miR-126a-5p-Dbp and miR-31a-Crot/Mrpl4 interaction pairs crucial for the development of hypertension and stroke

QINI ZHAO[1-3], HUAN SUN[1,2], LIQUAN YIN[4] and LIBO WANG[5]

[1]Department of Cardiology, [2]Jilin Provincial Key Laboratory for Genetic Diagnosis of Cardiovascular Disease, [3]Jilin Provincial Engineering Laboratory for Endothelial Function and Genetic Diagnosis of Cardiovascular Disease, Departments of [4]Rehabilitation Medicine and [5]Neurology, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, P.R. China

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Abstract. The present study aimed to integrate the mRNA and microRNA (miRNA) expression profiles of spontaneously hypertensive rats (SHR rats) and stroke-prone spontaneously hypertensive rats (SHRSP rats) to screen for potential therapeutic targets for hypertension and stroke. The datasets GSE41452, GSE31457, GSE41453 and GSE53363 were collected from the Gene Expression Omnibus (GEO) database to screen differentially expressed genes (DEGs). The GSE53361 dataset was obtained to analyze differentially expressed miRNAs (DEMs). The DEGs and DEMs were identified between SHR (or SHRSP) rats and normotensive Wistar-Kyoto (WKY) rats using the Linear Models for Microarray (limma) data method. Venn diagrams were used to show the SHR-specific, SHRSP-specific and SHR-SHRSP shared DEGs and DEMs, and these were utilized to construct the protein-protein interaction (PPI) and miRNA-mRNA regulatory networks. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to explore the function of the genes. Subsequently, the connectivity Map (CMAP) database was searched to identify small-molecule drugs. Comparisons between the GSE41452-GSE31457-GSE41453 merged and GSE53363 datasets identified 2 SHR-specific, 8 SHRSP-specific and 15 SHR-SHRSP shared DEGs. Function enrichment analysis showed that SHRSP-specific D-box binding PAR bZIP transcription factor (Dbp) was associated with circadian rhythm, and SHR-SHRSP shared carnitine O-octanoyltransferase (Crot) was involved in fatty acid metabolic processes or the inflammatory response via interacting with epoxide hydrolase 2 (EPHX2). SHR-SHRSP shared mitochondrial ribosomal protein L4 (Mrpl4) may exert roles by interacting with the threonine-tRNA ligase, TARS2. The miRNA regulatory network predicted that upregulated Dbp could be regulated by rno-miR-126a-5p, whereas downregulated Crot and Mrpl4 could be modulated by rno-miR-31a. The CMAP database predicted that small-molecule drugs, including botulin, Gly-His-Lys, and podophyllotoxin, may possess therapeutic potential. In conclusion, the present study has identified Dbp, Crot and Mrpl4 as potential targets for the treatment of hypertension and stroke. Furthermore, the expression of these genes may be reversed by the above miRNAs or drugs.

Correspondence to: Dr Libo Wang, Department of Neurology, China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Erdao, Changchun, Jilin 130033, P.R. China
E-mail: wang_libo@jlu.edu.cn

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Introduction

Hypertension, defined as systolic blood pressure (BP) ≥140 mmHg or diastolic BP ≥90 mmHg, has been a long-standing and common chronic disease in modern society due to the improvements in people's living standards and changes in their lifestyles (1). Sustained hypertension is an important risk factor for the development of cardiovascular disorders, such as transient ischemic attacks or stroke (2-4), which is the leading cause of death worldwide (5,6), imposing a heavy economic burden on family and society (7). Therefore, how to prevent hypertension-associated stroke has become a major public health problem.

Spontaneously hypertensive rats (SHR) and stroke-prone spontaneously hypertensive rats (SHRSP) are widely used animal models for studying the molecular mechanisms of severe hypertension and associated stroke (8) to provide potential therapeutic strategies (9). Previous studies have utilized high-throughput microarray technology to investigate gene expression in the brain (10), adrenal gland (11), kidneys (12), mesenteric artery (13) and liver (14) of SHR and SHRSP rats compared with the normotensive control strain, Wistar-Kyoto (WKY) rats. In these studies, obvious overlaps were identified in different tissue samples as SHRSP-specific genes, including angiotensinogen (Agt), which was found to be crucial for SHRSP in the adrenal gland (11) and kidneys (12), and angiotensin II receptor-associated protein (Agtrap), which was found to be crucial for SHRSP in the brain (10) and kidneys (12). These analyses indicated the common genes that may be

underlying targets for treatment of hypertension-associated stroke. However, to the best of the authors' knowledge, no studies have been performed to date to investigate the shared genes in all studied samples previously.

Furthermore, increasing evidence has reported that microRNAs (miRNAs), small non-coding RNA molecules of 18-25 nucleotides in length, are also essential in the development of hypertension and associated stroke. They function by negatively regulating the expression of their target genes at the post-transcriptional level. For example, Rubattu *et al* (15) demonstrated that uncoupling protein 2 (UCP2)-targeted rno-miR-503 was significantly upregulated in the brain of SHRSP rats. Downregulation of miR-503 level protected SHRSP from stroke occurrence. *In vitro* overexpression of miRNA-503 in endothelial cells suppressed UCP2 expression and led to a significant increase in cell mortality, with decreased cell viability (15). Matsuoka *et al* (16) observed that the miR-124 level was markedly lower in brains of SHRSP compared with WKY rats, whereas claudin domain-containing 1 (Cldnd1) mRNA and protein levels were significantly higher. Human brain endothelial cells transfected with a miR-124 mimic exhibited a significantly decreased mRNA expression level of Cldnd1 (16). However, the mechanism underpinning miRNA-mRNA interaction for SHRSP rats has yet to be fully elucidated (13).

The present study aimed to further screen for crucial miRNA-mRNA interactions in order to explain the etiology of SHRSP, and to explore novel treatment modalities by integrating all the mRNA and miRNA expression profiles associated with SHRSP that were obtained from the public databases using a serial of bioinformatic methods.

Materials and methods

Collection of microarray data. The microarray datasets of SHRSP were available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The GSE41452 (10), GSE31457 (11) and GSE41453 datasets (12) were analyzed by the research team of Watanabe *et al* (12) to respectively investigate the mRNA expression profiles in whole brains, adrenal glands and kidneys of 3 rat strains: Normotensive WKY, SHR, and SHRSP, at 3 and 6 weeks of age. GSE53363 and GSE53361 datasets were provided by Palao *et al* (13) to respectively analyze the mRNA and miRNA expression profiles in mesenteric arteries of normotensive WKY (including 3 sublines, WKY/NCrl, WKY/NHsd, WKY/NTac), SHR (including 2 sublines, SHR/NCrL and SHR/NHsd) and SHRSP rats at 6 weeks and 5 months of age. The characteristics of the microarray datasets are shown in Table I.

Differential expression analysis. The normalized series matrix files of each dataset were downloaded from GEO. Data from the GSE41452, GSE31457 and GSE41453 datasets were merged into one in order to use the same model samples. The series matrix data were extracted and quantile-normalized using the Linear Models for Microarray Data (Limma) package (version 3.38.3; https://bioconductor.org/packages/release/bioc/html/limma.html) in R (version 3.5.2; http://www.R-project.org/) (17). The differentially expressed genes (DEGs) and miRNAs (DEMs) between SHR (or SHRSP) and WKY were identified using the Limma empirical Bayes analysis pipeline. P-values were adjusted to the false discovery rate (FDR) using Benjamini and Hochberg multiple testing (18). DEGs and DEMs were defined as |log2 fold change (FC)|>0.5 and P<0.05, since the number of DEGs and DEMs would be fewer if FDR were to be considered, which may have been disadvantageous in terms of performing the following analyses. Hierarchical clustering of DEGs and DEMs, with results visualized as heat-maps, was performed using the pheatmap package (version 1.0.8; https://cran.r-project.org/web/packages/pheatmap) based on Euclidean distances. Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) were drawn to obtain the DEGs that were SHR-specific, SHRSP-specific and SHR-SHRSP shared, respectively, in two datasets (GSE41452-GSE31457-GSE41453 and GSE53363), as well as genes common to both datasets.

Protein-protein interaction (PPI) network. All genes for SHR-specific, SHRSP-specific and SHR-SHRSP shared in the two datasets were used for constructing the PPI network to reveal the possible interaction mechanisms of common DEGs. Underlying PPI associations were collected from the Search Tool for the Retrieval of Interacting Genes database, version 10.0 (STRING; http://string db.org/) (19), which is a comprehensive database that provides >200 million interactions among 5 million proteins, including experimental, predicted, transferred and text-mining interactions. Only interaction pairs with a combined score >0.4 (medium confidence) were used to establish the PPI network, and this was accomplished using Cytoscape software (version 3.4.0; www.cytoscape.org/) (20).

miRNA-target gene regulatory network. The target genes of DEMs were predicted using the miRwalk database (version 2.0; http: //www.zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2) (21), which is a comprehensive database for integrating the predicted results of 12 algorithms (miRWalk, Microt4, miRanda, mirbridge, miRDB, miRMap, miRNAMap, Pictar2, PITA, RNA22, RNAhybrid and Targetscan). Subsequently, the target genes of DEMs that were predicted by at least 5 of the databases were overlapped with the common DEGs in two datasets to obtain potentially new negative expression associations between DEMs and DEGs, and this procedure was followed to construct the miRNA-target gene regulatory network using Cytoscape software (20).

Function enrichment analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) [including the categories of 'biological process' (BP), 'molecular function' (MF) and 'cellular component' (CC)] enrichment analyses were performed to explore the underlying functions of the DEGs in the SHR-specific, SHRSP-specific and SHR-SHRSP shared PPI networks, as well as the miRNA-mRNA network, The Database for Annotation, Visualization and Integrated Discovery

Table I. Microarray datasets collected from the GEO database.

Accession number	Type	Platform		Sample size			Tissue source
				WKY	SHR	SHRSP	
GSE41452	mRNA	GPL14745	Agilent-028282 Whole Rat Genome microarray 4x44K v3	6	6	6	Whole brains
GSE31457	mRNA	GPL14745	Agilent-028282 Whole Rat Genome microarray 4x44K v3	6	6	6	Adrenal glands
GSE41453	mRNA	GPL14745	Agilent-028282 Whole Rat Genome microarray 4x44K v3	6	6	6	Kidneys
GSE53363	mRNA	GPL15084	Agilent-028279 SurePrint G3 Rat GE 8x60K microarray	6	4	2	Mesenteric artery
GSE53361	miRNA	GPL18115	Agilent-046066 Unrestricted Rat miRNA V19.0	6	6	4	Mesenteric artery

WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; GEO, Gene Expression Omnibus.

