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Original Article

Association of the Serpine1 with the Obesity Paradox in Ischemic Heart Failure

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Abstract

Background: Extreme obesity pathology is with a risk factor for heart failure (HF), whereas the obesity paradox in HF shows that obese subjects had a good prognosis. The mechanism underlying the obesity paradox in HF prognosis is unclear till now. We aimed to provide evidence for the molecular mechanisms of the obesity paradox in HF.

Methods: Differentially expressed genes (DEGs) in ischemic HF samples were identified in the GSE57338 and GSE5406 datasets. Weighted gene co-expression network analysis (WGCNA) modules and a protein-protein interaction network (PPI) were constructed. HF-associated DEGs and pathways were screened in the Comparative Toxicogenomics Database (CTD). The expression of hub genes in adipose tissues from obese patients and LV samples from HF patients were validated in microarray datasets.

Results: Three HF-associated WGCNA modules were identified and DEGs were associated with the 'hsa04115: *p53* signaling pathway'. *SERPINE1* was the only common gene between DEGs and HF-associated genes in the CTD database. The *SERPINE1* gene was downregulated in the white adipose tissues compared with brown adipose tissues (P = 3.90e-03) and was upregulated in the omental adipose tissues from obese patients compared with lean subjects (P = 3.85e-02).

Conclusion: The downregulation of *SERPINE1* expression might be responsible for the obesity paradox in HF via interacting with *ESR1*.

Keywords: Microarray; Plasminogen activator inhibitor 1; Heart failure; Obesity

Introduction

Heart failure (HF), characterized by the failing heart unable to pump blood sufficiently to meet the needs of the body's tissues, is a global public health issue (1). The one-year and five-year mortalities of HF are approximately 35% and 50%, respectively (2).The one-year hospital readmission rate is as high as 65% (2, 3). The early diagnosis of HF is crucial for improving treatment success (4). The clinical diagnosis of HF depends on myocardial function indexes and independent circulating blood predictors, including brain natriuretic peptide (BNP) and N-terminal-proBNP

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(5-7). These predictors have high sensitivity and accuracy in the diagnosis of HF.

Evidence shows that hypertension, obesity, or diabetes increase the risk of HF (8,9). Obesity is an epidemic problem in some countries and confers to the increasing morbidity of HF (7). A validated clinical phenomenon is the obesity paradox in patients with cardiovascular diseases (CVDs) (10). The obesity paradox in HF indicates that patients with lower body mass index (BMI) scores have higher mortality than patients with higher BMI (9,11,12). However, the molecular mechanisms underlying the obesity paradox in HF are not fully understood.

The serpin family E member 1 (*SERPINE1*) encodes a plasminogen activator inhibitor (PAI)-1 that associates with diabetes, obesity, and adiposity changes (13,14). Our present study showed that the downregulation of *SERPINE1* might be responsible for the obesity paradox in HF. The analysis of *SERPINE1*-mediated biological functions might give novel insights into the obesity paradox in HF.

Materials and Methods

Affymetrix microarray data

Gene expression microarray datasets GSE57338 and GSE5406 were obtained from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The searching terms were 'ischemic', 'heart failure' and 'Homo sapiens'. The inclusion criteria were: 1) genome-wide expression profile; 2) left ventricle (LV) myocardium from ischemic HF and normal controls; and 3) ≥ 100 samples. The GSE57338 and GSE5406 datasets were on the Affymetrix GPL11532 and GPL96 platforms, respectively. Gene expression profiles were obtained from LV myocardium from patients with ischemic HF (95 in GSE57338 and 108 in GSE5406) and non-failing LV control samples (unused donor hearts with normal LV functions; 136 in GSE57338 and 16 in GSE5406).

Data process and gene expression profiling

Standard data processing was performed using the Oligo software package (http://www.bioconductor.org/packages/release/bioc/html/oligo.html). The differentially expressed genes (DEGs) between ischemic HF and control samples in the two datasets were separately identified using the thresholds of $|\log2(\text{fold change, FC})| \geq 0.5$ and false discovery rate (FDR) < 0.05. The pheatmap (version 1.0.8, https://cran.r-project.org/package=pheatmap) was used to perform the hierarchical clustering for DEGs. Only DEGs common to the two datasets were used for further analysis.

Weighted gene co-expression network analysis (WGCNA)

WGCNA informs gene co-expression networks and identifies gene clusters or modules with consensus expression profiles (15). The co-expression networks of genes in the two datasets were identified using WGCNA integrated algorithm (version 1.61; https://cran.r-project.org/web/packages/WGCNA/index.html). Gene connectivity between the two datasets was calculated and genes with correlation coefficients ≥ 0.6 were used for WGCNA. The criteria were: cut height = 0.99 and ≥ 80 genes. GSE57338 and GSE5406 datasets were regarded as the training and validation dataset, respectively. WGCNA modules that were significantly associated with the clinical status of ischemic HF were identified with the thresholds of $P \leq 0.05$ and Z score > 5. DEGs in the WGCNA modules were identified using the hypergeometric algorithm of $f(k,N,M,n) = C(k,M)*C(n-k,N-M)/C(n,N)$ (16), where N and M represent total gene number and the number of genes in each module, respectively; n is the number of the common DEGs between the two datasets; k is the number of DEGs in each module. The common DEGs that had fold enrichment fold > 1 and $p < 0.05$ were retained.

Enrichment analysis

The functional properties of genes could be represented by Gene Ontology (GO) biological pro-

cesses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The GO biological processes and KEGG pathways associated with DEGs in WGCNA modules were identified from the DAVID (version 6.8, https://david.ncifcrf.gov/). The item with $p < 0.05$ was screened out and regarded as a significantly enrich item.

