_at	Copper-zinc containing superoxide dismutase	0.46	1.05	antioxidant activity, copper, zinc superoxide dismutase activity	
A1104924	rc_A1104924_f_at	α -Cardiac myosin heavy chain	0.48	1.06	ATP binding, actin binding, calmodulin binding, motor activity	
AA800190	rc_AA800190_g_at	Brain glycogen phosphorylase (Pygb)	0.43	0.89	catalytic activity, glycogen phosphorylase activity	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
Y00497	Y00497_s_at	Superoxide dismutase 2	0.41	0.84	copper, zinc superoxide dismutase activity	
A1229620	rc_A1229620_s_at	Cytochrome c oxidase subunit Vb	0.46	0.95	cytochrome-c oxidase activity, oxidoreductase activity	
X15030	X15030_at	CoxVa for mitochondrial cytochrome c oxidase subunit Va	0.48	0.97	cytochrome-c oxidase activity, oxidoreductase activity	
U95001	U95001UTR#1_s_at	Developmentally-regulated cardiac factor (DRCF-5)	0.44	0.88	diphosphoinositol-polyposphate diphosphatase activity	
K00750	K00750exon#2-3_g_at	Cytochrome c gene, nuclear gene for mitochondrial product	0.47	0.88	electron transporter activity, lipid binding	
A1176422	rc_A1176422_g_at	Electron-transferring-flavoprotein dehydrogenase	0.47	0.90	electron-transferring-flavoprotein dehydrogenase activity	
M83680	M83680_at	Sprague-Dawley (clone LRB13) RAB14 or GTPase Rab14	0.44	1.02	GTP binding, protein transporter activity	
AA799474	rc_AA799474_at	Similar to cytochrome c-1 (LOC300047)	0.37	0.93	heme binding, electron transporter	
D13120	D13120_s_at	ATP synthase subunit d	0.44	0.81	hydrogen ion transporter activity	22
AA875444	rc_AA875444_at	Dihydropyrimidinase-like 2 (Dpysl2)	0.33	0.85	hydrolase activity	
A1235358	rc_A1235358_at	Ubiquinol-cytochrome c reductase core protein II	0.43	0.89	metalloendopeptidase activity	
M20131	M20131cds_s_at	cytochrome P450, family 2, subfamily e, polypeptide 1	0.48	1.16	monooxygenase activity, oxidoreductase activity	
A1013297	rc_A1013297_g_at	NADH dehydrogenase (ubiquinone) Fe-S protein 4	0.47	0.83	NADH dehydrogenase (ubiquinone) activity	
AA819547	rc_AA819547_at	NADH dehydrogenase (ubiquinone) 1 α subcomplex	0.47	0.85	NADH dehydrogenase (ubiquinone) activity	
AA851403	rc_AA851403_at	NADH dehydrogenase (ubiquinone) 1 β subcomplex 8	0.49	1.04	NADH dehydrogenase (ubiquinone) activity	
AA891651	rc_AA891651_g_at	NADH dehydrogenase (ubiquinone) 1, α/β subcomplex, 1	0.45	0.83	NADH dehydrogenase (ubiquinone) activity, acyl carrier activity	
AF095927	AF095927_g_at	Protein phosphatase 2C	0.48	0.89	negative regulation of cell cycle	
D12769	D12769_g_at	Basic transcription element binding protein 1	0.38	0.91	nucleic acid binding, DNA binding, zinc ion binding	
AA875327	rc_AA875327_g_at	Eukaryotic translation initiation factor 4H	0.44	0.81	nucleotide binding, translation initiation factor activity	
A1136977	rc_A1136977_at	FK506 binding protein 4	0.37	0.87	peptidyl-prolyl <i>cis-trans</i> isomerase activity	
U90261	U90261UTR#1_at	Hypertension-regulated vascular factor-1 (HRVF-1)	0.45	0.99	protein binding	
D32209	D32209_at	LANP for leucine-rich acidic nuclear protein	0.48	0.94	protein binding	
AA892544	rc_AA892544_s_at	Neural precursor cell expressed, developmentally down-regulated gene 8	0.49	0.95	protein binding	
A1103838	rc_A1103838_g_at	Heat shock 20-kDa protein (Loc192245)	0.45	0.99	regulation of muscle contraction	
AF090867	AF090867_at	Guanosine monophosphate reductase (GMP-reductase)	0.31	1.12	response to cold	
D84480	D84480_s_at	PMSG-induced ovarian mRNA, 3' sequence, N2	0.35	0.84	RNA binding, structural constituent of ribosome	
AA900476	rc_AA900476_g_at	Transcription factor MRG1	0.44	0.96	transcription factor activity, transcription cofactor activity	
AA892554	rc_AA892554_g_at	AA891476BAC clone RP24-216M6 from 5	0.49	0.82	—	
D16554	D16554_at	Polyubiquitin (four repetitive ubiquitins in tandem)	0.49	0.92	—	
AA891476	rc_AA891476_at	Similarity to protein pdb: ILBG (E. coli) B Chain B	0.48	0.91	—	
AA946040	rc_AA946040_at	Similar to Uroplakin Ia (UPIa) (UPKa) (LOC365227)	0.49	0.79	—	
A1177256	rc_A1177256_at	EST220870 (ovary derived cDNA)	0.49	0.95	—	
A1011556	rc_A1011556_s_at	EST206007 (ovary derived cDNA)	0.48	1.05	—	
A1010292	rc_A1010292_s_at	EST204743 (Similar to mitochondrial cytochrome oxidase subunit II)	0.44	0.89	—	