(DAVID) (version 6.8; http://david.abcc.ncifcrf.gov) (22). P<0.05 was considered to indicate a statistically significant value.

Screening of small-molecule drugs. To better identify potential therapeutic drugs for treatment of SHRSP, the common DEGs (upregulated or downregulated) in two datasets were queried using the Connectivity Map online tool (version 2.0; https://portals.broadinstitute.org/cmap/) (23). The query small molecules were output with a connectivity score from +1 to -1. Small-molecule drugs may be therapeutic when their connectivity scores are near to -1; by contrast, small-molecule drugs may actually induce the disease if their connectivity scores are near to +1. Candidate small-molecule drugs were identified with a P-value <0.05 and a |connectivity score|≥0.8.

Results

Differential expression analysis. In the merged dataset (GSE31457-GSE41452-GSE41453), a total of 366 DEGs were identified between SHR and WHY rats, including 177 upregulated and 189 downregulated DEGs. Three hundred and ninety-three DEGs were screened between SHRSP and WHY, including 151 upregulated and 242 downregulated DEGs. Venn diagrams revealed that 75 upregulated DEGs were SHR-specific, 49 were SHRSP-specific, and 102 were SHR-SHRSP shared; for the downregulated DEGs, 44 were SHR-specific, 97 were SHRSP-specific, and 145 were SHR-SHRSP shared (Fig. 1A).

In the GSE53363 dataset, a total of 246 DEGs were identified between SHR and WHY, including 145 upregulated and 101 downregulated DEGs, whereas 480 DEGs were screened between SHRSP and WHY, including 300 upregulated and 180 downregulated DEGs. Venn diagrams revealed that 55 upregulated DEGs were SHR-specific, 210 were SHRSP-specific, and 90 were SHR-SHRSP shared; for the downregulated DEGs, 44 were SHR-specific, 123 were SHRSP-specific and 57 were SHR-SHRSP shared (Fig. 1A).

In the GSE53361 dataset, a total of 11 DEMs were identified between SHR and WHY, including 5 upregulated and 6 downregulated DEMs; 13 DEMs were screened between SHRSP and WHY, including 6 upregulated and 7 downregulated DEMS (Table II). Venn diagrams revealed that 2 upregulated DEMs were SHR-specific (rno-miR-146b-5p and rno-miR-132-3p), whereas 3 were SHRSP-specific (rno-miR-196b-5p, rno-miR-21-5p and rno-miR-196a-5p) and 3 were SHR-SHRSP shared (rno-miR-31a-5p, rno-miR-31a-3p and rno-miR-511-3p). For the downregulated DEMs, 2 were SHR-specific (rno-miR-3593-3p and rno-miR-150-3p), 3 were SHRSP-specific (rno-miR-126a-5p, rno-miR-126a-3p and rno-miR-483-3p), and 4 were SHR-SHRSP shared (rno-miR-1224, rno-miR-146a-5p, rno-miR-672-5p and rno-miR-150-5p) (Fig. 1A). Among these DEMs, rno-miR-31a-3p and rno-miR-31a-5p may be especially important, since their FDR was <0.05 (Table II).

Subsequently, the merged and GSE53363 datasets were compared. This analysis identified 2 SHR-specific DEGs [cholesterol 25-hydroxylase (Ch25h) and SEC16 homolog B, endoplasmic reticulum export factor (Sec16b)], 8 SHRSP-specific DEGs (7 with consistent expression in the two datasets {RGD1310414, phosphatidylinositol glycan anchor biosynthesis, class Z (Pigzl1), nuclear receptor subfamily 1, group D, member 2 (Nr1d2), Tef, LOC686388, D-box binding PAR bZIP transcription factor (Dbp) and transforming growth factor β induced (Tgfbi); and one gene with differing expression [Myoglobin (Mb)]} and 15 SHR-SHRSP shared DEGs (14 with consistent expression between the two datasets: (aldehyde dehydrogenase 5 family, member A1 (Aldh5a1), transmembrane protein 243 (RGD1562351), zinc finger protein 597 (Zfp597), endonuclease G (Endog), membrane-spanning 4-domains A6A (Ms4a11), torsin family 1 member B (Tor1b), retinol saturase (Retsat), carnitine O-octanoyltransferase (Crot), FKBP prolyl isomerase

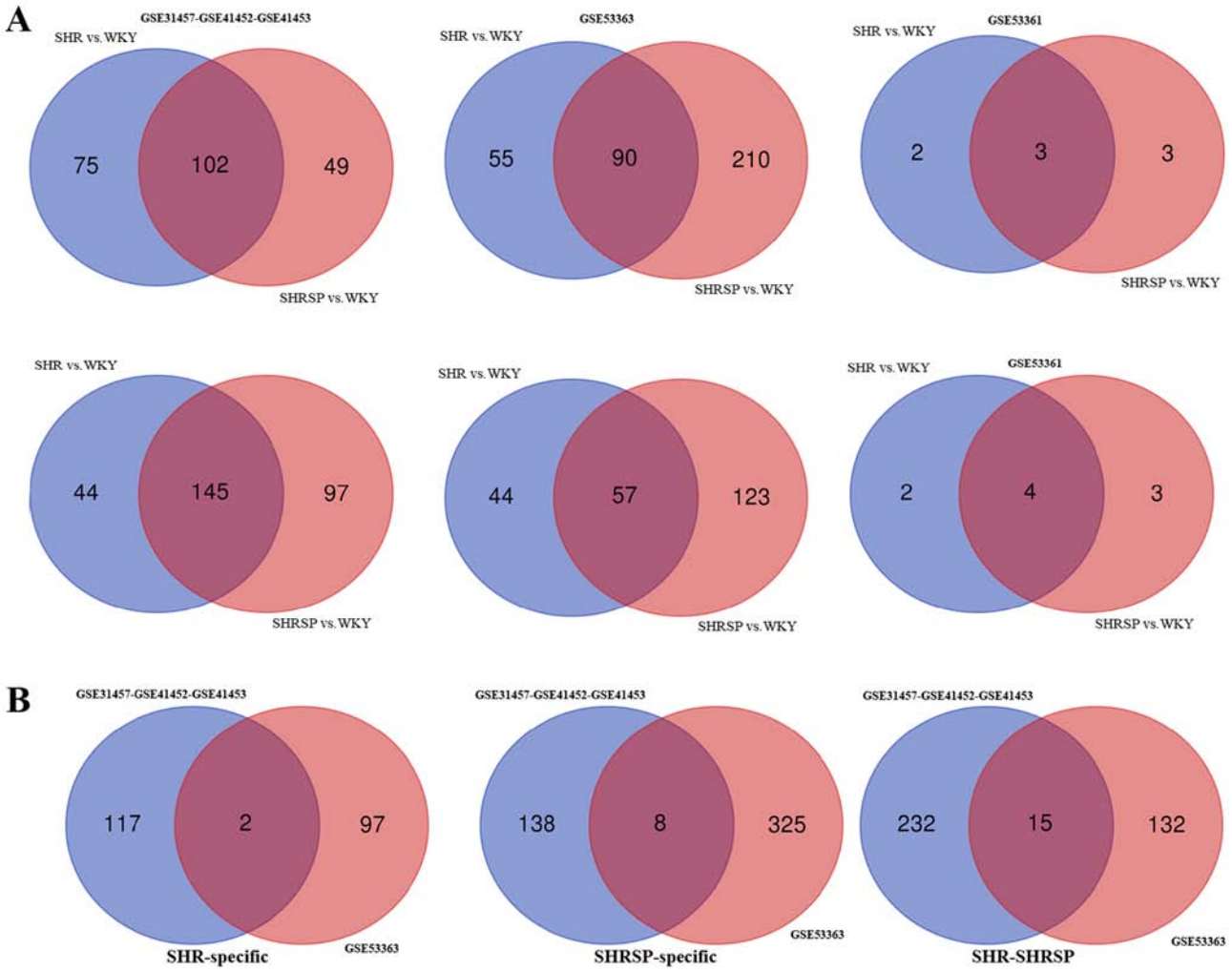

Figure 1. Venn diagrams to display the SHR-specific, SHRSP-specific and SHR-SHRSP shared differentially expressed genes or miRNAs. (A) Comparison of DEGs or miRNAs between SHR and SHRSP in three datasets (i.e., GSE31457-GSE41452-GSE41453, GSE53363 and GSE53361). The upper panel shows the upregulated DEGs, whereas the lower panel shows the DEGs that were downregulated. (B) Comparison of DEGs between two datasets. DEG, differentially expressed gene; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; WKY, Wistar-Kyoto.

(5Fkbp5), charged multivesicular body protein 1B (Chmp1b), mitochondrial ribosomal protein L4 (Mrpl4), threonyl-tRNA synthetase 2, mitochondrial (Tars2), RIO kinase 1 (Riok1) and transmembrane protein 14A (Tmem14a); and one gene with differing expression [Peroxisomal membrane protein 4 (Pxmp4)]} that were common in these two datasets (Fig. 1B; Table III). Among these DEGs, Tor1b and Mrpl4 may be especially important, since their FDR was <0.05 in all datasets as well as SHR and SHR-SP (Table III).

The expression of common DEGs in the GSE31457-GSE41452-GSE41453 (Fig. 2A) and GSE53361 (Fig. 2B) datasets, and all DEMs in the GSE53361 dataset (Fig. 2C) in the three sample groups, are shown in the heat-map.

PPI network and function enrichment analyses. PPI networks were respectively constructed for SHR-specific, SHRSP-specific and SHR-SHRSP shared DEGs by uniting the DEGs in two datasets due to the small number of common DEGs (Fig. 2). As a result, 123 interaction pairs between 115 DEGs (for example, Sec16b-Ccdc92) were obtained for the SHR-specific group (Fig. 3); 828 interaction pairs between 345 DEGs (for example, Mb-Hsph1) were obtained for the SHRSP-specific group (Fig. 4); and 454 interaction pairs between 254 DEGs (for example, Crot-Ephx2 and Mrpl4-Tars2) were obtained for the SHR-SHRSP shared group (Fig. 5).