Construction of protein-protein interaction (PPI) network

The interaction pairs among the DEGs in the WGCNA modules were retrieved from STRING (version 10.0; http://string-db.org/). We constructed the protein-protein interaction (PPI) network using the Cytoscape software (version 3.6.1; http://www.cytoscape.org/). DEGs in the PPI network were were used enrichment analysis.

Screening of the hub candidate genes and pathways

Before identifying hub genes in HF, the pathways and genes associated with ischemic HF were identified from the Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org/) using searching items of 'ischemic' AND 'heart failure". Common KEGG pathways overlapped between the CTD and DAVID databases were screened out and regarded as the hub pathways. Accordingly, common genes between the CTD database and DEGs in hub pathways were regarded as hub genes.

Validation of the expression profiles of hub genes in ischemic HF samples

The expression profiling of the *SERPINE1* gene was validated in datasets GSE26887, GSE76701, and GSE59867. GSE59867 (GPL6244 platform) consisted of 436 peripheral blood samples collected from 46 healthy controls and 390 samples collected from 130 patients with HF at 1, 4-5, and 30 days post myocardial infarction. GSE26887 (GPL6244 platform) included 24 LV cardiac biopsies collected from controls (n=5) patients with (n=7) and without (n=12) diabetic HF. GSE76701 (GPL570 platform) consisted of eight LV samples isolated from patients with ischemic HF (n=4) and ischemic patients (n=4) with non-failing hearts.

Expression profiles of the hub genes in adipose tissue

To investigate the potential association of *SERPINE1* expression with the obesity paradox, the expression profiles of the *SERPINE1* gene in adipose tissues were analyzed. Gene expression profiling datasets GSE113764, GSE15524, and GSE20950, including adipose tissues collected from individuals with normal weight or obese, were downloaded from the GEO database. GSE113764 (GPL16791, platform) included 15 pairs of brown and white adipose tissues from healthy subjects). GSE15524 (GPL4044 platform) contained 14 pairs of subcutaneous and omental abdominal adipose tissues from five non-obese and 11 obese subjects. The GSE20950 dataset (GPL570 platform) included 19 subcutaneous and 20 omental abdominal adipose tissues from insulin-sensitive (n=20) and insulin-resistant (n=19) patients. The expression profiling of the estrogen receptor 1 (*ESR1*) gene in the above datasets was also validated.

Statistical analysis

The differences in the expression levels of the *ESR1* and *SERPINE1* genes between genes between groups were compared using the non-parametric Mann-Whitney test. All the statistical analyses were performed in the SPSS software (version 22.0; IBM Corporation, Somers, NY, USA). $P < 0.05$ was considered as significant difference.

Results

Gene expression profiling

After normalization, 1926 and 1448 DEGs were identified in the GSE57338 and GSE5406 datasets, respectively (Fig. 1A and B), including 1102 common DEGs (Fig. 1C). Hierarchical clustering analysis showed that the distinct expression profiles of DEGs between the ischemic HF and controls samples (Fig. 1D and E).

1. cotransporter 2 inhibitors: proposed role

of ketone utilization. *Heart Fail Rev,* 25:403–408.