Table 1. (Continued)

Accession	Probe set ID	Gene name	VO	PO	Molecular function	Reference
VO↓ and PO↓						
J03481	J03481mRNA_g_at	Dihydropteridine reductase	0.49	0.49	6,7-dihydropteridine reductase activity, oxidoreductase activity	
J02791	J02791_at	Acetyl coenzyme A dehydrogenase medium chain	0.47	0.42	acyl-CoA dehydrogenase activity, oxidoreductase activity	
D00688	D00688_s_at	Monoamine oxidase	0.44	0.43	amine oxidase activity, oxidoreductase activity	
L19998	L19998_at	Minoxidil sulfotransferase	0.49	0.47	aryl sulfotransferase activity, sulfotransferase activity	
AA946532	rc_AA946532_at	Similar to ATP-binding cassette, sub-family D (ALD)	0.27	0.38	ATP binding, ATPase activity	
D49785	D49785_at	Protein kinase (MUK) norvegicus mitogen activated protein kinase	0.33	0.21	ATP binding, cAMP-dependent protein kinase activity	
A1007820	rc_A1007820_s_at	Heat shock 90 kDa protein 1, β	0.48	0.49	ATP binding, nitric-oxide synthase regulator activity	
A1176546	rc_A1176546_at	Heat shock protein 86	0.44	0.49	ATP binding, unfolded protein binding	
AA858640	rc_AA858640_s_at	Heat shock protein 1	0.46	0.46	ATP binding, unfolded protein binding	
A1235707	rc_A1235707_g_at	Calnexin	0.48	0.48	calcium ion binding, sugar binding, unfolded protein binding	
M26125	M26125_at	Epoxide hydrolase 1	0.48	0.47	catalytic activity, aminopeptidase activity	19
X60328	X60328_at	Cytosolic epoxide hydrolase	0.36	0.38	catalytic activity, epoxide hydrolase activity, hydrolase activity	
AA818226	rc_AA818226_s_at	Mitochondrial cytochrome c oxidase subunit	0.45	0.49	cytochrome-c oxidase activity, oxidoreductase activity	
A1136891	rc_A1136891_at	Zinc finger protein 36	0.45	0.45	DNA binding, nucleic acid binding	19
A1171355	rc_A1171355_s_at	cytochrome b, mitochondrial	0.38	0.46	electron transporter activity	19
AA945054	rc_AA945054_s_at	Cytochrome b5	0.39	0.41	electron transporter activity	19
A1103396	rc_A1103396_g_at	Mitochondrial gene for cytochrome b	0.49	0.49	electron transporter activity	19
A1234604	rc_A1234604_s_at	Heat shock cognate 71 kDa protein	0.46	0.49	heat shock protein activity	
AA818858	rc_AA818858_s_at	Peptidylprolyl isomerase A (Ppia)	0.47	0.47	isomerase activity, peptidyl-prolyl <i>cis-trans</i> isomerase activity	