Function analysis revealed that 30 significant GO terms (comprising 22 BP, 5 MF and 3 CC) were enriched for genes in the SHR-specific group, including GO:0006955~immune response, GO:0030174~regulation of DNA-dependent DNA replication initiation, and GO:0033262~regulation of nuclear cell cycle DNA replication; however, no common genes were involved (Table IV). In addition, 103 significant GO terms (including 64 BP, 11 MF and 28 CC) and 9 KEGG pathways were enriched for genes in the SHRSP-specific group, including GO:0030198~extracellular matrix organization (TGFBI), GO:0002062~chondrocyte differentiation (TGFBI), GO:0007623~circadian rhythm (DBP), GO:0001525~angiogenesis (TGFBI) and rno04141:Protein processing in endoplasmic reticulum [heat shock protein family H (Hsp110) member 1 (HSPH1)] (Table V). Furthermore, 62 significant GO terms (including 32 BP, 6 MF and 11 CC) and 13 KEGG pathways were enriched for genes in the SHR-SHRSP shared group,

Table II. Differentially expressed miRNAs comparing between SHR (or SHRSP) and WKY rats.

A, SHR vs. WKY

	SHR vs. WKY		
miRNA	logFC	P-value	FDR
rno-miR-31a-3p	1.66	**3.99E^{-06}**	**2.87E^{-03}**
rno-miR-31a-5p	2.35	**4.15E^{-05}**	**1.49E^{-02}**
rno-miR-150-5p	-0.51	**2.87E^{-04}**	6.88E^{-02}
rno-miR-511-3p	0.77	**6.86E^{-04}**	9.87E^{-02}
rno-miR-146b-5p	0.84	**5.78E^{-03}**	6.92E^{-01}
rno-miR-146a-5p	-0.56	**8.54E^{-03}**	7.62E^{-01}
rno-miR-672-5p	-0.74	**1.67E^{-02}**	7.62E^{-01}
rno-miR-132-3p	0.76	**1.70E^{-02}**	7.62E^{-01}
rno-miR-150-3p	-0.68	**2.63E^{-02}**	8.42E^{-01}
rno-miR-3593-3p	-0.56	**3.44E^{-02}**	8.42E^{-01}
rno-miR-1224	-0.89	**3.54E^{-02}**	8.42E^{-01}

B, SHRSP vs. WKY

	SHRSP vs. WKY		
miRNA	logFC	P-value	FDR
rno-miR-31a-3p	1.65	**1.27E^{-03}**	4.33 E^{-01}
rno-miR-126a-5p	-0.58	**1.91E^{-03}**	4.33 E^{-01}
rno-miR-196a-5p	2.11	**2.52E^{-03}**	4.33 E^{-01}
rno-miR-146a-5p	-0.85	**2.98E^{-03}**	4.33 E^{-01}
rno-miR-150-5p	-0.51	**3.05E^{-03}**	4.33 E^{-01}
rno-miR-31a-5p	2.12	**3.61E^{-03}**	4.33 E^{-01}
rno-miR-126a-3p	-0.53	**1.57E^{-02}**	1.00E^{-01}
rno-miR-511-3p	0.73	**2.10E^{-02}**	1.00E^{-01}
rno-miR-21-5p	0.70	**2.39E^{-02}**	1.00E^{-01}
rno-miR-1224	-1.43	**2.57E^{-02}**	1.00E^{-01}
rno-miR-672-5p	-1.01	**2.60E^{-02}**	1.00E^{-01}
rno-miR-196b-5p	1.98	**2.76E^{-02}**	1.00E^{-01}
rno-miR-483-3p	-0.66	**4.14E^{-02}**	1.00E^{-01}

Values in bold indicate P<0.05 or FDR<0.05. FDR, false discovery rate; FC, fold change; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats.

including GO:0006954~inflammatory response [epoxide hydrolase 2 (EPHX2)], GO:0055114~oxidation-reduction process (ALDH5A1), GO:0032355~response to estradiol (ENDOG), GO:0006631~fatty acid metabolic process (CROT), GO:0007417~central nervous system development (ALDH5A1), GO:0004829~threonine-tRNA ligase activity (TARS2) and rno00650:Butanoate metabolism (ALDH5A1) (Table VI).

miRNA-mRNA regulatory network. After integrating the target genes of DEMs with the common DEGs (consistent expression trend) in two datasets, 97 negative interaction pairs between 17 miRNAs and 18 DEGs were identified, including SHRSP-specific rno-miR-126a-5p-Dbp/Tor1b, rno-miR-196a-5p/rno-miR-196b-5p/rno-miR-21-5p-Tgfbi, and SHR-SHRSP shared rno-miR-672-5p-Zfp597 and rno-miR-31a-5p-Crot/Mrpl4, which were used for constructing the miRNA-mRNA regulatory network (Fig. 6).

Prediction of potential therapeutic agents. After uploading the upregulated and downregulated DEGs to the CMAP database, a serial of small-molecule drugs that may exert therapeutic potential for SHR were predicted, including botulin, Gly-His-Lys, benzathine benzylpenicillin, Prestwick-1103, PF-00539745-00, quinostatin, 5279552 and podophyllotoxin (Table VII).

Discussion

By integrating the results of the miRNA regulatory network, PPI network and function enrichment, the present study has demonstrated that changes in the expression of Dbp, Crot and Mrpl4 (all P<0.01, especially the latter one with FDR <0.05 in all datasets) may possibly be important genetic changes associated with SHRSP: Upregulated Dbp is associated with circadian rhythm and regulated by rno-miR-126a-5p, whereas downregulated Crot may participate in fatty acid metabolic processes or the inflammatory response by interacting with EPHX2. Downregulated Mrpl4 may exert roles by interacting with threonine-tRNA ligase TARS2. Expression of Crot and Mrpl4 may both be modulated by rno-miR-31a.

Accumulating evidence has demonstrated abnormal BP circadian rhythm is associated with the development of hypertension (24) and stroke (25). Normal circadian BP is in a 'dipper' pattern, with a decline in nocturnal BP of 10-20% compared with the day time (24). Patients with non-dipper (i.e., a lack of nocturnal BP fall) patterns of circadian BP rhythm are found to have a 4.222-fold increased risk for the development of hypertension (P=0.011) (26). Furthermore, non-dipper (risk ratio=1.42) or reverse-dipper (odds ratio=2.492) patterns of BP were also shown to be stronger risk factors for the occurrence of stroke in patients with essential hypertension (i.e., high BP that lacks a known secondary cause) (25,27,28). Therefore, genes that regulate the circadian rhythm may be potentially associated with the pathogenesis of hypertension and stroke. This hypothesis has been validated in a previously published study (29). For example, Leu *et al* (30) identified genetic polymorphisms in five circadian clock genes [neuronal PAS domain protein 2 (NPAS2), rs3888170; period circadian regulator 2 (PER2), rs6431590; retinoic acid receptor-related orphan receptor ββ (RORββ), rs1410225; brain and muscle ARNT-like 1 (BMAL1), rs3816358; and RORα, rs10519096], and these were significantly associated with the non-dipper phenotype in 372 young hypertensive patients. The study of Corella *et al* (31) revealed that CLOCK-rs4580704 single nucleotide polymorphism (SNP) was associated with an increased risk of stroke in type-2 diabetic subjects, with CC-carriers having a higher risk (31). Kurbatova *et al* (32) observed further that the transcript expression levels of

Table III. Differentially expressed genes between SHR (or SHRSP) and WKY.

