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ORIGINAL ARTICLE

Vascular Transcriptome Profiling Reveals Aging-Related Genes in Angiotensin Ⅱ-Induced Hypertensive Mouse Aortas

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Key words: hypertension; vascular aging; angiotensin Ⅱ; transcriptome sequencing

Objective Angiotensin Ⅱ (Ang Ⅱ)-induced vascular damage is a major risk of hypertension. However, the underlying molecular mechanism of Ang Ⅱ-induced vascular damage is still unclear. In this study, we explored the novel mechanism associated with Ang II-induced hypertension.

Methods We treated 8- to 12-week-old C57BL/6J male mice with saline and Ang Ⅱ (0.72 mg/kg·d) for 28 days, respectively. Then the RNA of the media from the collected mice aortas was extracted for transcriptome sequencing. Principal component analysis was applied to show a clear separation of different samples and the distribution of differentially expressed genes was manifested by Volcano plot. Functional annotations including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were performed to reveal the molecular mechanism of Ang Ⅱ-induced hypertension. Finally, the differentially expressed genes were validated by using quantitative real-time PCR.

Results The result revealed that a total of 773 genes, including 599 up-regulated genes and 174 down-regulated genes, were differentially expressed in the aorta of Ang Ⅱ-induced hypertension mice model. Functional analysis of differentially expressed genes manifested that various cellular processes may be involved in the Ang Ⅱ-induced hypertension, including some pathways associated with hypertension such as extracellular matrix, inflammation and immune response. Interestingly, we also found that the differentially expressed genes were enriched in

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vascular aging pathway, and further validated that the expression levels of insulin-like growth factor 1 and adiponectin were significantly increased ($P<0.05$).

Conclusion We identify that vascular aging is involved in Ang Ⅱ-induced hypertension, and insulin-like growth factor 1 and adiponectin may be important candidate genes leading to vascular aging.

HYPERTENSION is a key risk factor for health, leading to 40% of global deaths from cardiovascular diseases.[1] Hypertension has been indicated to result from an overactive sympathetic nervous system[2] as well as disorder in the renin-angiotensin system (RAS). Moreover, hypertension is thought to be caused by different factors, including genetic factors and environmental factors.[3, 4] Though the utilization of many drugs for treating this disease, lots of patients are not well controlled.[5] Additional factors or mechanisms that may be involved in hypertension are unrevealed by current therapy strategies. Therefore, it is necessary to elucidate the genes involved in the regulation of hypertension on a large scale, which may provide us with more information for developing anti-hypertension drugs which target normalizing gene expression.

The RAS is vital to regulate blood pressure (BP) and electrolyte homeostasis.[6] But RAS can also cause cardiac and vascular injury through triggering structural remodeling and inflammation under pathophysiological conditions.[7, 8] Angiotensin Ⅱ (Ang Ⅱ) is the main product of the RAS and the prime ligand of Ang Ⅱ receptor.[8] Ang Ⅱ is produced from the angiotensinogen and then cleaved by renin and angiotensin-converting enzyme.[9] Mechanically, Ang Ⅱ functions through Ang Ⅱ type 1 (AT_1) and Ang Ⅱ type 2 (AT_2) receptors, which are cell surface receptors belonging to the G-protein-coupled receptor family.[10] AT_1 receptor is the most important receptor in mediating the function of Ang Ⅱ.[11] Thus, Ang II is of critical importance to regulate BP.[8]

Transcriptome analysis, which can supply the level of gene expression of tissues at different conditions, has enabled a novel method to reveal the mechanism of diseases in recent years.[12] In our study, we established an Ang Ⅱ-induced hypertensive mouse model. The whole-transcriptome of the aortas obtained from mice treated with saline and Ang Ⅱ was sequenced. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) were performed to discover the mechanisms of Ang Ⅱ-induced hypertension.

MATERIALS AND METHODS

Study design and animal experiments

All animal treatments and experimental protocols were approved by the Animal Care and Use Committee at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College. Totally, 16 of 8- to 12-week old C57BL/6J male mice were maintained on a 12-hour day/night cycle with constant access to food and water, in which 8 mice were infused with Ang Ⅱ (Sigma-Aldrich, Cat No. A9525) subcutaneously *via* osmotic pump (Alzet model 2004) in a dose of 0.72 mg/kg·d, and the other received saline as the controls. The detailed procedure was performed as previously described.[13]

BP measurement

After 28 days of Ang Ⅱ infusion, BP, systolic BP, diastolic BP and heart ratio were measured by tail-cuff occlusion method according to the manufacturer's instructions (Kent Scientific Corporation, Torrington, CT, USA). In brief, mice were habituated to the device on a daily basis for 1 week. For BP measurements, mice were placed in a tail-cuff restrainer over a warmed surface. We recorded and averaged ten readings per mouse after BP was stabilized.[14]

Histological analysis

After the mice were executed, the aortas from the ascending aorta to the furcation of the common iliac artery were isolated. The aortas and adventitia were fixed with 4% paraformaldehyde-PBS for 24 hours and embedded in paraffin. Paraffin sections (5 μm) were stained with Elastin van Gieson (EVG) and the grade of elastin degradation was quantified by a researcher who was blinded to the group assignment according to the criteria previously described.[13] The grades were defined as follows: score 1, no degradation; score 2, mild elastin degradation; score 3, severe elastin degradation; score 4, aortic rupture. Meanwhile, paraffin sections were stained with hematoxylin-eosin (HE), and

the intimal and media thickness was measured and analyzed with Image-Pro Plus 6.0 (Media Cybernetics).