A1228674	rc_A1228674_s_at	Peptidyl-prolyl <i>cis-trans</i> isomerase A (PP1ase)	0.49	0.49	isomerase activity, peptidyl-prolyl <i>cis-trans</i> isomerase activity	
A1103874	rc_A1103874_at	FK506 binding protein 3	0.45	0.49	peptidyl-prolyl <i>cis-trans</i> isomerase activity, receptor activity	
A1230406	rc_A1230406_at	RAB10, member RAS oncogene family	0.42	0.45	protein binding, GTP binding, protein transporter activity	
A1007614	rc_A1007614_at	Mitogen-activated protein kinase kinase kinase 4	0.43	0.36	protein kinase activity, small GTPase regulator activity	19
AB016532	AB016532_at	rPER2, period homolog 2 (Per2)	0.41	0.44	signal transducer activity	
X62951	X62951mRNA_s_at	Endogenous retrovirus	0.41	0.37	—	
AB013454	AB013454_at	Similar to Ac2-210	0.34	0.32	—	
A1009132	rc_A1009132_at	Similar to chromosome 13 open reading frame 12	0.47	0.47	—	
AA892056	rc_AA892056_at	Similar to deoxycytidyl transferase	0.43	0.46	—	
A1104035	rc_A1104035_s_at	EST213324 (heart derived cDNA)	0.43	0.47	—	
AA945152	rc_AA945152_s_at	EST20065 (liver derived cDNA)	0.32	0.49	—	
A1009141	rc_A1009141_at	EST203592 (embryo derived cDNA)	0.37	0.42	—	

GeneBank accession numbers, probe set ID, gene name, and their functions are listed for each gene. The fold change in gene expression in PO and VO vs. sham samples is given. "Reference" indicates that the results were similar to the indicated references.

(LOXL) TaqMan Probe, AGGCAGCCTCCCCCAAGA AGCA; LOXL sense-primer, TTGAAAAGCAGGACCTGC TTC; LOXL antisense-primer, CTCCGGCTAGGCGGCT; pyruvate dehydrogenase kinases-1 (PDK-1) TaqMan Probe, CCGTCGCCACTCTCCATGAAGCA; PDK-1 sense-primer, GGAATTCTATGCGCGCTTCT; PDK-1 antisense-primer, ACTGACCCGAAGTCCAGGAA; transgelin TaqMan Probe, CCGCCCTCCATGGTCTTCAAGCA; transgelin sense-primer, GCCAGTGAAGGTGCCTGAGA; transgelin antisense-primer, AGAATTGAGCCACCTGTTCCA.

Subcloning of Rat MT

Rat MT cDNA was cloned from rat heart by RT-PCR. The following primers were used for this experiment: sense primer, 5'-ACTGCCTTCTTGTCGTTAC-3'; antisense primer, 5'-AGGGCAGCAGCACTGTTCGT-3'. This PCR product was subcloned into pCR2.1 and then transferred to pcDNA 3.1 (Invitrogen).