		GSE31457-GSE41452-GSE41453			GSE53363		
	Gene	logFC	P-value	FDR	logFC	P-value	FDR
SHR-specific	Ch25h	0.51	**4.25E^{-03}**	3.40E^{-01}	0.62	**6.53E^{-03}**	3.82E^{-01}
	Sec16b	0.67	**2.61E^{-02}**	9.16E^{-01}	0.50	**5.58E^{-03}**	3.71E^{-01}
SHRSP-specific	RGD1310414	0.57	**1.44E^{-06}**	**8.96E^{-04}**	0.53	**2.70E^{-03}**	1.64E^{-01}
	Nr1d2	0.58	**6.35E^{-04}**	1.10E^{-01}	0.54	**2.69E^{-03}**	1.64E^{-01}
	Dbp	0.97	**1.15E^{-03}**	1.63E^{-01}	0.87	**4.76E^{-03}**	2.05E^{-01}
	LOC686388	-0.53	**1.41E^{-03}**	1.81E^{-01}	-0.60	**6.61E^{-04}**	8.71E^{-02}
	Mb	-0.53	**3.18E^{-03}**	3.05E^{-01}	0.54	**4.04E^{-04}**	7.10E^{-02}
	Pigzl1	1.23	**4.50E^{-02}**	1.00E^{-01}	0.63	**3.13E^{-02}**	4.07E^{-01}
	Tgfbi	-0.57	**4.66E^{-02}**	1.00E^{-01}	-0.62	**2.09E^{-02}**	3.59E^{-01}
	Tef	0.822	**4.84E^{-02}**	1.00E^{-01}	0.74	**1.53E^{-02}**	3.20E^{-01}
SHR-SHRSP (SHRSP)	Endog	-1.52	**5.13E^{-09}**	**7.06E^{-06}**	-0.94	**9.03E^{-06}**	**6.20E^{-03}**
	Tor1b	1.29	**7.91E^{-09}**	**1.02E^{-05}**	1.37	**1.69E^{-08}**	**7.75E^{-05}**
	Chmp1b	-1.08	**1.16E^{-08}**	**1.40E^{-05}**	-0.70	**8.21E^{-04}**	9.27E^{-02}
	Riok1	0.96	**2.01E^{-06}**	**1.21E^{-03}**	0.66	**4.15E^{-04}**	7.14E^{-02}
	Fkbp5	1.25	**1.15E^{-05}**	**5.05E^{-03}**	1.76	**7.83E^{-06}**	**5.70E^{-03}**
	Mrpl4	-0.74	**4.99E^{-05}**	**1.58E^{-02}**	-0.87	**5.28E^{-06}**	**5.00E^{-03}**
	RGD1562351	-0.89	**3.21E^{-04}**	6.80E^{-02}	-0.50	**7.14E^{-03}**	2.42E^{-01}
	Tars2	-0.66	**5.01E^{-04}**	9.04E^{-02}	-0.93	**4.27E^{-07}**	**7.32E^{-04}**
	Crot	-0.77	**1.27E^{-03}**	1.74E^{-01}	-0.52	**9.78E^{-03}**	2.83E^{-01}
	Zfp597	1.37	**2.35E^{-03}**	2.52E^{-01}	1.00	**2.64E^{-05}**	**1.32E^{-02}**
	Ms4a11	0.76	**4.37E^{-03}**	3.72E^{-01}	1.09	**7.76E^{-03}**	2.52E^{-01}
	Aldh5a1	-0.59	**5.85E^{-03}**	4.45E^{-01}	-0.51	**2.75E^{-03}**	1.66 E^{-01}
	Retsat	-1.07	**1.10E^{-02}**	6.08E^{-01}	-0.66	**4.09E^{-02}**	4.45E^{-01}
	Pxmp4	-0.68	**1.42E^{-02}**	7.07 E^{-01}	0.93	**1.02E^{-05}**	**6.67E^{-03}**
	Tmem14a	0.67	**1.75E^{-02}**	7.93E^{-01}	0.80	**6.04E^{-06}**	**5.00E^{-03}**
SHR-SHRSP (SHR)	Endog	-1.54	**1.30E^{-09}**	**1.94E^{-06}**	-0.71	**4.88E^{-04}**	1.01E^{-01}
	Tor1b	1.27	**3.43E^{-08}**	**2.55E^{-05}**	1.04	**1.01E^{-04}**	**4.38E^{-02}**
	Chmp1b	-1.09	**7.64E^{-09}**	**7.37E^{-06}**	-0.88	**4.82E^{-05}**	**2.81E^{-02}**
	Riok1	0.98	**1.38E^{-07}**	**6.17E^{-03}**	0.56	**3.63E^{-03}**	3.00E^{-01}
	Fkbp5	1.20	**5.53E^{-06}**	**2.32E^{-03}**	1.22	**9.36E^{-04}**	1.67E^{-01}
	Mrpl4	-0.68	**1.82E^{-05}**	**6.17E^{-03}**	-0.90	**1.93E^{-06}**	**4.40E^{-03}**
	RGD1562351	-0.81	**1.163E^{-03}**	1.45E^{-01}	-0.60	**8.74E^{-04}**	1.60E^{-01}
	Tars2	-0.61	**8.61E^{-04}**	1.18E^{-01}	-0.721	**1.21E^{-04}**	**4.54E^{-02}**
	Crot	-0.61	**1.26E^{-02}**	6.10E^{-01}	-0.53	**4.67E^{-03}**	0.3.48E^{-01}
	Zfp597	1.35	**5.63E^{-03}**	3.92E^{-01}	0.94	**6.49E^{-05}**	**3.07E^{-02}**
	Ms4a11	0.80	**1.34E^{-02}**	6.33E^{-01}	0.71	**2.24E^{-02}**	5.98E^{-01}
	Aldh5a1	-0.55	**8.58E^{-03}**	5.12E^{-01}	-0.58	**3.00E^{-04}**	7.34E^{-02}
	Retsat	-0.96	**2.42E^{-02}**	8.81E^{-01}	-0.61	**3.19E^{-02}**	6.45E^{-01}
	Pxmp4	-0.79	**9.60E^{-03}**	5.41E^{-01}	0.77	**2.78E^{-04}**	6.95E^{-02}
	Tmem14a	0.81	**4.98E^{-03}**	3.72E^{-01}	0.93	**1.14E^{-03}**	1.84E^{-01}

Values in bold indicate P<0.05 or FDR<0.05. FDR, false discovery rate; FC, fold change; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR rats.

CLOCK, BMAL1 and PER were differentially regulated in hypertension patients with various genotypes (32). In the SHR rat, small interfering RNA-mediated knockdown of Per1 was shown to significantly reduce BP (33,34). Bmal1-deficient female mice were also observed to exhibit a significantly smaller infarct core volume compared with female *Bmal1*$^{+/+}$ mice at 14 days after the induction of photothrombosis (35). In line with these studies, the present study also revealed that Dbp, a clock-controlled transcription factor, was significantly upregulated in all analyzed tissues

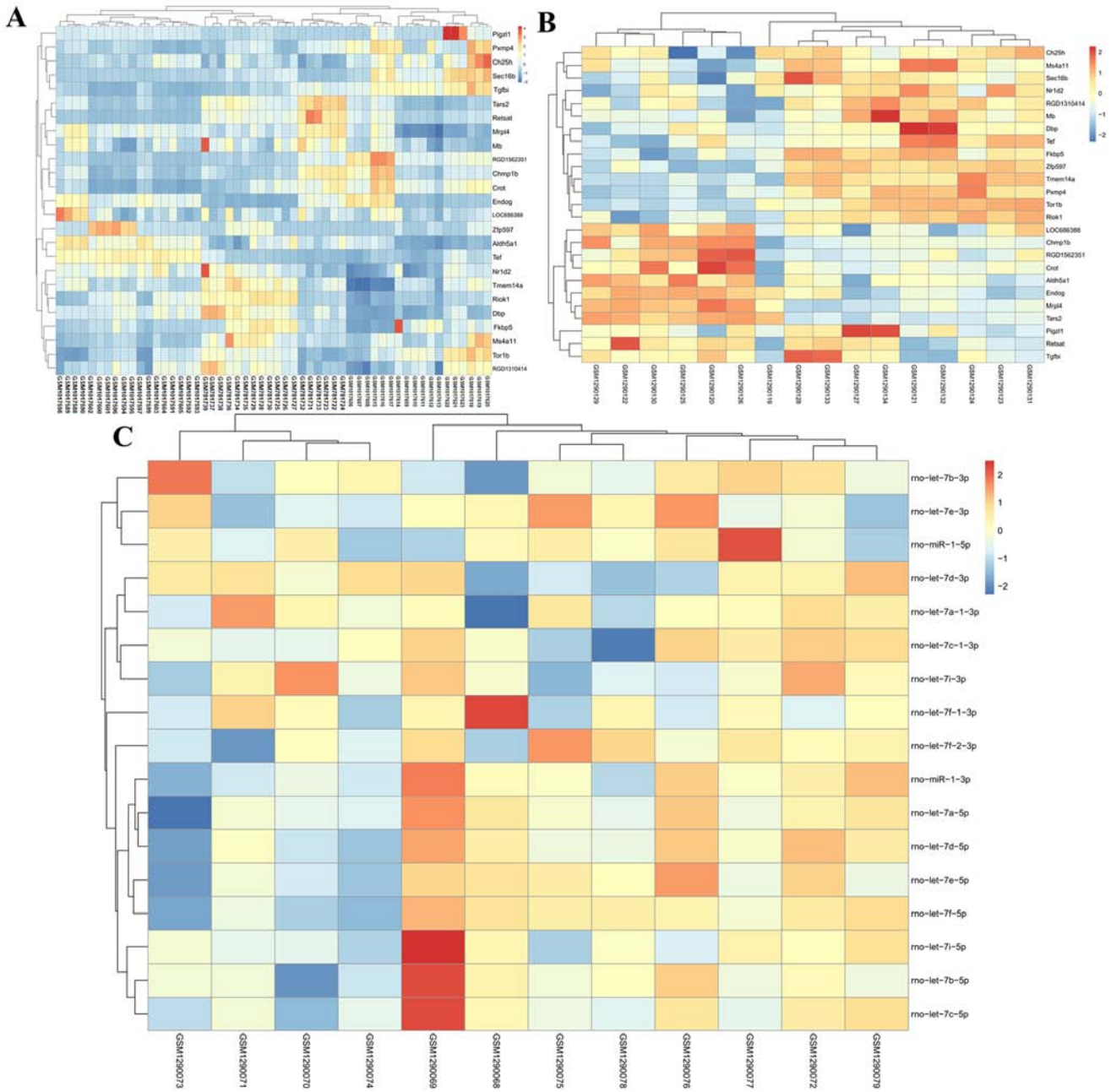

Figure 2. Hierarchical clustering and heat-map analysis of differentially expressed (A and B) genes and (C) miRNAs. (A) 3GSE31457-GSE41452-GSE41453; (B) GSE53361; (C) GSE53361. The deep-red color denotes high expression; dark blue indicates low expression.

of SHRSP rats compared with WKY rats. These results were dissimilar to a previous study performed with SHR rats, in which the expression of Dbp occurred differentially in heart (significant expression) and aortas (no expression), although it was also relatively higher compared with WKY rats (36). These findings further verified that Dbp may be a specific target for treatment of SHRSP, and that downregulation of Dbp may represent a potential therapeutic approach.

Relative to direct knockout of the specific target gene, the introduction of endogenous non-coding miRNAs that negatively regulate this gene may be safer. Therefore, miRNAs that could regulate the expression of Dbp were also investigated in our study. Our results predicted that miR-126 could bind with Dbp at the 3'-untranslated region, and thereby overexpression of miR-126 may represent a potential therapeutic approach for SHRSP via the suppression of Dbp expression. Other studies have also reported on the association of miR-126 with the development of stroke. Jin and Xing (37,38) reported that the plasma miR-126 expression level was lower in patients with acute ischemic stroke compared with those of controls, and its expression was negatively correlated with National Institutes of Health Stroke Scale (NIHSS) scores. Overexpression of miR-126 in the stem cells attenuated the infarct volume, improved functional recovery, enhanced neurogenesis, and inhibited neuroinflammation (39,40). These studies indirectly indicated that a negative correlation existed between miR-126 and Dbp in stroke. However, direct evidence that may have been used to investigate the regulatory relationship between them was lacking, and the present study may provide a novel mechanism for explaining the pathogenesis of SHRSP.