Quantitative real-time PCR

It has been revealed that vascular inflammation and fibrosis are associated with hypertension,[15] so we detected the expression levels of vascular cell adhesion molecular 1 (VCAM-1), proinflammatory cytokine interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP1), and fibrotic markers (collagen-Ⅰ, collagen-Ⅲ and fibronectin) with quantitative real-time PCR (qRT-PCR). The total RNA was extracted from the aortas using TRIzol reagent (Invitrogen) according to the manufacturer's protocols. The first-strand cDNA was synthesized using Reverse Transcription System (TaKaRa) according to manufacturer's instructions. PCR was performed using AceQ qPCR SYBR Green Master Mix (Vazyme) on an Eppendorf Mastercycler machine with cDNA as a templet.[16] To avoid genomic DNA amplification, primers were designed to span exon boundaries (**Table 1**).

We also used qRT-PCR to validate the result of RNA-seq data. Sequences of primers for PCR amplification are listed in **Table 2.**

RNA extraction and library preparation

The adventitia was carefully removed from the collected mice aortas, and the media were processed for RNA purification and sequencing. The total RNA was extracted with mirVana miRNA Isolation Kit (Ambion) following the manufacturer's protocol. An Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to evaluate RNA integrity. The samples with RNA Integrity Number (RIN) ⩾ 7 were chosen for further analysis.

The libraries were constructed using TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, after DNA was digested with DNase, mRNA was enriched with magnetic beads containing Oligo (dT) (Invitrogen). Afterward, the mRNA was fragmented into small pieces using divalent cations at 94°C for 8 min. The cleaved mRNA fragments were copied into the first-strand cDNA using First Strand Synthesis Act D Mix and SuperScript II Reverse Transcripts (Invitrogen). Then, end-repair control and second strand marking master mix were used for synthesizing second-strand cDNA. The second-strand DNA fragments were purified again by AMPure XP beads (BECKMAN COULTER) and then subjected to a terminal repair process, in which a single 'A' base was added and the adaptor was ligated. Then PCR was carried out to purify and enrich the product to construct the final cDNA library. The purified libraries were validated by an Agilent 2100 Bioanalyzer (Agilent Technologies) through checking size and purity of the library. Then these libraries were sequenced on an Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeqX Ten) and 125 bp/150 bp paired-end reads were generated.[17] The transcriptome sequencing and analysis were performed by OE Biotech Co., Ltd. (Shanghai, China).

Table 1. Sequences of primers for gene amplification

Genes	Sequences
IL-6	Forward: 5′-TAGTCCTTCCTACCCCAATTTCC-3′
	Reverse: 5′-TTGGTCCTTAGCCACTCCTTC-3′
MCP1	Forward: 5′- TTAAAAACCTGGATCGGAACCAA-3′
	Reverse: 5′-GCATTAGCTTCAGATTTACGGGT-3′
VCAM-1	Forward: 5′-AGTTGGGGATTCGGTTGTTCT-3′
	Reverse: 5′-CCCCTCATTCCTTACCACCC-3′
Collagen Ⅰ	Forward: 5′-GCTCCTCTTAGGGGCCACT-3′
	Reverse: 5′-ATTGGGGACCCTTAGGCCAT-3′
Collagen Ⅲ	Forward: 5′-CTGTAACATGGAAACTGGGGAAA-3′
	Reverse: 5′-CCATAGCTGAACTGAAAACCACC-3′
Fibronectin	Forward: 5′-TGTGACAACTGCCGTAGACC-3′
	Reverse: 5′-GACCAACTGTCACCATTGAGG-3′
β-actin	Forward: 5′-GGCTGTATTCCCCTCCATCG-3′
	Reverse: 5′-CCAGTTGGTAACAATGCCATGT-3′

IL-6: interleukin-6; MCP1: monocyte chemoattractant protein 1; VCAM-1: vascular cell adhesion molecular 1.

Table 2. Sequences of primers for amplifying differentially expressed genes associated with vascular aging

Genes	Sequences
IGF1	Forward :5′-AAGACTCAGAAGTCCCCGTC-3′
	Reverse :5′-AAAGGATCCTGCGGTGATGT-3′
Adipoq	Forward :5′-CCAAAAGGGCTCAGGATGCTA-3′
	Reverse :5′-AGGGACCAAAGCAGGAGCTA-3′
Hspa1l	Forward: 5′-CAGCACAGGCTCAAAGTTGC-3′
	Reverse :5′-CCGAGGCCTGGGATCTCTA-3′
Hspa2	Forward: 5′-CGCCCCTTGGTCTCCCT-3′
	Reverse :5′-GCAGACATCCTGACTGGTCG-3′
Adcy1	Forward: 5′-AGGCGCAATGGGCTCG-3′
	Reverse :5′-CACCGAGCGTCCTCCAGA-3′
Irs3	Forward: 5′-GCCTGCACTATTAGCAAGCG-3′
	Reverse :5′-TGGGGACTGAAACCCATAGC-3′
β-actin	Forward: 5′-GGCTGTATTCCCCTCCATCG-3′
	Reverse :5′-CCAGTTGGTAACAATGCCATGT-3′

IGF1: insulin like growth factor 1; Adipoq: adiponectin; Adcy1: adenylate cyclase 1; Hspa1l: heat shock protein family A (Hsp70) member 1 like; Hspa2: heat shock protein family A (Hsp70) member 2; Irs3: insulin receptor substrate 3.

