Signature-oriented investigation of the efficacy of multicomponent drugs against heart failure

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ABSTRACT: Increasing attention has been paid to the application of precision medicine in heart failure (HF), and biomarker-based personalized medical care has shown great progress in HF management. Additionally, multicomponent drugs represented by traditional Chinese medicine (TCM) are demonstrating safety and efficacy in the management of HF. However, the potential mechanism is unclear, and the ambiguous clinical locations of TCM remain a hindrance to its extensive application. To meet this requirement, a precise investigation on the efficacy of multicomponent drugs against HF using the signature biomarkers-oriented approach was developed and applied to a conventional drug for cardiac disease, YiXinShu (YXS). On the basis of in vitro and in vivo efficacy evaluation of YXS against HF, and with the aid of proteomics and network pharmacology approaches, specific signatures regulated by YXS against HF were identified. YXS may show distinctive treatment features for those HF patients showing the elevation of fatty acid–binding protein 3 and cytoskeleton-associated protein 5, as well as other classic HF biomarkers. This study provides the first precise investigation of the efficacy of multicomponent drugs against HF and offers a practicable and low-cost approach for personalized management of HF.—Wei, J., Guo, F., Zhang, M., Xian, M., Wang, T., Gao, J., Wu, H., Song, L., Zhang, Y., Li, D., Yang, H., Huang, L. Signature-oriented investigation of the efficacy of multicomponent drugs against heart failure. FASEB J. 33, 000–000 (2019). www.fasebj.org

KEY WORDS: personalized medicine • biomarker • traditional Chinese medicine

Heart failure (HF) is one of the most serious cardiac diseases and markedly impairs the quality of life of patients. As a result of the complicated pathologic cascades involved in HF, complex pharmacologic remedies for HF have been applied, including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-blockers, and mineralocorticoid receptor antagonists (1). Although these treatments have proven beneficial in HF management, more personalized medicine is urgently needed for clinical management of HF patients (2–6). Hence, biomarker-based precision medicine has emerged as a great development in the significant decrease of the clinical and financial burdens associated with HF (2).

Multicomponent drugs represented by traditional Chinese medicine (TCM) have drawn increasing attention in HF treatment (7–13). Potentially relevant TCM characteristics include its multiconstituent and multitarget features (12); in addition, TCM has fewer adverse effects in some cases than in controls or with Western medication in some studies (11). The efficacy and safety of a TCM, qili qiangxin capsules, which is a typical TCM representative, were investigated by a large-scale multicenter, randomized, double-blind, parallel-group, placebo-controlled clinical trial; the result showed a greater reduction in the NT-pro–brain natriuretic peptide (BNP) level from baseline in the TCM group compared to the placebo group. Additionally, the study also saw improved quality of life (12). Even though there was considerable benefit with such...
multicomponent drugs, further investigations are still required before definitive conclusions may be drawn regarding whether TCM really works in the treatment of HF (11). The potential mechanism and ambiguous clinical locations hinder the widespread use of TCM. Therefore, more randomized controlled trials have been performed to systematically review the efficacy of TCM in the management of HF and to try to reveal its precise localization in the clinic (14–17).

However, investigation remains a challenge; because of the rigorous criteria and the increased costs of randomized controlled trials, we need to get as much information as possible before such trial-based precise investigations into the treatment of HF by TCM, and before fundamental information about the potential targets or effector molecules of TCM, may be elucidated. To meet the requirements, an alternative method of using human induced pluripotent stem (iPS) cells for high-throughput drug testing (18–22) could be a promising approach. Because humanized iPS cells provide a unique human system, they can serve as a true in vitro model to understand the molecular mechanisms of disease and the corresponding effective drugs (18, 21). Consequently, by utilizing human iPS cell-derived cardiomyocytes for the precise monitoring of biomarkers associated with HF, the efficacy of a drug for the accurate management of patients with various types of HF could be easily evaluated. However, another challenge is the high-throughput and precise monitoring of biomarkers. In fact, the current view holds that monitoring should be based on protein panels (i.e., signatures) that include several classes of biomarkers (23, 24). Only if multiple HF biomarkers can be simultaneously monitored can the efficacy of a drug be comprehensively investigated, especially for multicomponent and multitarget drugs. Therefore, a high-throughput approach that can accurately monitor the signature biomarkers is also needed.