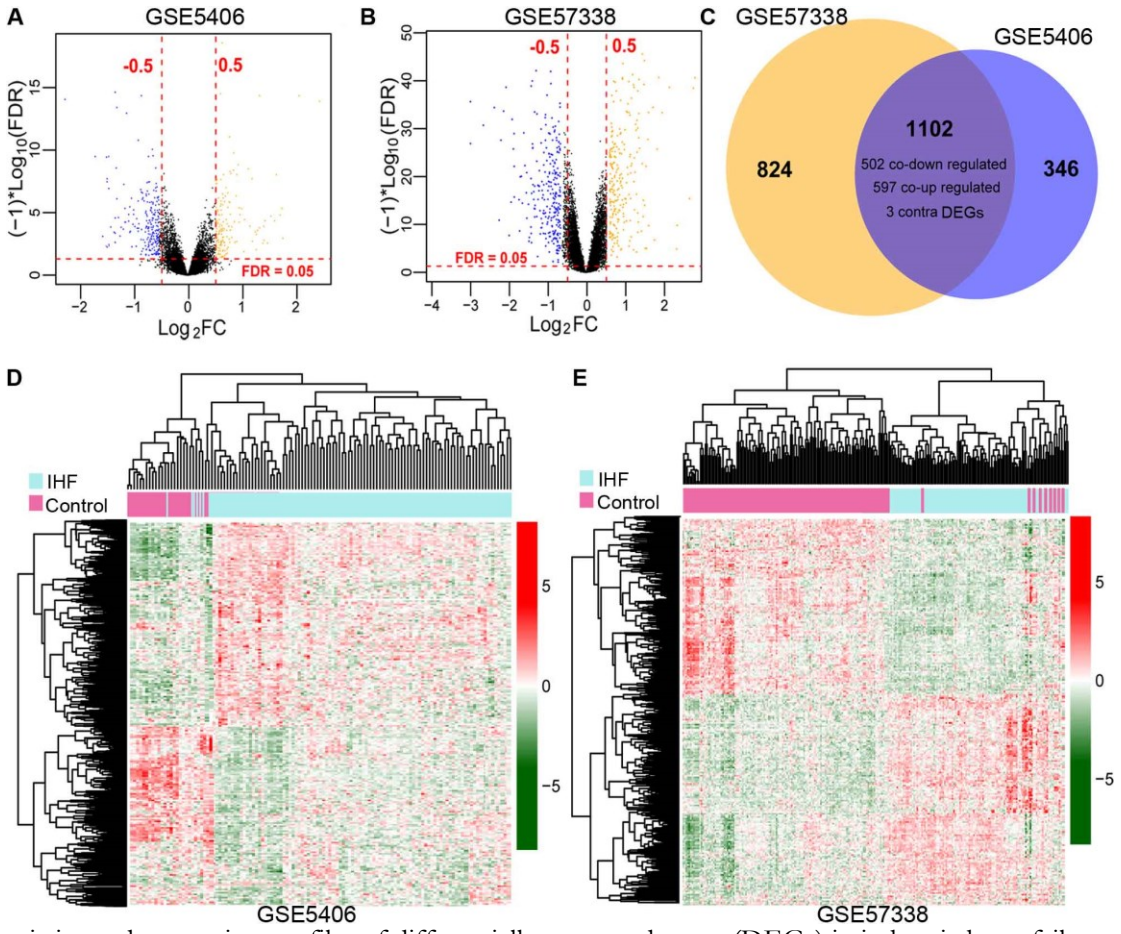

Fig. 1: Statistics and expression profiles of differentially expressed genes (DEGs) in ischemic heart failure. A and B, the volcano plots of DEGs between ischemic heart failure (IHF) samples and control samples in the GSE5406 and GSE57338 datasets, respectively. FDR, fold discovery rate. FC, fold change. C, the Venn diagram indicating the common DEGs between the two datasets. D and E, the sample clustering heatmaps of the common DEGs

WGCNA modules analysis

The genes in the two datasets had significant correlation (Cor = 0.58, $P < .27e\text{-}205$, Fig. 2A) and high connectivity (Cor = 0.18, $P = 1.92e\text{-}86$, Fig. 2B). Genes with correlation coefficients ≥0.6 (n = 3683) between the two datasets were retained and used to identify the WGCNA modules. The adjacency matrix soft-thresholding power = 12 when the correlation coefficient square (r^2) = 0.9 and mean connectivity = 1 (Fig. 2C and D). According to these criteria (soft-thresholding power = 12, gene number ≥ 80, and cutHeight = 0.99), nine WGCNA modules were obtained in the two datasets (Fig. 3A). These modules were significantly associated with ischemic HF clinic traits ($P < 0.05$, Fig. 3B). Three WGCNA modules (brown, green, and red) had stable correlation with HF traits (Z score > 5, enrichment fold ≥ 1, and $P \leq 0.05$; Table 1). The three modules contained 275 DEGs.