Quality control and mapping

Raw data (raw reads) were processed using Trimmomatic (Version 0.36).[18] To obtain clean reads, we removed ploy-N and low-quality reads. All subsequent analyses were based on clean data. Then the clean reads were mapped to the reference genome using hisat2 (Version 2.2.1.0).[17]

Gene expression level and DEGs analysis

Fragments per kilobase per million reads (FPKM)[19] value of each gene was calculated using cufflinks (Version 2.2.1),[20] and the read counts of each gene were obtained by htseq-count (Version 0.9.1).[20] DEGs were identified using the functions estimate SizeFactors of DESeqR package (2012, Version 1.18.0)[21] and nbinomTest.[21] *P* value < 0.05 and fold change >2 or fold change < 0.5 were set as the threshold for significantly differential expression. Fold change represents change in the expression level of the same gene between the Ang II group and the saline group.

GO and KEGG pathway enrichment analysis

Hierarchical cluster analysis of DEGs was performed to explore gene expression pattern. Functional enrichment was analyzed with GO database to identify underlying biological processes of the DEGs. The "biological process," "molecular function," "cellular component" were selected as background databases for testing. The pathways were ranked according to *P* value, and the most significantly impacted pathways were shown on the top. And KEGG database was also applied to reveal the pathways that may be related to the DEGs. GO and KEGG[22] pathway enrichment analysis of DEGs were respectively performed using R software based on the hypergeometric distribution.

Statistical analysis

All statistical analyses were carried out using GraphPad Prism 7.0 software.[23] Quantitative results were expressed as the means ± SD. All experiments were performed at least in triplicate unless otherwise stated. Normally distributed datasets were analyzed with the unpaired Student's *t*-test for two independent groups or paired *t*-test for two dependent groups. *P* value <0.05 was considered statistically significant.

RESULTS

Elevation of BP in Ang II-induced mice

As expected, after 28 days of Ang Ⅱ infusion, compared with the saline-treated mice (n=8), the Ang Ⅱ-infused mice (n=8) showed a markedly elevation in mean systolic BP about 40 mm Hg (t=11.69, P<0.0001, **Figure 1A**), an increase in mean BP about 30 mm Hg (t=7.681, P<0.0001, **Figure 1B**), and an enhancement in mean diastolic BP about 25 mm Hg (t=5.624, P<0.0001, **Figure 1C**). However, no significant difference in heart ratio was found (t=0.8254, P=0.4230, **Figure 1D**). These results demonstrated that Ang Ⅱ can increase BP significantly while not influence heart ratio, which is consistent with the results of the previously published study.[13]

Ang Ⅱ increases elastin degradation and intimal and media thickness of aorta

To validate the phenotype of Ang Ⅱ-induced hypertension, we performed histological analysis with HE and EVG staining. EVG staining demonstrated that the aortic elastin was degraded and disrupted in the Ang Ⅱ-treated group (t=3.000, P=0.0240, **Figure 2A and 2B**). HE staining showed that the intimal and media thickness of the Ang Ⅱ-treated group (225±14.63 μm) increased significantly compared with the saline group (166.5±11.3 μm, t=3.168, P=0.0194; **Figure 2C and 2D**).

Ang-Ⅱ induces vascular inflammation and fibrosis

As we expected, compared with the saline treated group, the expressions of inflammation-associated genes, including IL-6 (t=4.424, P=0.0115, **Fig-**

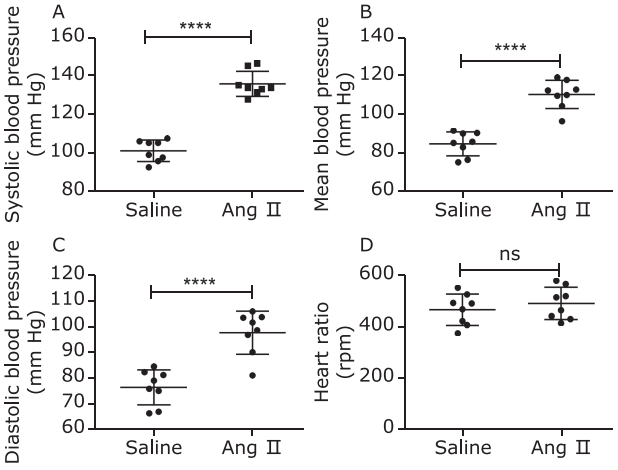

Figure 1. Blood pressure and heart ratio of the mice treated with angiotensin Ⅱ (Ang Ⅱ) *vs*. saline for 28 days. A. systolic blood pressure; B. mean blood pressure; C. diastolic blood pressure; D. heart ratio.
ns: no significance. $^{****}P$<0.0001, n=8 per group.