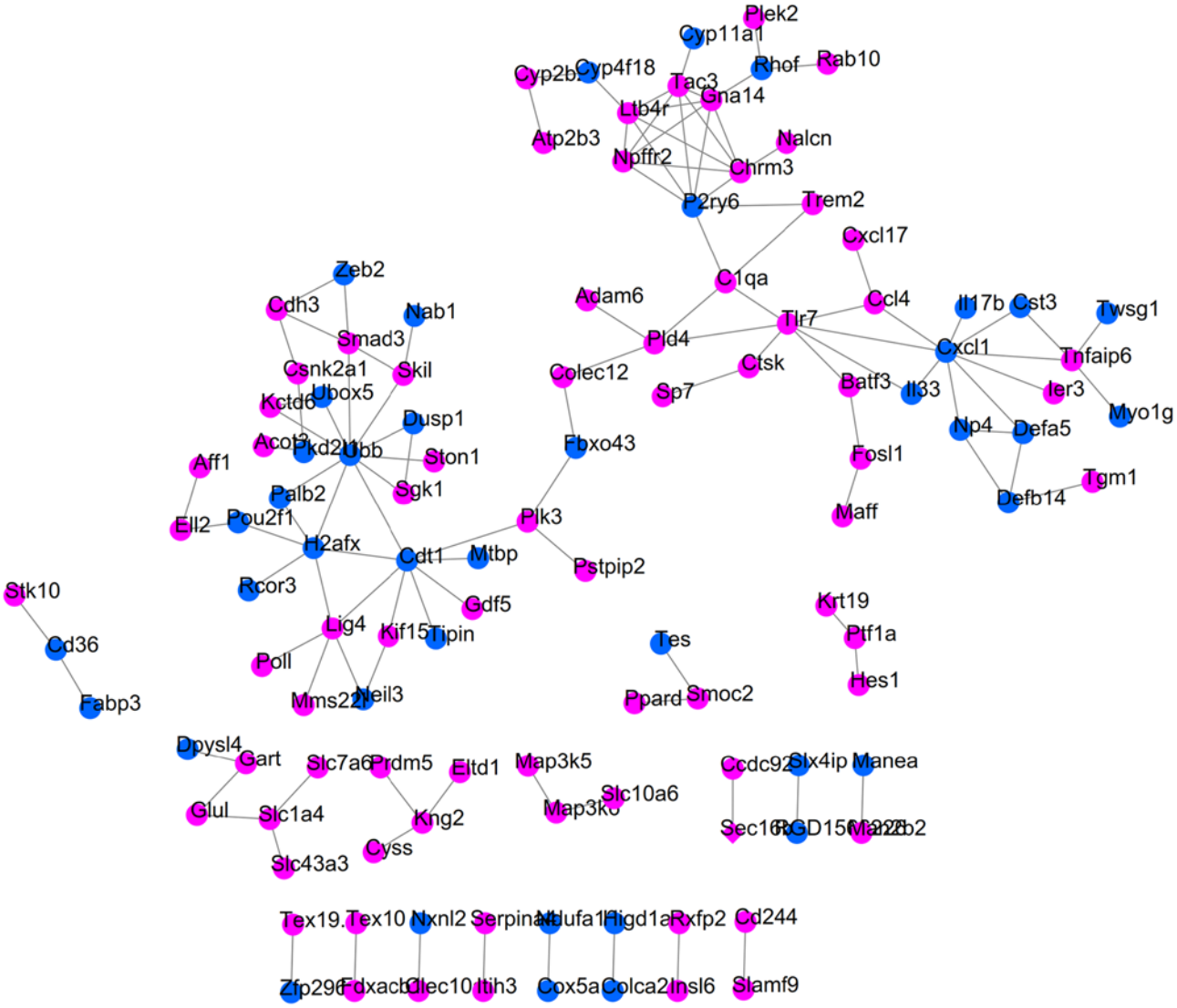

Figure 3. Protein-protein interaction network for SHR-specific genes. Dark pink represents upregulated genes; blue illustrates the downregulated genes. SHR, spontaneously hypertensive rats.

The CROT gene encodes a member of the carnitine/choline acetyltransferase family that converts 4,8-dimethylnonanoyl-CoA into its corresponding carnitine ester, and facilitates the transport of medium- and long-chain acyl-CoA molecules out of the peroxisome to the cytosol and mitochondria as a feature of the fatty acid oxidation process. Downregulation of CROT may contribute to impaired fatty acid oxidation and thus promote fat accumulation, leading to the development of obesity (41-43). Obesity is considered to be important risk factors for hypertension (44) and stroke (45). Thus, CROT may also be expressed at a lower level in SHR and SHRSP rats, a finding which was, for the first time to the best of our knowledge, confirmed in our study. Furthermore, it has also been reported that obesity may induce hypertension and stroke according to a pro-inflammatory mechanism (46,47). Therefore, CROT was also hypothesized to inhibit inflammation. Although some experimental evidence has been provided in support of this hypothesis (41), our hypothesis was that CROT may indirectly exert an anti-inflammatory effect by interacting with EPHX2, a member of epoxide hydrolase family, since it was enriched in the inflammatory response process in our study. Furthermore, the study of Ulu *et al* (48) demonstrated that an epoxide hydrolase inhibitor enhanced the anti-hypertensive and anti-inflammatory effects of omega-3 polyunsaturated fatty acids, providing further indirect support for our hypothesis. Moreover, few previous studies have been concerned with miRNAs that regulate the expression of CROT, other than miR-33 (41). The present study has, for the first time, revealed that miR-31 may also regulate CROT, and this process may be involved in the development of SHR and SHRSP. Although no direct evidence has been provided to prove that miR-31 is important for stroke and hypertension, its roles in mediating the production of inflammatory cytokines (49) may reveal the possible interaction between miR-31 and CROT.

In addition, the present study has identified that miR-31 regulates Mrpl4, and therefore Mrpl4 may also be associated with inflammation in SHR and SHRSP. Although studies on Mrpl4 are relatively rare, its potential roles in inflammation have been investigated previously. For example, MRPL4 was reported to be a downregulated downstream target of

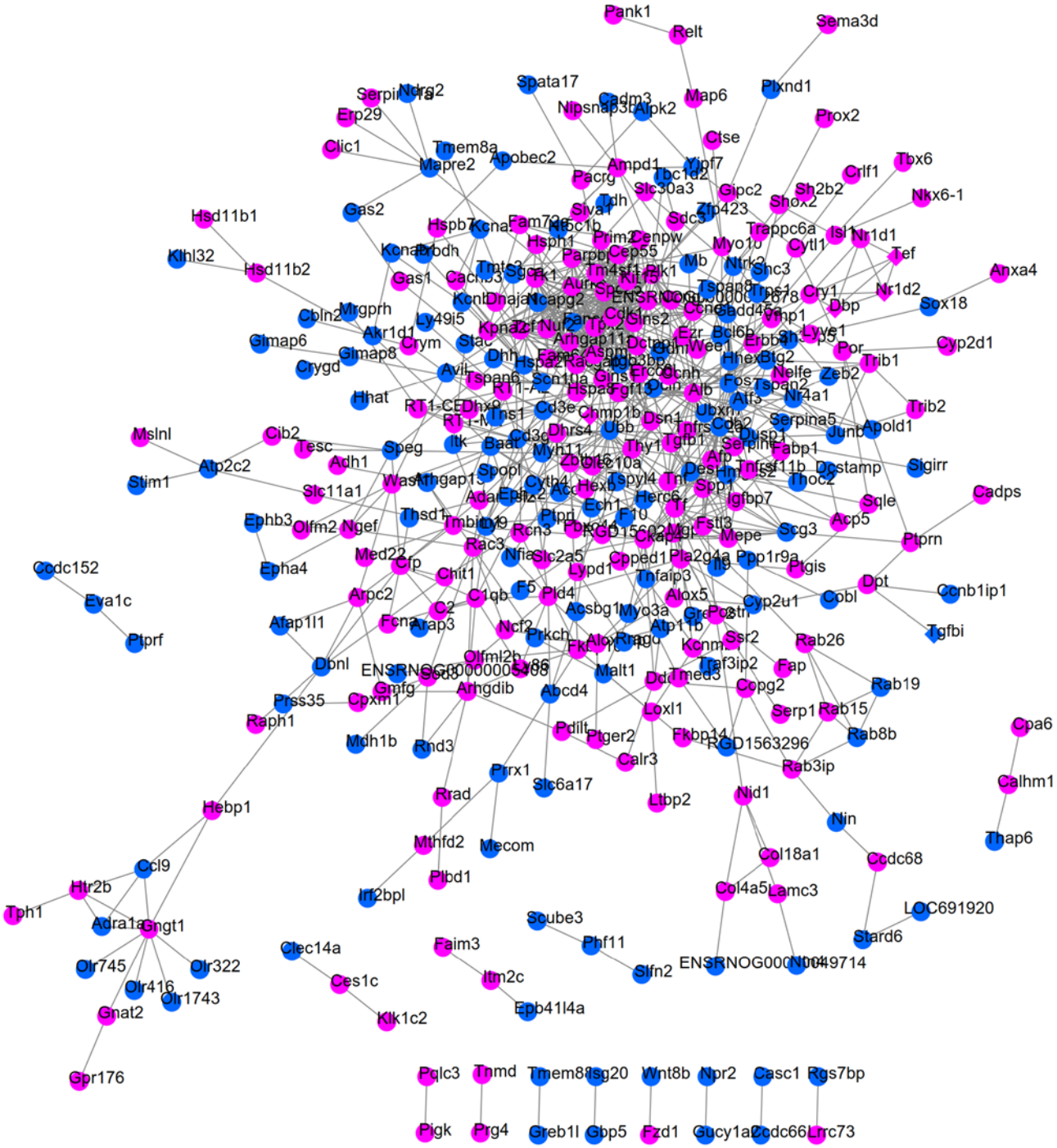

Figure 4. Protein-protein interaction network for SHRSP-specific genes. Dark pink represents upregulated genes; blue illustrates the downregulated genes. SHRSP, stroke-prone SPR rats.

hypoxia-inducible factor-1α (HIF-1α) (50). Accumulation of HIF-1α was observed in neurons of 9-month-old SHR and SHRSP rats (51). Deletion of HIF-1α was shown to significantly reduce vascular high pressure and vascular inflammation, attenuate atherosclerosis (52), and improve neuronal survival and sensorimotor function in ischemic stroke (53). Furthermore, our prediction was also that Mrpl4 could interact with TARS2. It was previously reported that secreted TARS stimulated endothelial cell migration and angiogenesis (54), which was beneficial for neurogenesis and functional recovery in patients with stroke (55). Accordingly, the present study has suggested miR-31 may be involved in SHRSP by regulating MRPL4 to mediate inflammation and angiogenesis inhibition.