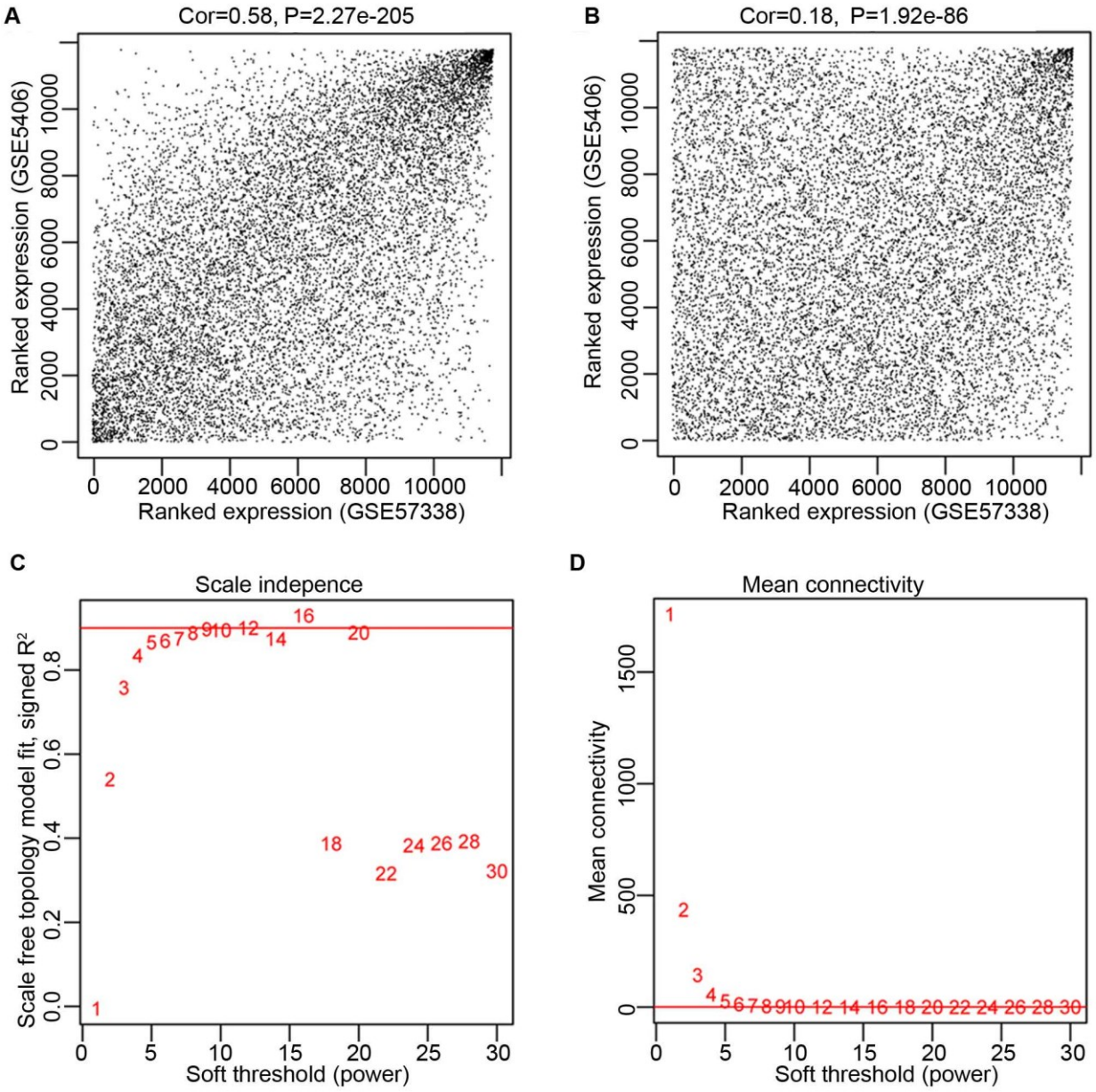

Fig. 2: The analysis of topology parameters of weighted gene co-expression network analysis (WGCNA). A and B, the scatter diagrams showing the correlation and connection of the gene expression levels between the two datasets, respectively. C and D, the adjacency matrix analysis in the training dataset (GSE57338). Red line in Fig. C indicates correlation coefficient square (r^2) = 0.9. Red line in Fig. D reveals connectivity = 1. Red numbers in both boxes are the soft-thresholding powers

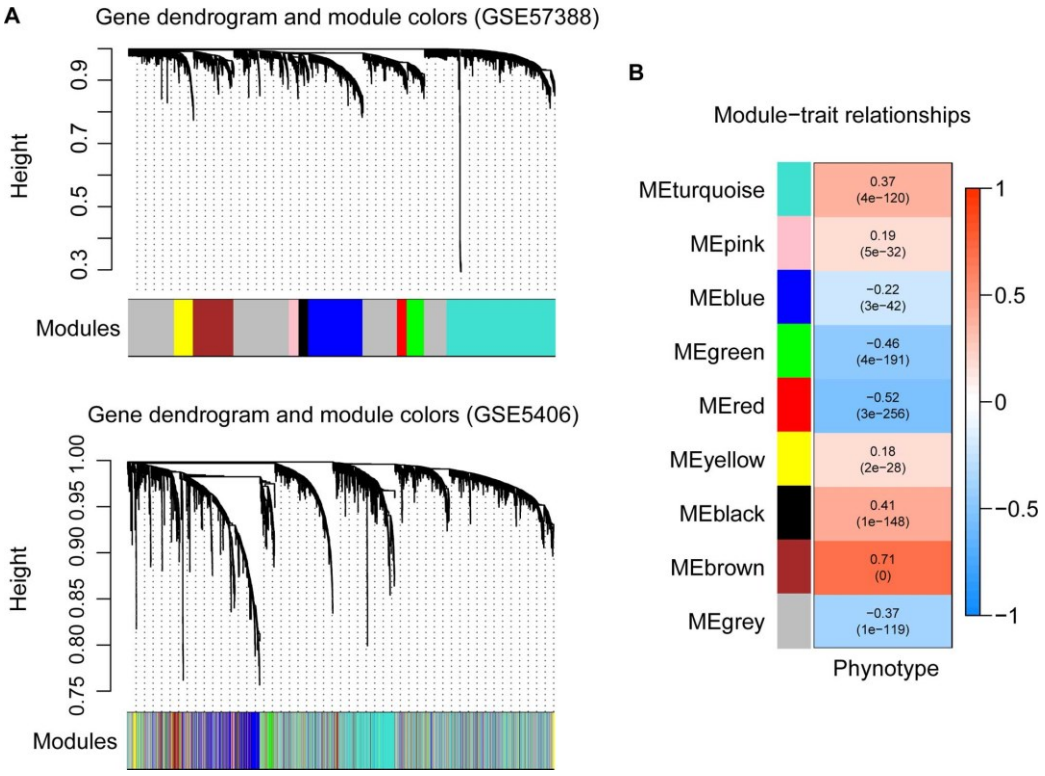

Fig. 3: The identification of weighted gene co-expression network analysis (WGCNA) modules. A, identification of WGCNA modules in GSE57338 (the training dataset; upper) and GSE5406 (the validation dataset; bottom). B, the heatmap indicating the correlation of WGCNA modules with the clinic traits in patients with ischemic heart failure (HF). The red and blue boxes note the positive and negative correlation, respectively

Table 1: Preservation traits of modules and identification of significant stable modules

Module Color	Module Size	Preservation			DEG No.	Enrichment fold (95%CI)	Phyper
		Z-score	Cor.	P value			
black	83	3.956	0.08	0.46	16	1.116[0.606-1.937]	0.668
blue	469	27.288	0.51	2.00×10^{-32}	66	0.815[0.612-1.072]	0.152
*brown	349	8.494	0.35	1.70×10^{-11}	168	2.786[2.261-3.426]	2.2×10^{-16}
*green	149	12.096	0.32	6.90×10^{-5}	61	2.370[1.709-3.255]	2.29×10^{-7}
grey	1374	2.694	0.18	1.80×10^{-11}	114	0.481[0.386-0.593]	2.78×10^{-13}
pink	82	11.455	0.48	5.00×10^{-6}	5	0.353[0.111-0.863]	1.39×10^{-2}
*red	84	12.973	0.46	1.10×10^{-5}	46	3.170[2.141-4.644]	8.57×10^{-9}
turquoise	930	6.841	0.3	8.50×10^{-21}	147	0.915[0.749-1.113]	0.38
yellow	163	3.755	−0.09	0.26	13	0.462[0.239-0.819]	5.87×10^{-3}

DEG, differentially expressed genes. ***** stable WGCNA modules with enrichment fold >1 for all DEGs (lower limit of 95% CI ≥1) and P_{hyper} <0.05. Module size, the number of total eigengenes

Enrichment analysis for the significant stable modules

Functional enrichment analysis showed that the 275 DEGs were significantly involved in 26 GO biological processes, including 'GO:0008284: positive regulation of cell proliferation', 'GO:0001568~blood vessel development', and 'GO:0006954: inflammatory response' ($P < 0.05$; Fig. 4A), and eight KEGG pathways, including 'hsa04614: Renin-angiotensin system', 'hsa04115:

p53 signaling pathway', and 'hsa04350: TGF-beta signaling pathway' ($P < 0.05$; Fig. 4B).