ure 3A), VCAM-1 (t=3.616, P=0.0111, **Figure 3B**) and MCP-1 (t=3.248, P=0.0117, **Figure 3C**), were all increased after Ang Ⅱ treatment. And the fibrotic markers, collagen Ⅰ (t=2.216, P=0.0468, **Figure 3D**), collagen Ⅲ (t=3.712, P=0.0075, **Figure 3E**) and fibronectin (t=2.588, P=0.0322, **Figure 3F**), were also elevated in the aortas of our hypertensive mouse model. Taken together, these data suggest that Ang Ⅱ can induce vascular inflammation and fibrosis in our hypertensive model, which was in agreement with our previous studies.[24]

DEGs between the Ang Ⅱ-treated group and saline group

Next, we analyzed the DEGs through RNA-seq and found 773 DEGs with P value<0.05 and $|\log_2$fold-change$|$>1, including 599 up-regulated genes and 174 down-regulated genes. Principal component analysis manifested a clear separation of samples with the two treatments (**Figure 4A**). Notably, the Volcano plot showed that Ang Ⅱ treatment had a significant impact on gene expression (**Figure 4B**). The heat map of the genes' expression is presented in **Figure 4C**, suggesting that the samples can be distinguished by these DEGs. These results revealed that Ang II can obviously influence on vascular gene expression.

Functional analysis of DEGs

To further explore the pathways and biological processes associated with Ang Ⅱ treatment, we performed GO and KEGG pathway analysis. GO analysis demonstrated that the DEGs were mostly enriched in these pathways including positive regulation of natural killer cell chemotaxis, extracellular region, chemokine activity, inflammatory response and immune response (**Figure 5A**). Previous studies have showed that persistent immune system activation contributes to the process of various forms of hypertension.[25] Except that, degradation of elastin and remodeling of extracellular matrix are major features in hypertension,[26] which is in accordance with our study.

The top 20 KEGG pathways for DEGs are shown in **Figure 5B**. We found that the aging pathway was enriched unexpectedly (**Figure 5C**). Taken together, these results suggest that, besides inflammatory pathway and extracellular matrix, vascular aging may contribute to hypertension in response to Ang Ⅱ.

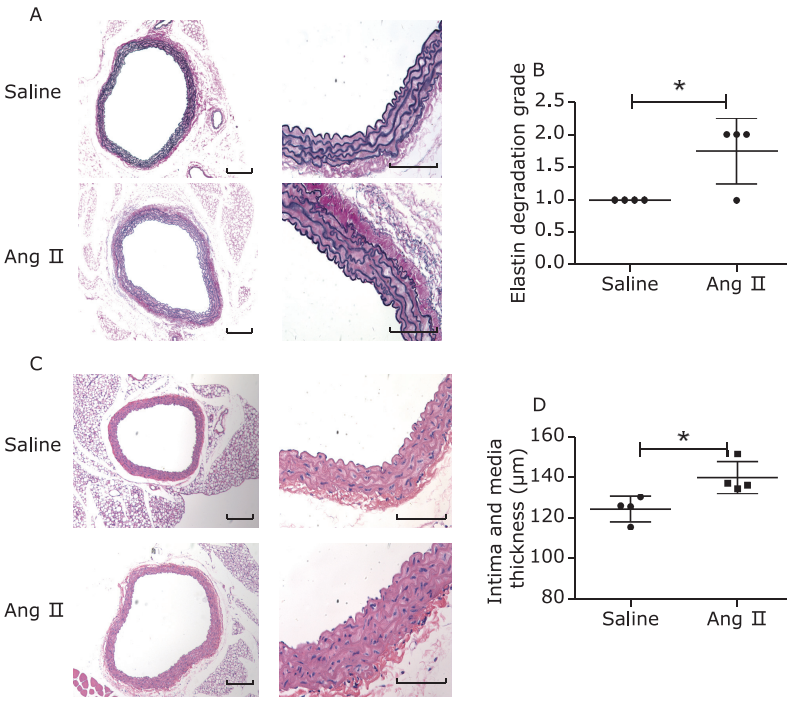

Figure 2. Ang Ⅱ can increase elastin degradation grade and the intima and media thickness of the mice aorta.
Representative images of Elastin van Gieson staining of the aorta from the saline- and Ang Ⅱ-induced mice. B. Semi-quantitative analysis of elastin degradation. C. HE staining of aortic structure in the indicated groups. D. Quantitative analysis of the intima and media thickness.
Bars: (left) 400 μm, (right) 200 μm, n=4. All values are shown as means±SD, $^*P$<0.05.

Validation of DEGs involved in aging

There are 6 DEGs in the aging pathway, including Adcy1, Adipoq, Hspa1l, Hspa2, Irs3 and Igf1. Then we used qRT-PCR to validate the result of RNA-seq data.