Cell Culture and Transfection

H9c2 cells (a cardiac myoblast cell line) were obtained from the American Type Culture Collection (Rockville, USA). Cells were transfected with rat MT-pcDNA3.1 or pcDNA 3.1 (empty vector) by Lipofectamine 2000 (Amersham Biosciences, Piscataway, USA). At 24 h after the transfection, cells were exposed to norepinephrine (NE; 10^{-4} mol/l) and cultured for an additional 24 h. The efficiency of the transfection was assessed by the co-transfection of pQBI50, which encodes blue fluorescent protein (Takara, Tokyo, Japan).

Assessment of Apoptosis and Caspase 3 Assay

Apoptosis was assessed by TUNEL staining (*In Situ* Cell Death Detection kit, Roche Diagnostics, Mannheim, Germany). Caspase-3 activity was measured by a CaspACE assay system (Promega, Madison, USA).

Statistical Analysis

Data are presented as the mean $\pm$ SEM. Comparisons of mean values were performed by one-way ANOVA followed by Scheffe's test. Values of $p < 0.05$ were considered to indicate statistically significant differences.

Results

Figure 1 shows the echocardiographic findings 4 weeks after the surgery. Left ventricular diastolic diameter (LVDd) was significantly higher in the rats subjected to VO compared with those subjected to PO and those undergoing sham operation (hereafter the VO, PO, and sham groups, respectively) (PO: $96 \pm 8\%$ of the sham-group value; VO: $119 \pm 9\%$ of the sham-group value). While interventricular septal wall thick-

ness (IVST) was increased in both types of hypertrophy (PO, $126 \pm 12\%$ of the sham-group value; VO, $120 \pm 11\%$ of the sham-group value), the IVST of the PO group was greater than that of the VO group. Thus, PO produced concentric hypertrophy and VO produced eccentric hypertrophy. There was no difference among the PO, VO and sham groups in percent fractional shortening (%FS). The left atrial diameter (LAD) of both hypertrophy groups was larger than that of the sham group. As shown in Fig. 2, the left ventricular weight/body weight ratio and heart weight/body weight ratio were significantly increased in both hypertrophy groups, compared with the sham group. These ratios were slightly larger in the VO than the PO group (PO: $139 \pm 11\%$ of the sham-group value; VO: $157 \pm 16\%$ of the sham-group value).

Figure 3 and Table 1 show results of DNA chip analysis. A total of 33 genes were upregulated in both forms of hypertrophy, whereas 31 were downregulated. The genes that were upregulated in both forms included BNP, atrial natriuretic peptide (ANP), and skeletal α -actin, which are known to be upregulated in the hypertrophied myocardium. In the PO group, 52 genes were selectively upregulated and 41 genes were downregulated. In the VO group, 88 genes were selectively upregulated and 46 genes were downregulated.

Subsequently, we performed hierarchical clustering with the average linkage method. Figure 4 shows the cluster of upregulated genes (A) and downregulated genes (B) in both hypertrophy groups. The differentially expressed genes were grouped by functional category classification according to the GeneOntology (<http://www.geneontology.org/>) terms based on the NCBI LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink/>) database. The gene cluster that showed the most marked increase was the natriuretic peptide family. The hydrogen-transporting two-sector ATPase group, the cytoskeletal organization and biogenesis group, and the mitochondrial substrate carrier group were upregulated in both hypertrophied hearts. On the other hand, the calcium ion-binding group and the basic-leucine zipper transcription factor group were downregulated in both hypertrophy groups. We observed selective upregulation of the genes that bind to actin in the VO group (*e.g.*, tropomyosin 4, thymosin β -4, and transgelin).

Next, we performed real-time RT-PCR analysis to confirm the results of the DNA chip analysis (Fig. 5). Real-time RT-PCR revealed that BNP, MT and LOXL mRNAs were all upregulated in both the VO and PO groups, compared with the sham group. MT mRNA expression in the VO group was significantly higher than that in the PO group. The levels of BNP and LOXL mRNAs in the VO group were also higher than those in the PO group, but these differences did not reach the level of statistical significance. DNA chip analysis showed that GMP reductase mRNA was selectively downregulated in the VO group. This was confirmed by real-time RT-PCR.