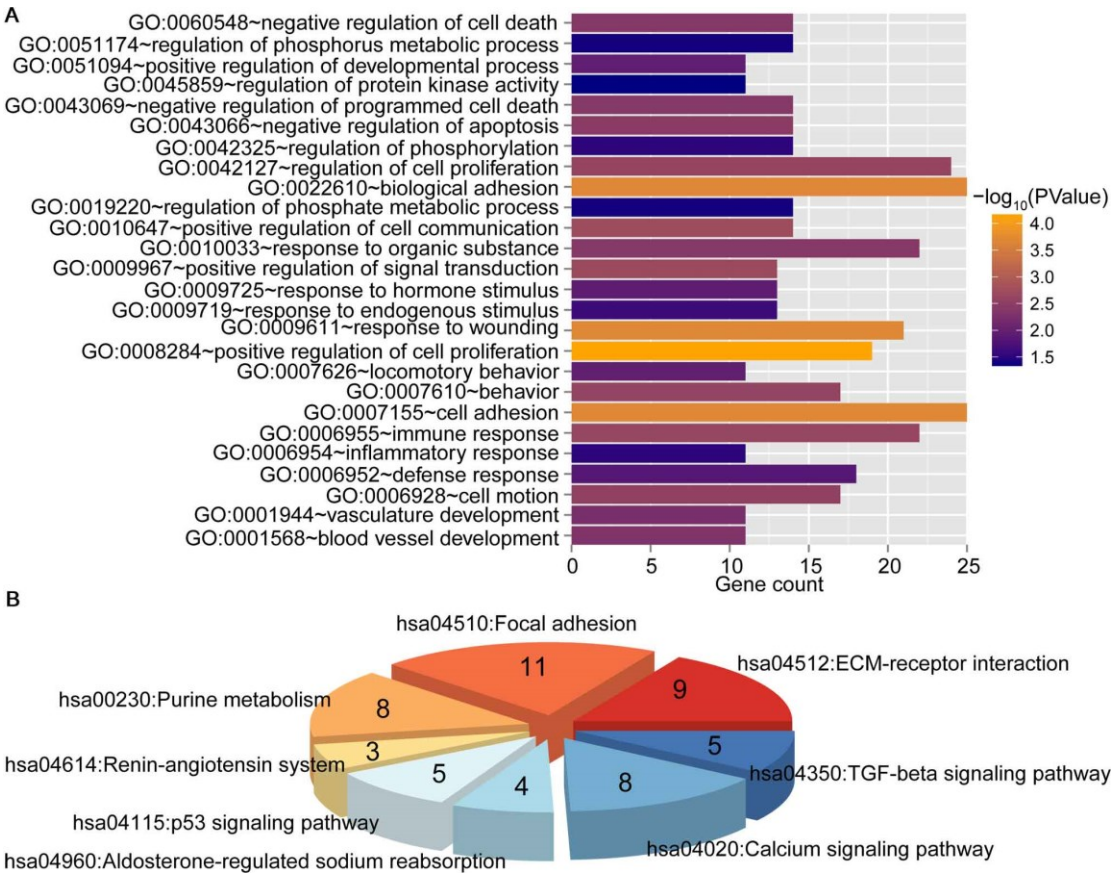

Fig. 4: Gene set enrichment for the differentially expressed genes (DEGs) in three significant stable modules. A, the 26 Gene Ontology (GO) biological processes associated with DEGs. Bar length reveals gene count in each biological process. B, the pie chart of the pathways (n = 8) that associate with DEGs. The number of genes that are enriched in each pathway is presented

PPI network construction

The PPI network consisted of 196 nodes (82 downregulated and 114 upregulated DEGs) and 526 edges (interactions; Fig. 5). *ESR1* and *SERPINE1* had relatively higher interaction degrees of 21 and 15, respectively. Six significant KEGG pathways were associated with DEGs in the PPI network ($P<0.05$; Table 2). Downregulated DEGs including integrin subunit alpha 3 (*ITGA3*), *ITGA5*, and thrombospondin 1 (*THBS1*) were enriched in the 'hsa04512: ECM-receptor interaction'. These genes interplayed with *SERPINE1*.