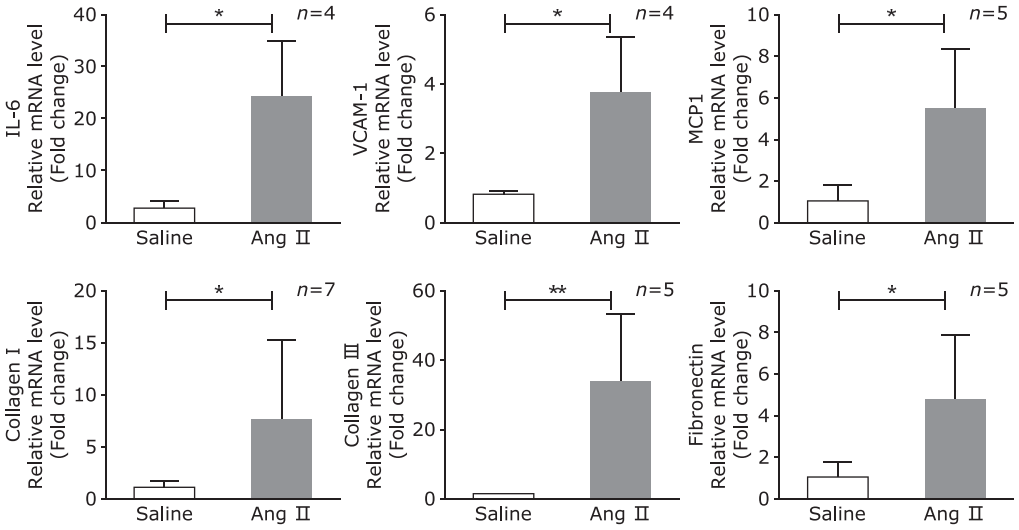

Figure 3. Ang Ⅱ can induce inflammatory and fibrotic gene expressions.

The mRNA expression levels of inflammatory factors IL-6, VCAM-1, MCP1 and fibrotic factors collagen Ⅰ (D), collagen Ⅲ, fibronectin in the aortic tissues were tested by qRT-PCR.

Data are expressed as means±SD. Student's *t*-tests were performed to compare the difference in mRNA expression between the two groups: $^{*}P<0.05$, $^{**}P<0.01$.

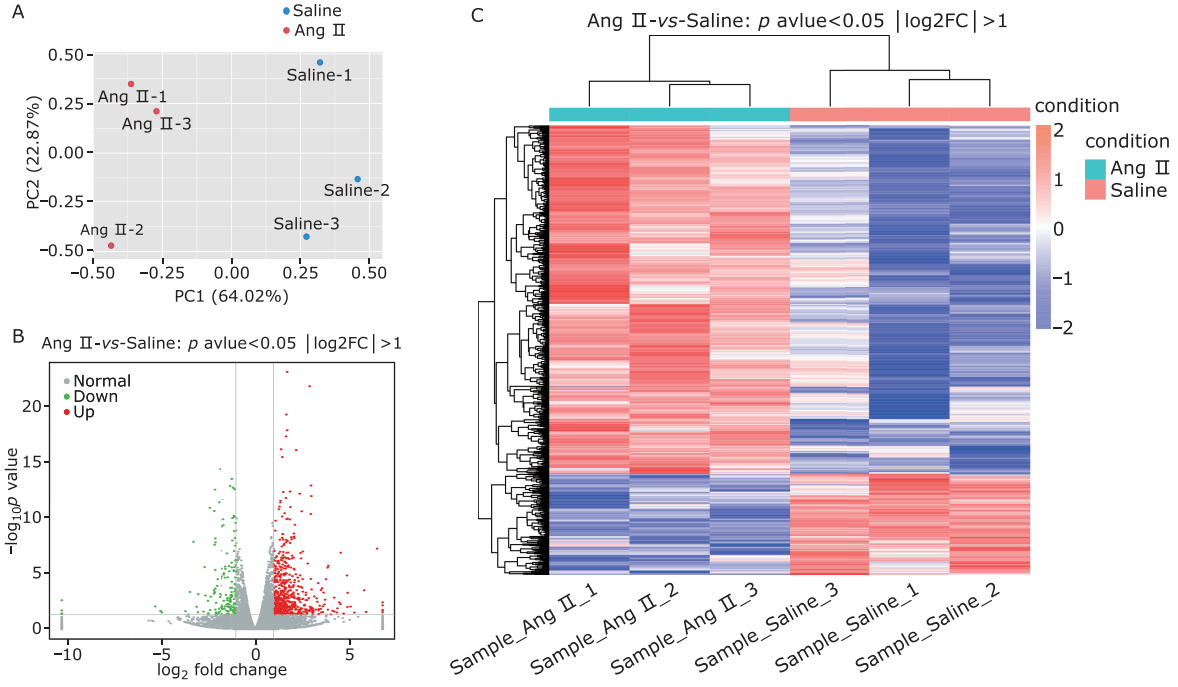

Figure 4. Differential analysis of gene expression.

A. Plot of the 1st and 2nd principal component of sample variations by using principal component analysis. Red dots represent Ang Ⅱ induced samples and green dots denote saline treated samples. B. Volcano plot of gene expression differences between the two groups, up- (red) or down-regulated genes (green) upon Ang Ⅱ treatment. C. Heat map of 773 differentially expressed genes (DEGs) in the Ang Ⅱ-induced samples and controls. X axis represents samples and Y axis represents genes.

Figure 5. Functional analysis and pathway enrichment analysis of DEGs.

A. The top 30 Gene Ontology terms for DEGs are shown in three categories: the 10 terms at the bottom of the list represent molecular function, the medium 10 represent cellular component and the top 10 represent biological process. B. The top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for DEGs. C. KEGG pathway classification of the Ang Ⅱ *vs*. Saline groups. Aging pathway is enriched.