Therefore, in this study, an integrated approach based on signature biomarkers was developed to precisely investigate the efficacy of multicomponent drugs against HF. First, the efficacy of a drug against HF was evaluated on human iPS cell-derived cardiomyocytes induced by endothelin-1 (ET-1), which has been proven to be directly correlated with the progression of HF (25, 26). Then multiple HF biomarkers from cardiomyocytes were simultaneously monitored by high-throughput proteomics approach. Because of the superior comprehensiveness of proteomics and their powerful quantitative ability (27, 28), this approach offers an opportunity to identify the changes of the signatures and biomarkers when perturbed with a drug. Next, after a systems-level explanation of the interactions of multiple drug components by a network pharmacology–based (29–31) approach and final validation in HF rat models, the signature biomarkers regulated by the multicomponent drugs against HF can be confirmed and the corresponding precise clinical localization deduced. The newly developed approach was then applied to a multicomponent conventional drug for cardiac disease, YiXinShu (YXS), a sheng mai san–based TCM formula (32–34). This study provides the first precise investigation of the efficacy of multicomponent drugs against HF; it also offers a practicable, low-cost approach for personalized management of HF.

MATERIALS AND METHODS

In vitro efficacy evaluation of YXS on ET-1–induced dysfunctional hiPS-C cells

First, the efficacy of YXS against in vitro HF was evaluated on ET-1–induced dysfunctional hiPS-C cells as previously described (35). Briefly, the cells were exposed to ET-1 for 24 h. Then changes in the BNP content were assessed by ImageXpress XLs widefield high-content analysis system (Molecular Devices, San Jose, CA, USA) (35). YXS intestinal absorption liquid was also prepared for efficacy evaluation as previously described (35, 36). Bosentan (MilliporeSigma) was set as the positive control.

Proteomic analysis

Three individual samples were analyzed in each group. Approximately 1 × 10⁶ hiPS-C cells in each sample were collected and washed gently with PBS (KC1 0.2 g, KH₂PO₄ 0.2 g, NaCl 8.0 g, Na₃HPO₄ 12 H₂O 3.9054 g, pH 7.4, 1000 ml) buffer and then lysed with 8 M urea containing the protease inhibitor PMSF for 30 min at 4°C. The lysate was centrifuged at 24,000 g for 60 min at 4°C, and the supernatant was collected. The protein concentration was determined by the bicinchoninic acid assay. Then proteins were reduced by adding 0.1 M DTT for 4 h at 37°C and alkylated by adding 0.5 M iodoacetamide for 60 min at room temperature in the dark. The protein sample was finally digested using trypsin in 50 mM ammonium bicarbonate (pH 8.0) at a mass ratio of 1:50 enzyme/protein for 24 h at 37°C.

Orbitrap Fusion (Thermo Fisher Scientific, Waltham, MA, USA) liquid chromatography–tandem mass spectrometry analyses were performed on an Easy-nLC 1000 liquid chromatography system (Thermo Fisher Scientific) coupled to an Orbitrap Fusion via a nano-electrospray ion source. Tryptic peptides were dissolved with loading buffer (acetonitrile and 0.1% formic acid); tryptic peptides were eluted from a 150-μm ID × 2 cm C18 trap column and separated on a homemade 150-μm ID × 12 cm column (C18, 1.9 μm, 120 A; Dr. Maisch GmbH, Ammerbuch, Germany) with a flow rate of 500 nl/min. Survey scans were acquired after accumulation of 5e (5) ions in Orbitrap (Thermo Fisher Scientific) for m/z 300–1400 using a resolution of 120,000 at m/z 200. The top speed data-dependent mode was selected for fragmentation in the higher-energy collision dissociation cell at normalized collision energy of 32%, and then fragment ions were transferred into the ion trap analyzer with the AGC target at 5e (3) and maximum injection time at 35 ms. The dynamic exclusion of previously acquired precursor ions was enabled at 18 s. Spectral data were searched against human protein RefSeq database (https://www.ncbi.nlm.nih.gov/refseq) in Proteome Discoverer1.4.1.14 suites with Mascot 2.3.01 software (Matrix Science, Torrance, CA, USA) to achieve a false-discovery rate of <1%. The mass tolerance was set to be 10 ppm for precursor, and it was set 0.5 Da for the tolerance of product ions. Oxidation (M) and acetyl (protein–N term) were chosen as variable modifications and carbamidomethylation (C) as fixed modification; 2 missed cleavage sites for trypsin were allowed.