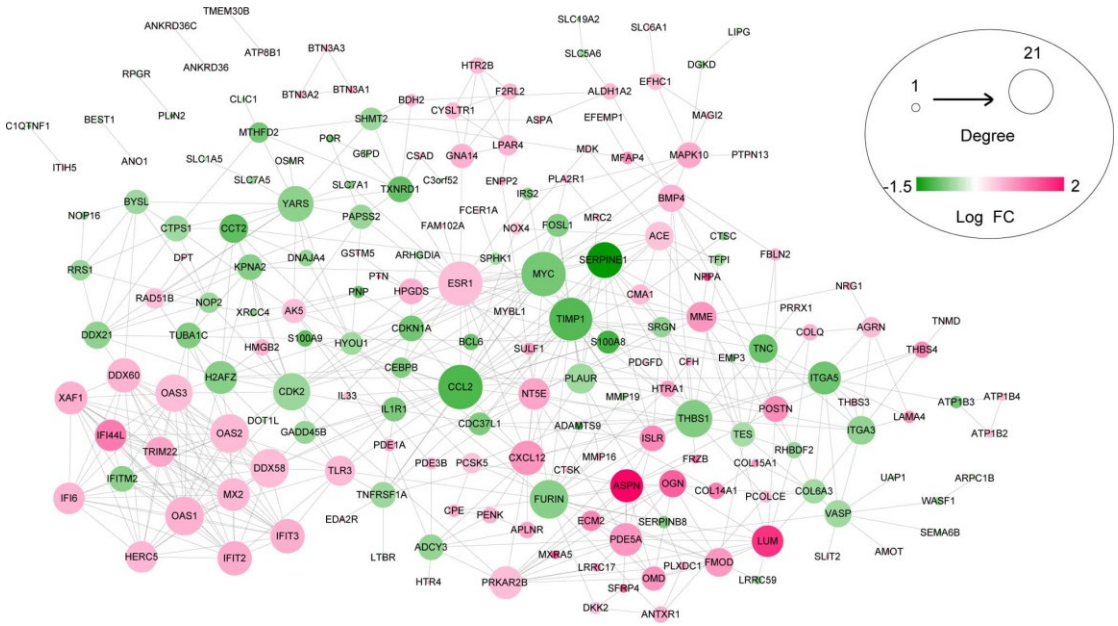

Fig. 5: The protein-protein interaction network of the differentially expressed genes (DEGs). Red and blue color represents upregulation and downregulation, respectively. Nodes are gene products. The higher the interaction degree, the larger the node is

Table 2: KEGG pathways obviously associated with differentially expressed genes in the protein-protein interaction network

Term	Count	P Value	DEGs
hsa04512:ECM-receptor interaction	9	8.02×10^{-5}	LAMA4, ITGA5, ITGA3, THBS1, THBS3, THBS4, et al
hsa04510:Focal adhesion	11	2.09×10^{-3}	LAMA4, ITGA5, ITGA3, MAPK10, THBS1, THBS3, et al
hsa00230:Purine metabolism	8	1.52×10^{-3}	PDE5A, PDE3B, AK5, NT5E, PAPSS2, PNP, et al
*hsa04115:p53 signaling pathway	5	2.84×10^{-2}	CDKN1A, SERPINE1, GADD45B, THBS1, CDK2
hsa04960:Aldosterone-regulated sodium reabsorption	4	3.23×10^{-2}	IRS2, ATP1B3, ATP1B2, ATP1B4
hsa04614:Renin-angiotensin system	3	3.33×10^{-2}	ACE, MME, CMA1

*the key pathway that is identified in the Comparative Toxicogenomics Database and the Database for Annotation, Visualization and Integrated Discovery (DAVID)

Identification of hub pathway and DEG in ischemic HF

A total of 243 and 204 KEGG pathways and 176 and 107 genes in the CTD database were associated with 'ischemic' and 'heart failure', respectively, The 'hsa04115:p53 signaling pathway' was the only common KEGG pathway between the CTD and DAVID databases. The *SERPINE1* gene was the only overlapped gene between DEGs and ischemic HF-related genes in CTD. Accordingly, the 'hsa04115:p53 signaling pathway' and *SERPINE1* gene was regarded as the hub pathway and gene in ischemic HF, respectively.

Validation of the SERPINE1 gene expression

The expression profiling of the *SERPINE1* gene in the datasets is shown in Fig. 6. The significant downregulation of the *SERPINE1* gene was identified in the HF samples versus controls in the GSE5406 (*P* = 1.57e-03) and GSE57338 dataset (*P* = 7.28e-11). Besides, it was significantly downregulated in the LV from non-diabetic HF patients compared with controls (*P* =3.90e-03), but not in the diabetic HF patients in the GSE26887 dataset (*P* = 7.32e-02) and not in the GSE76701 dataset (*P* = 0.4.86e-01). Also, there was no difference in the *SERPINE1* expression profile in the peripheral blood samples in the GSE59867 dataset (Fig. 6). These findings showed that the expression profiling of the *SERPINE1* gene was disease and site-specific.