We then examined the expressions of some of the genes that were differentially altered in VO and PO hearts. DNA

Fig. 4. Hierarchical cluster analysis of expression profiles. Regulated genes are depicted using a color scale (red, upregulated genes; blue, downregulated genes). **A:** The one of the cluster consisting of genes that were increased in both forms of hypertrophy. **B:** The one of the cluster consisting of genes that were decreased in both forms of hypertrophy.

chip analysis revealed that transgelin was increased in the VO group but downregulated in the PO group, and PDK1 was decreased in VO but upregulated in PO. Real-time RT-PCR confirmed these changes (Fig. 5E, F).

In our DNA chip analysis, BNP and ANP showed the most remarkable upregulations in hypertrophied LVs. MT showed the next highest level of upregulation. Accordingly, we examined the role of MT in the development of cardiac hypertrophy (Fig. 6). MT is a free radical scavenger and is suggested to play a protective role in many cell types. Therefore, we hypothesized that MT plays an anti-apoptotic role in cardiac hypertrophy and performed the following experiments. MT cDNA or control vector (pcDNA3.1) was overexpressed in H9c2 cells. The cells were then exposed to a high concentration of NE for 24 h, and TUNEL staining was performed. In cells overexpressing the control vector, NE increased TUNEL-positive cells. Overexpression of MT inhibited the increase of TUNEL-positive cells (Fig. 6). To confirm the

anti-apoptotic effects of MT, we next examined the level of caspase-3 activity, which is critically involved in apoptosis, in these cells (Fig. 7). Caspase-3 activity was increased in cells treated with NE. The overexpression of MT reduced the augmentation of caspase-3 activity by $42 \pm 9.9\%$ compared with that in the cells transfected with the control vector (pcDNA3.1; Fig. 7).

Discussion

We examined the similarity and difference of the gene expression profiling between the PO and VO hearts. We found many genes that are regulated similarly or differentially between the two groups. The alterations of some specific genes were confirmed by real-time RT-PCR. Among the genes that were upregulated in both forms of hypertrophy, we examined the role of MT in apoptosis, and found that this gene played a novel role in anti-apoptosis.

Fig. 5. Real-time RT-PCR. A: B-type natriuretic peptide (BNP). B: Metallothionein-1 (MT). C: Lysyl oxidase-like protein 1 (LOXL). D: GMP reductase. E: Transgelin. F: Pyruvate dehydrogenase kinases-1 (PDK-1). Values are the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

Fig. 6. Effects of overexpression of MT on the percentage of TUNEL-positive cells. A: TUNEL staining. B: Percentage of TUNEL-positive cells. H9c2 cells were transfected with MT-pcDNA3 or pcDNA3 alone. At 24 h after the transfection, cells were exposed to norepinephrine (10^{-4} mol/l) and cultured for an additional 24 h. TUNEL staining was performed as described in Methods. Values are the mean $\pm$ SD. ** $p < 0.01$.

Fig. 7. Effects of overexpression of MT on caspase-3 activity. H9c2 cells were transfected with MT-pcDNA3 or pcDNA3 alone. At 24 h after the transfection, cells were exposed to norepinephrine (10^{-4} mol/l) and cultured for an additional 24 h. The caspase-3 assay was performed as described in Methods. Data are from four independent experiments. Values are the mean $\pm$ SD. ** $p < 0.01$.

Methodological Considerations

In this study, we used two different procedures to induce cardiac hypertrophy: abdominal constriction for pressure overload and aortocaval shunt for volume overload. These two procedures produced the same magnitude of hypertrophy with distinct morphological differences as shown by echocardiography. Thus the similarities in gene profiling may be attributable to hypertrophy *per se* and the dissimilarity to the different morphologies of the heart, *i.e.*, concentric hypertrophy or eccentric hypertrophy. In both forms of hypertrophy, cardiac function assessed by fractional shortening was normal, indicating that changes in gene profiling were not attributable to those of the myocardium at the failing stage.