Among the 6 genes, IGF1 (t=3.745, P=0.0038, **Figure 6A**) and Adipoq (t=3.405, P=0.0093, **Figure 6B**) of the Ang Ⅱ-treated group exhibited a significant increase, which is consistent with the RNA-seq findings. No statistical differences in the other four genes between the two groups were found (t=0.3473, P=0.7363; t=0.5733, P=0.5805; t=2.077, P=0.0676; t=2.125, P=0.0712; **Figure 6C-6F**). These results suggest that IGF1 and Adipoq may play an important role in Ang Ⅱ-induced hypertension involved in vascular aging.

DISCUSSION

In the present study, we performed the vascular transcriptome analysis to identify changes related to the vascular damage induced by Ang Ⅱ. Consistently, some genes have been proved to play a causal role in hypertension and other cardiovascular diseases. Interestingly, we found that the vascular aging pathway was associated with Ang Ⅱ-induced hypertension. And IGF1 and Aqipoq, which were involved in the vascular aging from the transcriptome sequence, were up-regulated significantly. Our findings provided new insights into the risk factors of Ang Ⅱ-induced hypertension and vascular injury.

IGF1 signaling pathway plays an important role in postnatal (prepubertal and pubertal) somatic growth.[27] Previous studies have shown IGF1 can cause vascular damage in different ways, including repressing inflammation and cell apoptosis.[28, 29] IGF1 has been shown to induce nitric oxide which mainly locates in smooth muscle cells and endothelial cells, and the mechanism is linked to activate nitric oxide synthetase through phosphorylated AKt.[30] The relationship between IGF1 and hypertension is also studied, but the effects of IGF1 on hypertension remain controversial. And Andronico *et al*.[31] found that IGF1 is highly expressed in patients with hypertension. However, the role of paracrine IGF1, which can protect vasculature and heart, is manifested in recent studies.[32, 33] Besides, it is interesting to note that one of the major nutrition sensing pathways of caloric restriction is IGF1-mediated system, and caloric restriction can induce IGF1 system expression in the heart.[34] So the definitive relationship between IGF1 and hypertension is unclear, especially there is no data about IGF1 and Ang Ⅱ-induced hypertension. In our study, we performed vascular transcriptome sequencing and found that vascular aging pathway was enriched, and IGF1 was significantly up-regulated in this pathway, therefore, suggesting that Ang Ⅱ may cause vascular aging though IGF1.

Adipoq, a hormone secreted from adipocyte, is important to energy homeostasis.[35] However, low level of adipoq in plasma was detected in different diseases, including obesity and type Ⅱ diabetes,[36, 37] which suggest a potential link between adipoq and hypertension. But in our hypertensive model, the adipoq level was significantly up-regulated, which was opposite to the previous finding.[38] So we speculate that adipoq may have a different function in response to Ang Ⅱ . And adipoq is a hormone derived from fat, which fulfills a critical role as a messenger to communicate between adipose tissue and other

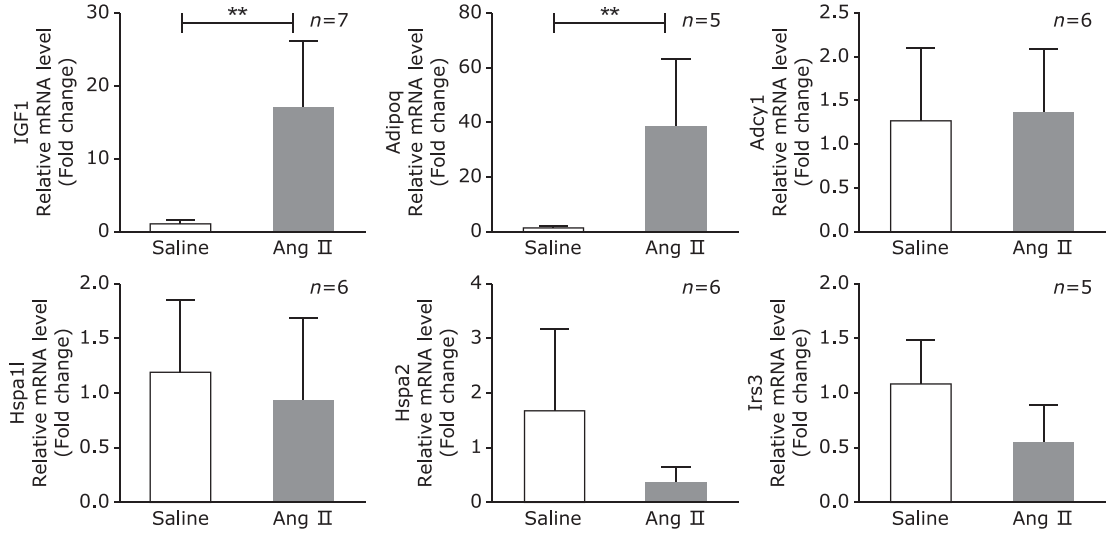

Figure 6. Validation of the DEGs associated with vascular aging by using quantitative real-time PCR. Data are expressed as means±SD. Student's t-tests were used in comparison: $^{**}P<0.01$.

organs.[39] So this reminds us vascular adipose tissue may have a key role in hypertension. Besides, vascular microenvironment homeostasis is important in maintaining vascular function and structure, but the underlying mechanism still needs to be further investigated.