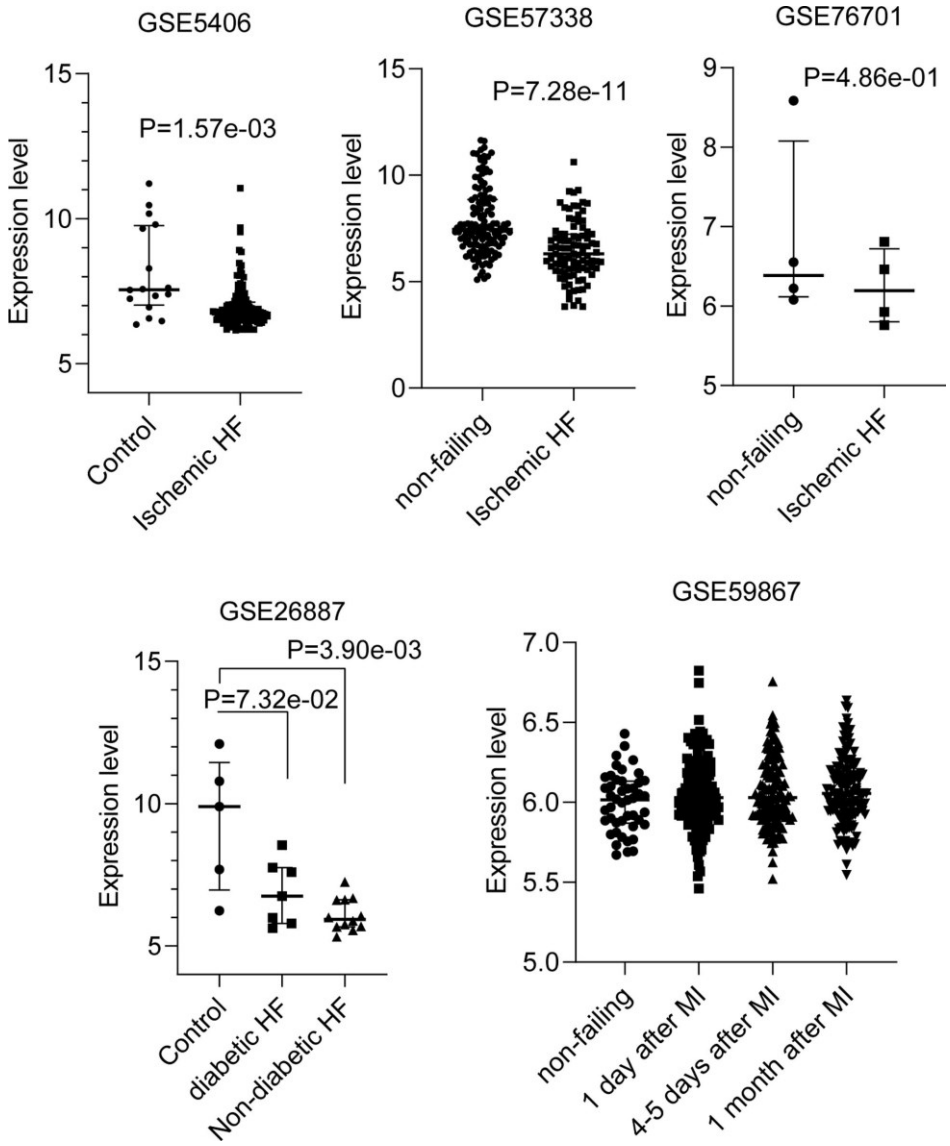

Fig. 6: The expression profiling of the SERPINE1 gene in the heart failure samples and controls in microarray datasets. All the datasets are available at the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The difference between the heart failure samples and control samples (blood samples in the GSE59867 dataset, and left ventricle samples in the other datasets) was identified using the non-parametric Mann-Whitney test. P < 0.05 was considered as a significant difference

Expression profiles of the SERPINE1 and ESR1 genes in adipose tissues

The expression profiles of the SERPINE1 and ESR1 genes changed in adipose tissues from subjects with different conditions (Fig. 7). The *SERPINE1* gene had a lower expression level in the white adipose tissues compared with the brown adipose tissues isolated from healthy subjects in the GSE113764 dataset (P = 3.90e-03; Fig. 7A) and a higher expression level in the omental abdominal adipose tissues collected from the obese subjects compared with the lean subjects (GSE15524, P = 3.85e-02; Fig. 7B). The *ESR1* gene significantly elevated in the white adipose tissues compared with the brown adipose tissues in the GSE113764 dataset (P = 4.06e-01; Fig. 7A). Also, we found that the *SERPINE1* and *ESR1* genes were both upregulated in the omental and subcutaneous abdominal adipose tissues from patients with insulin-sensitive obesity compared with the patients with insulin-resistant obesity (GSE20950; Fig. 7C).