Furthermore, the analysis of small-molecule drugs in the present study revealed that botulin, Gly-His-Lys and podophyllotoxin may potentially be agents for treatment of SHR and SHRSP. Although no evidence exists based on the study of their therapeutic effects on SHR and SHRSP, their anti-inflammatory activities may indirectly reveal their therapeutic potential. For example, botulinum toxin type A treatment was demonstrated to reduce persistent

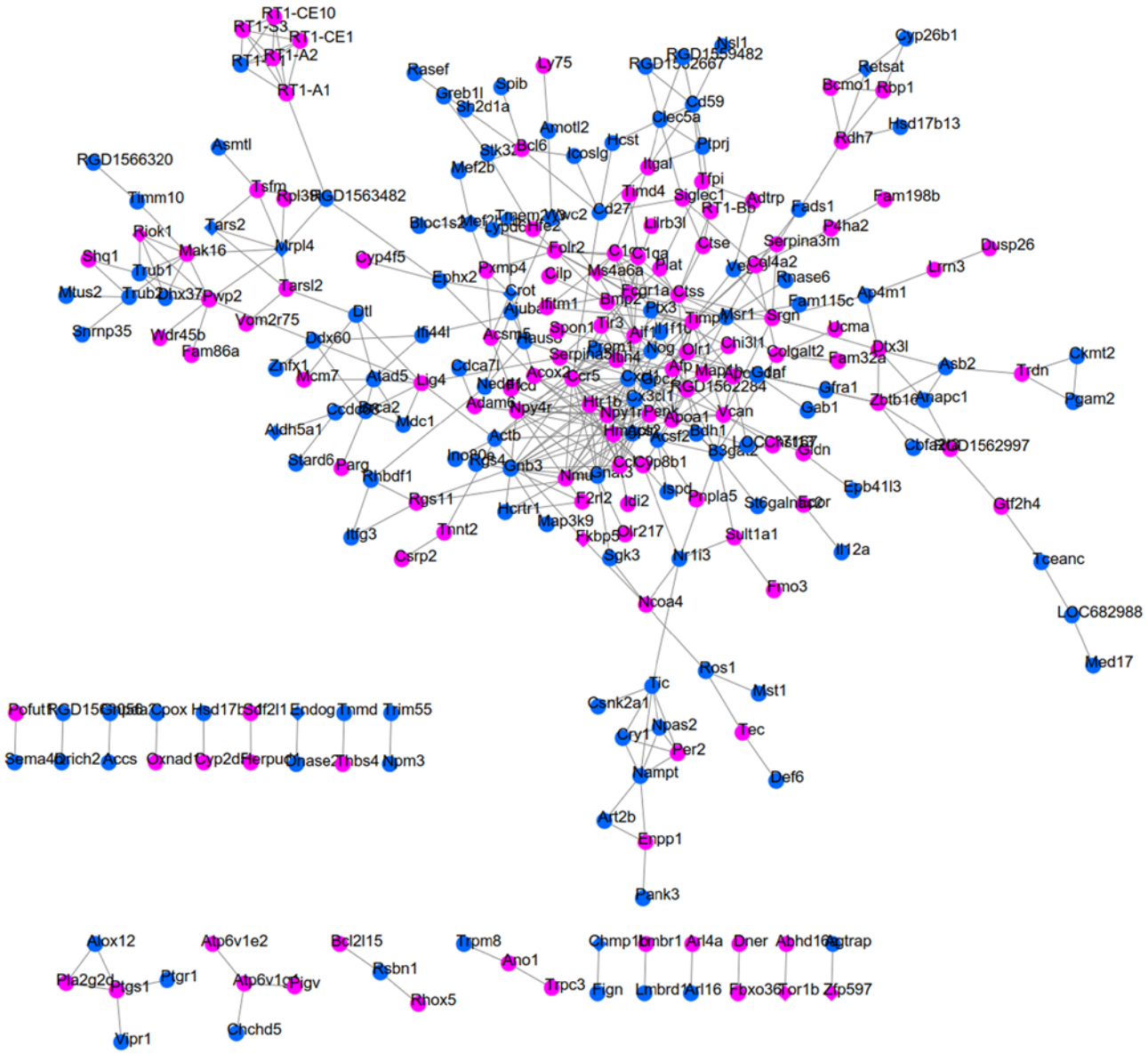

Figure 5. Protein-protein interaction network for SHR-SHRSP shared genes. Dark pink represents upregulated genes; blue illustrates the downregulated genes. SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats.

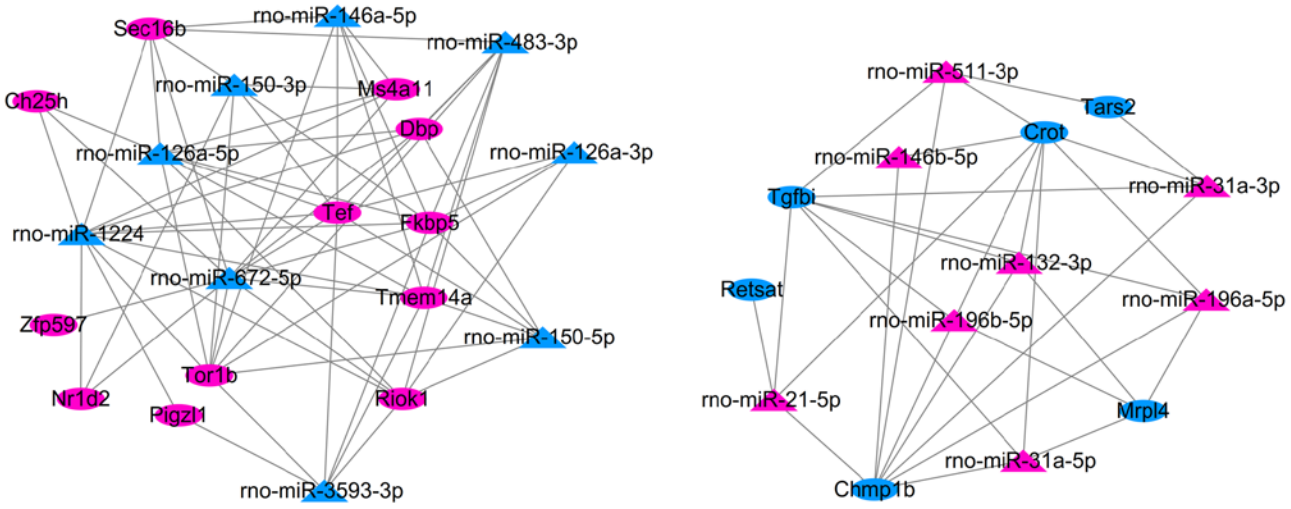

Figure 6. The miRNA-mRNA regulatory network. Dark pink represents upregulated genes; blue illustrates the downregulated genes; triangles denote miRNAs, whereas ovals indicate mRNAs.

Table IV. Function enrichment analysis for genes of the SHR-specific group of the PPI network.

Category	Term	P-value	Genes
GO BP	GO:0008584~male gonad development	2.69E^{-04}	TEX19.1, ADAM6, CYP11A1, RXFP2, CST3, UBB, INSL6
GO BP	GO:0050728~negative regulation of inflammatory response	1.76E^{-03}	CXCL17, IER3, TNFAIP6, PPARD, SMAD3
GO BP	GO:0043066~negative regulation of apoptotic process	4.11E^{-03}	IER3, PPARD, SGK1, PLK3, DUSP1, RXFP2, SMAD3, PALB2, INSL6, HIGD1A
GO BP	GO:0031640~killing of cells of other organism	1.80E^{-02}	DEFA5, NP4
GO BP	GO:0030513~positive regulation of BMP signaling pathway	2.00E^{-02}	HES1, TWSG1, GDF5
GO BP	GO:0006955~immune response	2.30E^{-02}	CXCL1, CD244, CD36, SMAD3, COLEC12, CCL4
GO BP	GO:0001562~response to protozoan	2.40E^{-02}	IER3, CYSS
GO BP	GO:0030174~regulation of DNA-dependent DNA replication initiation	2.40E^{-02}	TEX10, CDT1
GO BP	GO:0014070~response to organic cyclic compound	2.47E^{-02}	HES1, CYP2B2, CYP11A1, CYSS, CST3, FOSL1
GO BP	GO:0001701~in utero embryonic development	2.76E^{-02}	HES1, MAFF, SMAD3, LIG4, PALB2, FOSL1
GO BP	GO:0007431~salivary gland development	3.00E^{-02}	CYSS, CST3
GO BP	GO:0033262~regulation of nuclear cell cycle DNA replication	3.00E^{-02}	TIPIN, CDT1
GO BP	GO:0010332~response to gamma radiation	3.10E^{-02}	CXCL1, CYP11A1, LIG4
GO BP	GO:0046677~response to antibiotic	3.28E^{-02}	CYP11A1, CYSS, SKIL
GO BP	GO:0006952~defense response	3.41E^{-02}	CXCL1, NP4, CST3
GO BP	GO:0006954~inflammatory response	3.43E^{-02}	CXCL1, IL17B, KNG2, LTB4R, CCL4, TLR7
GO BP	GO:0032755~positive regulation of interleukin-6 production	3.67E^{-02}	CD36, IL33, TLR7
GO BP	GO:0042493~response to drug	4.10E^{-02}	CD36, CYP11A1, CYSS, CST3, FABP3, NDUFA10, CDH3, FOSL1
GO BP	GO:0000076~DNA replication checkpoint	4.16E^{-02}	TIPIN, CDT1
GO BP	GO:0044539~long-chain fatty acid import	4.16E^{-02}	CD36, FABP3
GO BP	GO:0001756~somitogenesis	4.39E^{-02}	SMAD3, ZEB2, PALB2
GO BP	GO:0071356~cellular response to tumor necrosis factor	4.68E^{-02}	HES1, MAP3K5, CYP11A1, CCL4
GO MF	GO:0003677~DNA binding	6.91E^{-04}	BATF3, POLL, RCOR3, PTF1A, TIPIN, SMAD3, ZEB2, LIG4, CDT1, HES1, POU2F1, H2AFX, SP7, SKIL, PALB2, FOSL1
	GO:0070538~oleic acid binding	1.63E^{-02}	CD36, FABP3
	GO:0004869~cysteine-type endopeptidase inhibitor activity	2.31E^{-02}	KNG2, CYSS, CST3
	GO:0001078~transcriptional repressor activity, RNA polymerase II core promoter proximal region sequence-specific binding	2.69E^{-02}	BATF3, HES1, PRDM5, SKIL
	GO:0004559~alpha-mannosidase activity	4.29E^{-02}	MAN2B2, MANEA
GO CC	GO:0005615~extracellular space	7.23E^{-04}	CXCL1, TWSG1, KNG2, DEFB14, GDF5, CST3, IL33, CCL4, CXCL17, TNFAIP6, CTSK, CD36, IL17B, DEFA5, SERPINA4, NP4, CYSS, FABP3, UBB
	GO:0043231~intracellular membrane-bounded organelle	7.83E^{-03}	SEC16B, CYP2B2, GLUL, ATP2B3, KNG2, CD36, POU2F1, TIPIN, TREM2, FOSL1, PKD2L1
	GO:0000785~chromatin	3.77E^{-02}	HES1, PLK3, MTBP, H2AFX

BP, biological process; MF, molecular function; CC, cellular component; GO, Gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; PPI, protein-protein interaction; SHR, spontaneously hypertensive rats.