Hypertension is inherently associated with accelerated vascular aging.[40] Early vascular aging, defined as dysfunction of vascular stiffness and remodeling,[41-43] is a key feature of hypertension. And vascular fibrosis and inflammation are the important features for hypertension accompanied by vascular aging.[44] Although significant progress has been achieved in characterizing aging-induced changes in vascular function and phenotype, more innovative strategies based on recent achievements in the biology of aging to improve vascular health span[45] are needed. Vascular aging used to be thought to coincide with physiological aging in the past, but vascular aging can occur at any stage in fact, especially in the process of some diseases.[46] Therefore, Ang Ⅱ may result in vascular aging, albeit more evidence is still needed.

In summary, our study investigated the changes of gene expression in Ang Ⅱ-induced hypertensive mouse model by vascular transcriptome profiling and found that the aging pathway was enriched, especially IGF1 and adipoq significantly increased in our model, which suggested that they may play a key role in vascular aging and hypertension.

Conflict of Interests Statement

The authors declare that they have no competing interest.

REFERENCES

1. Xu F, Zhu J, Sun N, et al. Development and validation of prediction models for hypertension risks in rural Chinese populations. J Glob Health 2019; 9(2):020601. doi: 10.7189/jogh.09.020601.
2. Guyenet PG. The sympathetic control of blood pressure. Nat Rev Neurosci 2006; 7(5):335-46. doi: 10.1038/nrn1902.
3. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell 2001; 104(4):545-56. doi: 10.1016/s0092-8674(01)00241-0.
4. Bruno RM, Di Pilla M, Ancona C, et al. Environmental factors and hypertension. Curr Pharm Des 2017; 23(22):3239-46. doi: 10.2174/1381612823666170321162233.
5. Oparil S, Schmieder RE. New approaches in the treatment of hypertension. Circ Res 2015; 116(6):1074-95. doi: 10.1161/CIRCRESAHA.116.303603.
6. Takimoto-Ohnishi E, Murakami K. Renin-angiotensin system research: from molecules to the whole body. J Physiol Sci 2019; 69(4):581-7. doi: 10.1007/s12576-019-00679-4.
7. Karnik SS, Unal H, Kemp JR, et al. International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin receptors: interpreters of pathophysiological angiotensinergic stimuli [corrected] [published correction appears in Pharmacol Rev. 2015 Oct;67(4):820]. Pharmacol Rev 2015; 67(4):754-819. doi: 10.1124/pr.114.010454.
8. Forrester SJ, Booz GW, Sigmund CD, et al. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. Physiol Rev 2018; 98(3):1627-738. doi: 10.1152/physrev.00038.2017.
9. Sparks MA, Crowley SD, Gurley SB. Classical renin-angiotensin system in kidney physiology. Compr Physiol 2014; 4(3):1201-28. doi: 10.1002/cphy.c130040.
10. Carey RM, Siragy HM. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. Endocr Rev 2003; 24(3):261-71. doi: 10.1210/er.2003-0001.
11. de Gasparo M, Catt KJ, Inagami T. International Union of Pharmacology. XXIII. The angiotensin II receptors. Pharmacol Rev 2000; 52(3):415-72.
12. Wang Z, Gerstein M, Snyder M, et al. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009; 10(1):57-63. doi: 10.1038/nrg2484.
13. Satoh K, Nigro P, Matoba T, et al. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nat Med 2009; 15(6):649-56. doi: 10.1038/nm.1958
14. Xu H, Qing T, Shen Y, et al. RNA-seq analyses the effect of high-salt diet in hypertension. Gene 2018; 677:245-50. doi: 10.1016/j.gene.2018.07.069.
15. Pan X, Shao Y, Wu F, et al. FGF21 prevents angiotensin II-induced hypertension and vascular dysfunction by activation of ACE2/angiotensin-(1-7) axis in mice. Cell Metab 2018; 27(6):1323-37.e5. doi: 10.1016/j.cmet.2018.04.002.
16. Tang X, Chen XF, Wang NY, et al. SIRT2 acts as a cardioprotective deacetylase in pathological cardiac hypertrophy. Circulation 2017; 136(21):2051-67. doi: 10.1161/CIRCULATIONAHA.117.028728.
17. Song Y, Milon B, Ott S, et al. A comparative analysis of library prep approaches for sequencing low input translatome samples. BMC Genomics 2018;