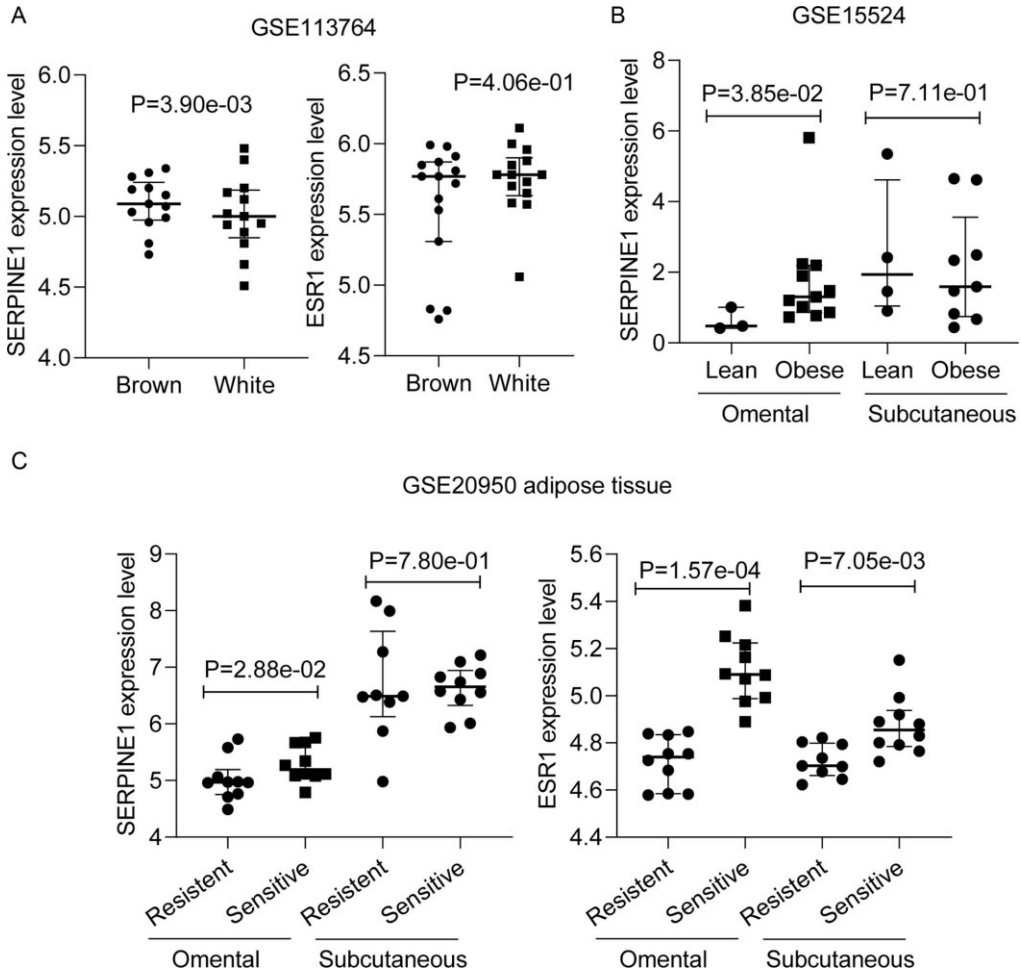

Fig. 7: Expression profiles of the *SERPINE* and *ESR1* genes in adipose tissues. A, the expression profiles of the *SERPINE1* and *ESR1* genes in the brown and white adipose tissues collected from obese patients without disease. B, the expression profiles of the *SERPINE1* gene in subcutaneous and omental abdominal adipose tissue from non-obese and obese subjects. C, the expression profiles of the *SERPINE1* and *ESR1* genes in omental and subcutaneous adipose tissue samples obtained from insulin-sensitive obese and insulin-resistant obese patients undergoing gastric bypass surgery. The differences between groups were analyzed using the non-parametric Mann-Whitney test

Discussion

The contribution of obesity to HF is undoubted. However, the association of obesity with the prognosis of HF patients is not well documented till now (12,17). Our present study identified a downregulated *SERPINE1* gene, a PAI-1 protein-coding gene related to obesity was associated with ischemic HF through the KEGG pathway 'hsa04115: p53 signaling pathway'. Besides, *SERPINE1* had direct interaction with genes enriched with the pathways 'hsa04614: Renin-angiotensin system' and 'hsa04512: ECM-receptor interaction'.

PAI-1 is primarily produced in human adipose tissue (18) and could be produced by endothelial cells and hepatocytes *in vitro* (19). The *SERPINE1* gene was significantly associated with total fat mass, body weight, insulin resistance, cholesterol and triglycerides levels in patients with metabolic syndrome (13,19) . PAI-1 inhibits tissue-type plasminogen activator (tPA) (20), the PAI-1 level was responsive to hypoxia-inducible factor (HIF)-1α (21,22). *SERPINE1*/PAI-1 overexpression induces the accumulation of extracellular matrix (ECM), promotes vascular remodeling, vascular fibrosis, and therefore promoting hypertension, HF, and other CVDs (23-25). By contrast, the knockout or inhibition of the PAI-1-encoding gene prevented vascular fibrosis and hypertension in mice (23). These studies suggested that PAI-1/*SERPINE1* elevation contributed to the pathogenesis of CVDs, including HF, while the downregulation of them might be a protective effect against these diseases.

The *SERPINE1* gene was downregulated in the LV myocardial tissues from patients with ischemic HF compared with healthy donors. We then paid close attention to the DEGs that interacted with *SERPINE1* in the PPI network and found that the upregulated *ESR1* gene interplayed with *SERPINE1* directly or indirectly. ESR1 is a sex hormone receptor that mediates estrogen responses coupling with membrane-bound G-protein (26). The inhibition of G-protein coupled estrogen receptor 1 (GPER) led to LV dysfunction in mouse (27). *SERPINE1* is a target of conjugated estrogen Premarin (14). Previous clinical studies showed that estrogen treatment in postmenopausal women decreased *SERPINE1* expression and PAI-1 (28). We speculated that the upregulated *ESR1* was associated with or responsible for the downregulation of *SERPINE1*.

There is one thing that we should keep in mind is that obese patients have more adipose tissues and white adipose tissue in particular. White adipose tissue contains a high level of ESRs (29,30) and therefore obese patients benefit more from estrogen drug treatment. GPER mainly expresses in adipocytes (29). Accordingly, we assumed that obese HF patients might have a higher utilization rate of estrogens due to the higher levels of ESRs and GPER in adipose tissues. Moreover, the increased GPER activity might inhibit *SERPINE1* via direct interaction, decrease HF severity, and benefit prognosis. Also, insulin-resistance is an independent risk factor for LV dysfunction in individuals with normal weight but no in obese individuals (31) and the prognosis of HF patients with insulin resistance was relatively poor compared with patients who were insulin-sensitive (32,33). Our present study showed that the *SERPINE1* and *ESR1* gene was downregulated and upregulated in white adipose tissues compared with the brown adipose tissues, respectively. However, the elevated expression levels of the *SERPINE1* and *ESR1* genes in the adipose tissues from insulin-sensitive obese patients compared with insulin-resistant obese patients might show that obese HF patients had a good response to treatment. Accordingly, we suggested that the downregulation of *SERPINE1* might be responsible for the obesity paradox in HF.

The limitation existed in this study was the lack of patients' demographic characteristics including BMI/weight, insulin resistance, and treatment responses in the GSE5406 and GSE57338 data. Hence, the expression profiles of the *SERPINE1* and *ESR1* genes in the HF patients who are supposedly obese were not clear and there is no di-

rect evidence showing the association between the obesity paradox and *SERPINE1* expression.

Conclusion

The *SERPINE1* gene might have a crucial role in ischemic HF. The downregulation of *SERPINE1* might be responsible for the obesity paradox in HF. Obese HF patients with a low expression level of the *SERPINE1* gene might have a good prognosis. However, the association of *SERPINE1* expression with HF prognosis should be validated in multiple clinical trials after adjusting for the risk factors for HF.

Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

No funding was received in this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

Availability of data and material

GSE26887, GSE76701, GSE59867, GSE57338, GSE5406, GSE113764, GSE15524, and GSE20950 are available from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/).

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