Table V. Function enrichment analysis for the genes of the SHRSP group of the PPI network.

Category	Term	P-value	Genes
GO BP	GO:0032570~response to progesterone	$1.00E^{-05}$	CCNE1, FOS, PTGER2, NCF2, ERBB4, ADH1, HSPA8, JUNB, TGFB1
GO BP	GO:0010033~response to organic substance	$1.69E^{-05}$	TNF, CYP2D1, MGP, TGFB1, AMPD1, AFP, C1QB, PLA2G4A, BTG2, DUSP1, ALB, SQLE, SPP1
GO BP	GO:0007067~mitotic nuclear division	$7.62E^{-05}$	ITGB3BP, SPC25, CDK1, PLK1, NUF2, CENPW, MAPRE2, FABP1, CEP55, CCNG1, WEE1
GO BP	GO:0000122~negative regulation of transcription from RNA polymerase II promoter	$7.95E^{-04}$	TNF, PRRX1, ZEB2, AURKB, ZBTB16, TGFB1, CCNE1, EZR, NR1D1, SOX18, CRY1, TBX6, ISL1, PROX2, JUNB, NKX6-1, SHOX2, HHEX, ATF3, BTG2, PLK1, IRF2BPL, TRPS1, BCL6B, NELFE, NFIA, CRYM
GO BP	GO:0051301~cell division	$1.34E^{-03}$	ITGB3BP, SPC25, CDK1, CCNE1, CHMP1B, DSN1, NUF2, CENPW, MAPRE2, CCNG1, WEE1
GO BP	GO:0032496~response to lipopolysaccharide	$1.50E^{-03}$	FOS, SLC11A1, TNFRSF11B, PLA2G4A, PTGER2, TNF, RELT, NCF2, SERPINE1, ACP5, C2, LOXL1, JUNB, TRIB1
GO BP	GO:0030198~extracellular matrix organization	$1.67E^{-03}$	COL18A1, TNFRSF11B, TNF, ADAMTSL2, OLFML2B, TGFBI, NID1, POSTN
GO BP	GO:0001755~neural crest cell migration	$1.69E^{-03}$	ERBB4, SEMA3D, ZEB2, ISL1, HTR2B, GDNF
GO BP	GO:0045880~positive regulation of smoothened signaling pathway	$1.83E^{-03}$	SHOX2, SCUBE3, PRRX1, GAS1, POR
GO BP	GO:0030316~osteoclast differentiation	$2.07E^{-03}$	TF, TNF, ACP5, DCSTAMP, JUNB
GO BP	GO:0002062~chondrocyte differentiation	$8.72E^{-03}$	SHOX2, TRPS1, TGFBI, CYTL1, TGFB1
GO BP	GO:0001889~liver development	$1.47E^{-02}$	AFP, CCNE1, HHEX, COBL, BAAT, HMGCS2, DBP, TK1
GO BP	GO:0007623~circadian rhythm	$2.06E^{-02}$	DHX9, TNF, NR1D1, DBP, NTRK2, CRY1, TPH1
GO BP	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	$2.50E^{-02}$	TNF, HEXB, PRRX1, CYTL1, FSTL3, ZEB2, GDNF, TGFB1, HSPH1, SLC11A1, FOS, TEF, SOX18, TBX6, CCNH, NR4A1, ISL1, MECOM, PROX2, JUNB, SHOX2, HHEX, ATF3, IRF2BPL, DBP, TRPS1, NELFE, NFIA
GO BP	GO:0001525~angiogenesis	$4.50E^{-02}$	COL18A1, SERPINE1, TGFBI, APOLD1, SOX18, PLXND1, EPHB3, THY1
GO BP	GO:0042542~response to hydrogen peroxide	$4.94E^{-02}$	CDK1, PLA2G4A, DUSP1, ERBB4, MB
GO MF	GO:0042803~protein homodimerization activity	$6.49E^{-04}$	CADM3, ERBB4, HEXB, ZBTB16, GREM2, GDNF, TGFB1, SLC11A1, ADH1, FAP, ALOX5AP, TEF, CIB2, ZFP423, TESC, OLFML2B, ERP29, EPHX2, FZD1, NR4A1, MECOM, HHEX, C1QB, PPP1R9A, ATF3, NTRK2, CTSE, CRYM
	GO:0005515~protein binding	$2.81E^{-03}$	COBL, ERBB4, KCNAB1, WASF1, KCNA2, CACNB3, ZBTB16, CDH2, RAB3IP, SDC3, FOS, EZR, HSPA2, NR1D1, ARPC2, SCG3, GUCY1A2, DNAJA1, C2, VMP1, HSPA8, SCN10A, NGEF, CDK1, RAB8B, KCNB1, ERP29, FZD1, NR4A1, NID1, ISL1, THY1, SH3BP5, RT1-A2, EPHA4, PPP1R9A, BTG2, NTRK2, FABP1, ALOX5, MAP6, HTR2B, NFIA
	GO:0005525~GTP binding	$5.84E^{-03}$	TF, RAB8B, GBP5, GIMAP6, GIMAP8, RRAD, NPR2, RRAGD, GNAT2, RND3, RAB19, RAC3, GUCY1A2, RAB15, RAB26
	GO:0050840~extracellular matrix binding	$1.06E^{-02}$	OLFML2B, TGFBI, NID1, SPP1
	GO:0046982~protein heterodimerization activity	$1.50E^{-02}$	ZFP423, CD3G, CD3E, KCNB1, HEXB, CRLF1, FZD1, NR4A1, RRAGD, TGFB1, FOS, ATF3, ALOX5AP, TEF, GUCY1A2, ADRA1A, CENPW, SOX18

Table V. Continued.

Category	Term	P-value	Genes
GO CC	GO:0005615~extracellular space	9.32E^{-06}	TF, DHH, TNF, LTBP2, IGFBP7, HEXB, PLBD1, SERPINB1A, CCL9, FSTL3, ACP5, POSTN, GREM2, GDNF, TGFB1, CHIT1, MTHFD2, TNFRSF11B, PTGIS, EZR, ALB, SERPINA5, FAP, SERPINE1, TGFBI, SEMA3D, C2, CES1C, HSPA8, SPP1, DPT, WNT8B, COL18A1, CPA6, PRG4, IL9, MGP, CLIC1, SOD3, AFP, CBLN2, F5, IRF2BPL, CPXM1, ALOX5, UBB
	GO:0031012~extracellular matrix	1.48E^{-04}	COL18A1, LTBP2, IGFBP7, CKAP4, MGP, NID1, POSTN, TGFB1, SOD3, CFP, TGFBI, SERPINE1, LOXL1, CLEC14A, DPT
	GO:0070062~extracellular exosome	1.59E^{-04}	LTBP2, IGFBP7, HEXB, TSPAN6, TSPAN8, HSPH1, DES, SLC2A5, SERPINA5, SERPINE1, TGFBI, SCN10A, MB, PTPRJ, DBNL, CDK1, PTPRF, ERP29, MGP, PRKCH, CLIC1, THY1, C1QB, RND3, CHMP1B, DHRS4, BTG2, RELT, RAB19, CTSE, RAB15, UBB, TNFAIP3, AKR1D1, TF, ECH1, SERPINB1A, ACP5, CDH2, GIPC2, ITM2C, EZR, HSPA2, ARPC2, ALB, RAC3, DNAJA1, C2, NDRG2, HSPA8, SPP1, ARHGDIB, DPT, COL18A1, RAB8B, CPPED1, CKAP4, EPHX2, NID1, TMBIM1, RACGAP1, ANXA4, SOD3, NKX6-1, LYVE1, TMEM8A, HEBP1, MYH11, FABP1, CRYM, CLEC14A
	GO:0005604~basement membrane	2.12E^{-04}	COL18A1, TF, LAMC3, ALB, TGFBI, NTN4, NID1, LOXL1, COL4A5
	GO:0030027~lamellipodium	3.81E^{-04}	DBNL, TESC, PPP1R9A, MYO10, WASF1, KCNA2, FAP, CDH2, ARAP3, PLXND1, RAB3IP
KEGG	rno04010:MAPK signaling pathway	3.86E^{-03}	TNF, NR4A1, CACNB3, FGF13, MECOM, TGFB1, FOS, PLA2G4A, HSPA2, DUSP1, NTRK2, GADD45A, HSPA8
KEGG	rno04610:Complement and coagulation cascades	1.22E^{-02}	C1QB, F10, F5, SERPINA5, SERPINE1, C2
KEGG	rno05142:Chagas disease (American trypanosomiasis)	1.69E^{-02}	C1QB, FOS, CD3G, TNF, CD3E, SERPINE1, TGFB1
KEGG	rno04110:Cell cycle	3.54E^{-02}	CDK1, CCNE1, CCNH, PLK1, GADD45A, WEE1, TGFB1
KEGG	rno04380:Osteoclast differentiation	3.78E^{-02}	FOS, TNFRSF11B, TNF, NCF2, ACP5, JUNB, TGFB1
KEGG	rno04612:Antigen processing and presentation	3.89E^{-02}	RT1-A2, TNF, HSPA2, RT1-CE4, RT1-M2, HSPA8
KEGG	rno04141:Protein processing in endoplasmic reticulum	4.30E^{-02}	HSPH1, HSPA2, CKAP4, ERP29, DNAJA1, DDOST, SSR2, HSPA8
KEGG	rno04115:p53 signaling pathway	4.53E^{-02}	CDK1, CCNE1, SERPINE1, CCNG1, GADD45A
KEGG	rno04514:Cell adhesion molecules (CAMs)	4.77E^{-02}	RT1-A2, CADM3, OCLN, PTPRF, RT1-CE4, RT1-M2, CDH2, SDC3

BP, biological process; MF, molecular function; CC, cellular component; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; SHRSP, stroke-prone spontaneously hypertensive rats.

inflammatory hypernociception induced by arthritis in the temporomandibular joint of rats by decreasing the expression of the pro-inflammatory cytokine, interleukin-1β (IL-1β) (56). Wang *et al* (57) also observed that intra-articular botulinum toxin type A administration caused anti-neurogenic inflammation by blocking the infiltration of inflammatory cells (57). The tripeptide Gly-Gly-His was shown to inhibit secretion of pro-inflammatory IL-6 in fibroblasts (58). The study of Kalita *et al* (59) demonstrated that a combination of podophyllotoxin and rutin is a safe and effective protective agent to attenuate radiation-induced gastrointestinal injury by negatively regulating NF-κB/p53 signaling (59).