These mechanical stresses activate many molecules in the heart. There are two peaks in the activations of biochemical signals after an exposure to hemodynamic load (18). Acute responses occur within a few minutes, and hypertrophic responses follow over the next several days or weeks. As a pilot study, we measured the time-dependent change of the expression of BNP mRNA in rat PO hearts. The left ventricular weight/body weight ratio increased gradually until day 28 after the initiation of PO and continued at a plateau level thereafter. BNP mRNA levels showed a biphasic response, *i.e.*, an initial increase and a later gradual increase. The first peak of the expression was at 24 h and the subsequent peak was at day 28 (data not shown). Thus, at day 28 acute gene responses were no longer observed and hypertrophic gene responses were maximal and stable. This is the time when hypertrophy was morphologically established. In order to investigate gene responses with hypertrophy but without

heart failure, we chose day 28.

We examined gene profiling at the stage of established cardiac hypertrophy but did not examine it chronologically. Thus the gene profiling in our study may not represent that of the developmental stage of hypertrophy, but merely that of the compensation of hypertrophy. Moreover, we examined gene profiling of the whole heart. Thus our results do not necessarily indicate gene profiling of cardiomyocytes, but may also include profiling for the interstitial tissues.

Gene Profiling in PO and VO

The present study revealed gene expression profiles that may be associated with hypertrophy *per se* or different types of remodeling. In agreement with previous reports (13, 14, 19, 20), we found changes in gene profiling in PO. As shown in Fig. 3, most genes were unaltered in the hypertrophic myocardium induced by PO. Only 85 genes were upregulated. Thus, the number of genes upregulated in the hypertrophied myocardium induced by PO was less than that of several previous reports. This discrepancy may be ascribable to the criteria used for the gene selection and the use of different kinds of chips. The present study is the first to describe gene expressions in VO. As shown in Fig. 3 and Table 1, the levels of most of the genes were unaltered, just as in PO. Only 121 genes were upregulated. It may be interesting to note that some genes were regulated differentially between VO and PO. Among those genes, extracellular matrix-related proteins, *e.g.*, osteopontin, secreted acidic cysteine rich glycoprotein, and tropoelastin, were predominantly upregulated in VO. The upregulation of these genes may be related to the specific remodeling in VO, but not to hypertrophy *per se*. In addition, the selective upregulation of genes in VO that bind to actin (*e.g.*, tropomyosin 4, thymosin β -4, and transgelin) may explain the difference in structural ventricular remodeling between VO and PO. Several gene profiles have been reported in other models of hypertrophy (21, 22). These reports have shown that the common genetic events in hypertrophy include upregulations of ANP, BNP, and skeletal α -actin. These genes were increased in both forms of hypertrophy in the present study as well.

GMP reductase was predominantly downregulated in VO. Because GMP reductase increases the adenine nucleotide pool through synthesis of inosine monophosphate (23), the decrease of GMP reductase may have some deteriorative effects on myocardial metabolism. We also found that the expressions of PDK-1 and transgelin were differentially regulated in VO and PO hearts (Fig. 5E, F). Transgelin (also known as SM22 α) is a 22-kDa protein that is associated with cytoskeletal actin filament bundles in contractile smooth muscle cell (SMC) (24, 25). It is expressed in cardiac, smooth, and skeletal muscle cells during early embryogenesis but becomes restricted to visceral and vascular SMC at late embryonic stages and throughout adulthood. Transgelin was upregulated in VO and downregulated in PO hearts in the

present study (Fig. 5E), but the significance of these changes is unknown. Transgelin may regulate several genes that are associated with vascular development, remodeling, and alterations. The differential expression of this gene may reflect the difference of vascular remodeling between concentric and eccentric cardiac hypertrophy. PDK-1 was downregulated in VO but upregulated in PO (Fig. 5F). PDKs phosphorylate the mitochondrial pyruvate dehydrogenase complex, which catalyzes the oxidative decarboxylation of pyruvate, and thereby inactivate it (26). Although the significance of alterations of this enzyme in the hypertrophied hearts is unclear, these changes may be associated with the difference of the myocardial energy production in both forms of hypertrophy.