19(1):696. doi: 10.1186/s12864-018-5066-2.
18. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014; 30(15):2114-20. doi: 10.1093/bioinformatics/btu170.
19. Roberts A, Trapnell C, Donaghey J, et al. Improving RNA-Seq expression estimates by correcting for fragment bias. Genome Biol 2011; 12(3):R22. doi: 10.1186/gb-2011-12-3-r22.
20. Trapnell C, Williams BA, Pertea G, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010; 28(5):511-5. doi: 10.1038/nbt.1621.
21. Anders S, Huber W. Differential expression of RNA-Seq data at the gene level–the DESeq package. Heidelberg, Germany: European Molecular Biology Laboratory (EMBL) 2012; 10 f1000research.
22. Kanehisa M, Araki M, Goto S, et al. KEGG for linking genomes to life and the environment. Nucleic Acids Res 2008; 36(Database issue):D480-D4. doi: 10.1093/nar/gkm882.
23. Mitteer DR, Greer BD, Fisher WW, et al. Teaching behavior technicians to create publication-quality, single-case design graphs in graphpad prism 7. J Appl Behav Anal 2018; 51(4):998-1010. doi: 10.1002/jaba.483.
24. Yan YF, Pei JF, Zhang Y, et al. The paraoxonase gene cluster protects against abdominal aortic aneurysm formation. Arterioscler Thromb Vasc Biol 2017; 37(2):291-300. doi: 10.1161/ATVBAHA.116.308684.
25. Lopez Gelston CA, Mitchell BM. Recent advances in immunity and hypertension. Am J Hypertens 2017; 30(7):643-52. doi: 10.1093/ajh/hpx011.
26. Thenappan T, Chan SY, Weir EK. Role of extracellular matrix in the pathogenesis of pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol 2018; 315(5):H1322-H31. doi: 10.1152/ajpheart.00136.2018.
27. Vélez EJ, Perelló M, Azizi S, et al. Recombinant bovine growth hormone (rBGH) enhances somatic growth by regulating the GH-IGF axis in fingerlings of gilthead sea bream (Sparus aurata). Gen Comp Endocrinol 2018; 257:192-202. doi: 10.1016/j.ygcen.2017.06.019.
28. Higashi Y, Sukhanov S, Shai SY, et al. Insulin-like growth factor-1 receptor deficiency in macrophages accelerates atherosclerosis and induces an unstable plaque phenotype in apolipoprotein E-deficient mice. Circulation 2016; 133(23):2263-78. doi: 10.1161/CIRCULATIONAHA.116.021805.
29. Li Y, Higashi Y, Itabe H, et al. Insulin-like growth factor-1 receptor activation inhibits oxidized LDL-induced cytochrome C release and apoptosis *via* the phosphatidylinositol 3 kinase/Akt signaling pathway. Arterioscler Thromb Vasc Biol 2003; 23(12):2178-84. doi: 10.1161/01.ATV.0000099788.31333.DB
30. Burgos JI, Yeves AM, Barrena JP, et al. Nitric oxide and CaMKII: critical steps in the cardiac contractile response to IGF-1 and swim training. J Mol Cell Cardiol 2017; 112:16-26. doi: 10.1016/j.yjmcc.2017.08.014.
31. Andronico G, Mangano MT, Nardi E, et al. Insulin-like growth factor 1 and sodium-lithium countertransport in essential hypertension and in hypertensive left ventricular hypertrophy. J Hypertens 1993; 11(10):1097-101. doi: 10.1097/00004872-199310000-00014.
32. Vinciguerra M, Santini MP, Claycomb WC, et al. Local IGF-1 isoform protects cardiomyocytes from hypertrophic and oxidative stresses *via* SirT1 activity. Aging (Albany NY) 2009; 2(1):43-62. doi: 10.18632/aging.100107.
33. Vinciguerra M, Santini MP, Martinez C, et al. mIGF-1/JNK1/SirT1 signaling confers protection against oxidative stress in the heart. Aging Cell 2012; 11(1):139-49. doi: 10.1111/j.1474-9726.2011.00766.x
34. Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, et al. Caloric restriction and growth hormone receptor knockout: effects on expression of genes involved in insulin action in the heart. Exp Gerontol 2006; 41(4):417-29. doi: 10.1016/j.exger.2006.01.009.
35. Zhu W, Cheng KK, Vanhoutte PM, et al. Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. Clin Sci (Lond) 2008; 114(5):361-74. doi: 10.1042/CS20070347.
36. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000; 20(6):1595-9. doi: 10.1161/01.atv.20.6.1595.
37. Pischon T, Girman CJ, Hotamisligil GS, et al. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004; 291(14):1730-7. doi: 10.1001/jama.291.14.1730.
38. Iwashima Y, Katsuya T, Ishikawa K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension 2004; 43(6):1318-23. doi: 10.1161/01.HYP.0000129281.03801.4b.
39. Wang ZV, Scherer PE. Adiponectin, the past two

decades. J Mol Cell Biol 2016; 8(2):93-100. doi: 10.1093/jmcb/mjw011.

40. Lakatta EG. Central arterial aging and the epidemic of systolic hypertension and atherosclerosis. J Am Soc Hypertens 2007; 1(5):302-40. doi: 10.1016/j.jash.2007.05.001.
41. Cunha PG, Boutouyrie P, Nilsson PM, et al. Early vascular ageing (EVA): definitions and clinical applicability. Curr Hypertens Rev 2017; 13(1):8-15. doi: 10.2174/1573402113666170413094319.
42. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. J Hypertens 2008; 26(6):1049-57. doi: 10.1097/HJH.0b013e3282f82c3e.
43. Nilsson PM, Boutouyrie P, Laurent S. Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension 2009; 54(1):3-10. doi: 10.1161/HYPERTENSIONAHA.109.129114.
44. Guzik TJ, Skiba DS, Touyz RM, et al. The role of infiltrating immune cells in dysfunctional adipose tissue. Cardiovasc Res 2017; 113(9):1009-23. doi: 10.1093/cvr/cvx108.
45. Ungvari Z, Tarantini S, Donato AJ, et al. Mechanisms of vascular aging. Circ Res 2018; 123(7):849-67. doi: 10.1161/CIRCRESAHA.118.311378.
46. Jin J, Liu Y, Huang L, et al. Advances in epigenetic regulation of vascular aging. Rev Cardiovasc Med 2019; 20(1):19-25. doi: 10.31083/j.rcm.2019.01.3189.