In conclusion, our study has provided some preliminary evidence to suggest that Dbp, Crot and Mrpl4 may

Table VI. Function enrichment for the genes of SHR-SHRSP shared group in the PPI network.

Category	Term	P-value	Genes
GO BP	GO:0006954~inflammatory response	$9.20E^{-05}$	CXCL1, BMP2, OLR1, AIF1, PTGS1, CHI3L1, EPHX2, CCL19, TLR3, CX3CL1, CYP4F5, CCR5, CYP26B1, CD27
GO BP	GO:0006955~immune response	$1.33E^{-04}$	CXCL1, RT1-A2, RT1-A1, RT1-CE1, ENPP1, CCR5, IL12A, RT1-CE10, IFI44L, CX3CL1, CTSS, CD27, RT1-BB
GO BP	GO:0007631~feeding behavior	$8.99E^{-04}$	HCRTR1, HTR1B, NPY4R, NPY1R, APLN
GO BP	GO:0002474~antigen processing and presentation of peptide antigen via MHC class I	$1.00E^{-03}$	RT1-A2, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3
GO BP	GO:0055114~oxidation-reduction process	$1.33E^{-03}$	HSD17B11, PTGR1, BMP2, CYP2D5, ALDH5A1, FADS1, HSD17B13, PTGS1, RDH7, P4HA2, CYP4F5, CPOX, CYP26B1, FMO3, OXNAD1, CYP8B1, BDH1, RETSAT, ALOX12
GO BP	GO:0001916~positive regulation of T cell mediated cytotoxicity	$1.59E^{-03}$	RT1-A2, RT1-A1, IL12A, RT1-S3
GO BP	GO:0032355~response to estradiol	$3.72E^{-03}$	CXCL1, PENK, FCGR1A, ENDOG, MAP1B, TFPI, BRCA2, NPY1R, BDH1
GO BP	GO:0045785~positive regulation of cell adhesion	$5.49E^{-03}$	PTPRJ, DUSP26, IL12A, CX3CL1, ALOX12
GO BP	GO:0007568~aging	$6.20E^{-03}$	CCR5, PENK, FADS1, ENDOG, PTGS1, GFRA1, EPOR, NPY1R, CX3CL1, TIMP1, ALOX12
GO BP	GO:0006631~fatty acid metabolic process	$6.58E^{-03}$	ACOX2, PER2, ACSF2, ACSM5, CROT
GO BP	GO:0007417~central nervous system development	$8.57E^{-03}$	NOG, ALDH5A1, DNER, VCAN, LIG4, ZBTB16
GO BP	GO:0001666~response to hypoxia	$2.00E^{-02}$	AJUBA, VEGFB, PLAT, BMP2, PENK, EPOR, CX3CL1, CBFA2T3, AGTRAP
GO BP	GO:0009612~response to mechanical stimulus	$4.08E^{-02}$	ACTB, BMP2, ENDOG, MAP1B, CHI3L1
GO MF	GO:0042605~peptide antigen binding	$1.09E^{-03}$	RT1-A1, RT1-CE1, RT1-CE10, RT1-S3, RT1-BB
	GO:0005102~receptor binding	$1.83E^{-03}$	ACOX2, PLAT, BMP2, RT1-CE1, HFE2, EPHX2, RT1-S3, HCST, TRDN, RT1-A1, RT1-CE10, CROT, TEC
	GO:0008083~growth factor activity	$8.50E^{-03}$	VEGFB, CXCL1, BMP2, IL12A, GDNF, TIMP1, THBS4
	GO:0004829~threonine-tRNA ligase activity	$3.67E^{-02}$	TARS2, TARSL2
	GO:0001602~pancreatic polypeptide receptor activity	$3.67E^{-02}$	NPY4R, NPY1R
GO CC	GO:0005615~extracellular space	$2.96E^{-06}$	CXCL1, NAMPT, NOG, HFE2, ENPP1, GLDN, MST1, CX3CL1, C1QC, GDNF, TIMP1, GPC2, APOA1, SERPINA5, PTX3, APLN, SRGN, SPON1, THBS4, ACTB, PLAT, BMP2, UCMA, IL1F10, CHI3L1, CILP, CCL19, CTSS, VEGFB, PROM1, AFP, SERPINA3M, CD59, TFPI, IL12A, SEMA4B, GFRA1, VCAN
	GO:0009986~cell surface	$9.64E^{-06}$	PLAT, PTPRJ, ITGAL, BMP2, HFE2, ENPP1, TNMD, TLR3, RT1-S3, CX3CL1, CTSS, RT1-BB, HCST, PROM1, APOA1, CCR5, FOLR2, CD59, TFPI, VCAN, ROS1, CLEC5A, CD27
	GO:0009897~external side of plasma membrane	$3.39E^{-05}$	LY75, ITGAL, TRPM8, ANO1, CCL19, RT1-BB, RT1-A2, CCR5, SERPINA5, FCGR1A, GFRA1, EPOR, CD27, ICOSLG
	GO:0042612~MHC class I protein complex	$1.76E^{-04}$	RT1-A2, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3
	GO:0005576~extracellular region	$1.70E^{-03}$	HSD17B11, COL4A2, OLR1, ENPP1, HSD17B13, CTSS, CX3CL1, GDNF, C1QC, TIMP1, DNASE2B, VEGFB, APOA1, PENK, TFPI, VCAN, APOL11A, PLA2G2D, NMU, THBS4

Table VI. Continued.

Category	Term	P-value	Genes
KEGG	rno04145:Phagosome	1.97E^{-06}	ACTB, MSR1, RT1-CE1, OLR1, CTSS, ATP6V1G1, RT1-S3, RT1-BB, RT1-A2, RT1-A1, FCGR1A, ATP6V1E2, RT1-CE10, RT1-N1, THBS4
KEGG	rno05416:Viral myocarditis	8.38E^{-05}	ACTB, RT1-A2, ITGAL, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3, RT1-N1, RT1-BB
KEGG	rno05330:Allograft rejection	1.12E^{-04}	RT1-A2, RT1-A1, RT1-CE1, IL12A, RT1-CE10, RT1-S3, RT1-N1, RT1-BB
KEGG	rno04940:Type I diabetes mellitus	1.89E^{-04}	RT1-A2, RT1-A1, RT1-CE1, IL12A, RT1-CE10, RT1-S3, RT1-N1, RT1-BB
KEGG	rno04514:Cell adhesion molecules (CAMs)	3.86E^{-04}	RT1-A2, SIGLEC1, ITGAL, RT1-A1, RT1-CE1, RT1-CE10, VCAN, RT1-S3, RT1-N1, ICOSLG, RT1-BB
KEGG	rno05332:Graft-versus-host disease	5.91E^{-04}	RT1-A2, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3, RT1-N1, RT1-BB
KEGG	rno04612:Antigen processing and presentation	8.43E^{-04}	RT1-A2, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3, CTSS, RT1-N1, RT1-BB
KEGG	rno05320:Autoimmune thyroid disease	1.34E^{-03}	RT1-A2, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3, RT1-N1, RT1-BB
KEGG	rno05168:Herpes simplex infection	2.21E^{-03}	RT1-A2, RT1-A1, RT1-CE1, CSNK2A1, PER2, IL12A, RT1-CE10, TLR3, RT1-S3, RT1-N1, RT1-BB
KEGG	rno04610:Complement and coagulation cascades	5.57E^{-03}	PLAT, C1QA, CD59, SERPINA5, TFPI, C1QC
KEGG	rno00650:Butanoate metabolism	9.57E^{-03}	HMGCS2, ALDH5A1, BDH1, ACSM5
KEGG	rno05150:Staphylococcus aureus infection	1.04E^{-02}	C1QA, ITGAL, FCGR1A, C1QC, RT1-BB
KEGG	rno05169:Epstein-Barr virus infection	3.01E^{-02}	RT1-A2, ITGAL, RT1-A1, RT1-CE1, RT1-CE10, RT1-S3, RT1-N1

BP, biological process; MF, molecular function; CC, cellular component; GO, Gene ontology; KEGG, Kyoto encyclopedia of genes and genomes. SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR rats.

Table VII. Candidate small-molecule drugs.

CMAP name	Enrichment	P-value
Betulin	-0.96	2.20E^{-04}
Gly-His-Lys	-0.93	5.40E^{-04}
Benzathine Benzylpenicillin	-0.90	1.60E^{-04}
Prestwick-1103	-0.89	4.00E^{-04}
PF-00539745-00	-0.88	3.43E^{-03}
Quinostatin	-0.88	2.99E^{-02}
5279552	-0.86	3.77E^{-02}
Podophyllotoxin	-0.86	7.00E^{-04}
Cefuroxime	0.80	3.00E^{-03}
STOCK1N-35215	0.81	1.37E^{-02}
NS-398	0.91	1.38E^{-02}

be potential targets for treatment of SHRSP. Their expression may be reversed by miRNAs (rno-miR-126a-5p and rno-miR-31a) or small-molecule drugs (botulin, Gly-His-Lys and podophyllotoxin). However, further *in vitro* and *in vivo* experiments are required in order to confirm these conclusions.

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Availability of data and materials

The sequencing data GSE41452, GSE31457, GSE41453, GSE53363 and GSE53361 were downloaded from the GEO database in NCBI (http://www.ncbi.nlm.nih.gov/geo/).

Authors' contributions

QZ and LW conceived the design of the original study. QZ and HS conducted the statistical analysis. LY was involved with the interpretation of the data. QZ drafted the manuscript. LW participated in critical revisions of the

manuscript. All authors approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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