Upregulated Genes in Both Forms of Hypertrophy

Our DNA chip analysis showed upregulation of 33 genes in both forms hypertrophy (Table 1). These genes may be associated with hypertrophy *per se*. Among these genes, we confirmed the upregulations of BNP, MT and LOXL mRNA. Wang *et al.* (13) and Weinberg *et al.* (14) have reported that MT is upregulated significantly in aortic banding mice. In the present study, MT was one of genes showing the highest level of upregulation in both forms of hypertrophied heart. Accordingly, we examined the role of MT in cardiac hypertrophy. MT is a cysteine-rich protein with a strong affinity for Zn²⁺ (reviewed in Palmiter (27), and Kang (28)). MT is known to detoxify the heavy metals. Recent studies revealed that MT binds to rare metals *in vivo* and regulates metalprotein and metal-dependent transcriptional factors. Moreover, MT is a potent free radical scavenger. And in the hearts of mice with adriamycin-induced cardiomyopathy, MT expression has been shown to be increased (29). Moreover, it has been reported that overexpression of MT in the hearts of transgenic mice suppresses doxorubicin cardiotoxicity and ischemia-reperfusion injury (30, 31). Thus MT plays cytoprotective roles in many cell types. In this study, MT suppressed the NE-induced increase of TUNEL-positive cells. This inhibition was associated with the suppression of caspase-3 activity, suggesting that MT inhibits the apoptosis of cardiomyocytes. It has been reported that the myocardium of both rat models of hypertrophy shows apoptotic cardiomyocyte death (32, 33). Therefore, we consider that MT may exert anti-apoptotic effects under physiological conditions. Aronow and coworkers showed that MT was universally increased in hypertrophied hearts produced by four different mechanisms, suggesting that the upregulation of MT is a highly conserved genetic event in hypertrophy (21). Interestingly, the expression of MT was greater in the hypertrophy model than in the heart failure model in their study (21). This suggests that apoptosis of the hypertrophied myocardium, which leads to heart failure, is prevented by the expression of MT. Taken together, these findings indicate that MT may have a protective effect on the heart through its anti-apoptotic effects.

LOXL was also significantly upregulated in both forms of hypertrophy. LOXL is a member of the lysyl oxidase gene family, which is an extracellular, copper-dependent enzyme family that initiates the cross-linking of collagens and elastin (34, 35). It may be possible that LOXL is associated with the remodeling of the extracellular matrix in the hypertrophied myocardium. Among the upregulated genes, GATA-4 is known to be essential for the induction of several cardiac-specific genes (36). The upregulation of GATA-4 supports the idea that this protein may be the common regulator of hypertrophic responses.

In conclusion, we found many genes that are similarly or dissimilarly regulated in the hearts of VO and PO. The altered expressions of BNP, MT, GMP reductase, LOXL, transgelin, and PDK-1 were confirmed by real-time RT-PCR. Gene profiling by DNA chip technology represents a useful tool for identification of genes that may contribute to morphological and functional differences between PO and VO. Moreover, the overexpression of MT in H9c2 cells attenuated the norepinephrine-induced apoptosis. MT may play a novel protective role in the development of cardiac hypertrophy. Although we found that many genes were altered in the hypertrophied hearts, it remains uncertain whether these changes played a role in or were secondary to the development of hypertrophy. To clarify these questions, functional studies should be performed for each molecule.

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