www.fasebj.org by Kaohsiung Medical University Library (163.15.154.53) on September 20, 2018. The FASEB Journal Vol. {article.issue.getVolume()}, No. {article.issue.getIssueNumber()}, primary_article.
The intensity-based absolute quantification (iBAQ)-based protein quantifications (37) were performed using in-house software. Briefly, the iBAQ intensities were obtained by dividing the protein intensities by the number of theoretical peptides, which were calculated by in silico protein digestion with a Perl script, and all fully tryptic peptides between 6 and 30 aa were counted, while the missed cleavages were neglected.

In vivo animal study

Male Sprague Dawley rats (Vital River Laboratories, Beijing, China), 9 wk of age, were anesthetized with 3% pentobarbital sodium. Then ischemia-induced HF was produced by occlusion of the left anterior descending coronary artery near its origin from the left coronary artery for up to 4 wk (38–40). Sham-treated rats underwent the same procedures except for the left coronary artery occlusion. Then all rats were randomly allocated into treatment groups and were orally administered high-dose YXS (0.64 g/kg daily, n = 10) or medium-dose YXS (0.32 g/kg daily, n = 10) or saline (model control, n = 10) for 6 consecutive weeks. Doppler echocardiography measurements were performed with VisualSonics Vevo 770 Ultrasound System (Toronto, Canada) before administration and at 2, 4, and 6 wk after intake of drugs. A 17.5 MHz probe was used. The left ventricular end-diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) were calculated, and then the ejection fraction (EF) was calculated as \([\frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \times 100\%]\) (39). On the last day of the experiment, after 24 h of the animals’ being unfed, plasma samples were collected and frozen at \(-20^\circ\text{C}\) for analysis. All the animal experiments were approved by the Committee on the Animal Care and Use of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, and were carried out in accordance with the approved guidelines.

Experimental design and statistical rationale

In the in vitro study, each sample contained \(1 \times 10^4\) hiPS-C cells, and 3 individual samples were analyzed in each group. Dynamic changes were identified by 1-way ANOVA of the measured protein amounts from each individual sample (24). In the in vivo study, each group contained 10 rats, and the results from each rat were analyzed by 1-way ANOVA and were considered significant at \(P < 0.05\).

Network pharmacology analysis

Known therapeutic targets for cardiac hypertrophy

In total, 282 known therapeutic targets for cardiac hypertrophy were collected in this study from the Online Mendelian Inheritance in Man database (https://omim.org/) and the Human Phenotype Ontology database (http://human-phenotype-ontology.github.io/).

Protein–protein interaction data from STRING

Protein–protein interaction relationships with interaction scores larger than 0.4 were retrieved from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) 10 database (http://www.string-db.org/).

Prediction of potential targets for YXS

Potential targets of YXS were predicted by BATMAN-TCM (41), which is a web service for the target prediction of TCM (http://bionet.ncpsb.org/batman-tcm/). The potential targets of YXS were obtained from the integrated known drug–target interaction data set and predicted by the similarity-based target prediction method. This drug–target prediction method ranks putative drug–target interactions on the basis of their similarity to the known drug–target interactions. Validation methods, including “leave one interaction out” cross-validation, “leave one drug out” cross-validation, and validation of the independent test set were applied to evaluate the performance of the prediction model in BATMAN-TCM and confirmed the excellent prediction performance of this prediction model.

Results

Scheme for signature-oriented precise investigation of YXS against HF

The pharmacologic effect of YXS against HF was evaluated in vitro on hiPS-C and in vivo in ischemia-induced HF rats (Fig. 1). Then the changes of the differential proteins in ET-1-induced dysfunctional hiPS-C cells, as well as those intervened by YXS, were measured by high-throughput proteomic analysis, followed by network pharmacology prediction and ELISA-based verification on hiPS-C cells. As a result, signature biomarkers corresponding to the specific efficacy of YXS against HF can be obtained. Finally, after further validation in the HF rat blood treated by YXS, signatures regulated by YXS against HF can be confirmed, and its precise clinical localization can be reasonably deduced.

YXS improved ET-1-induced dysfunctional hiPS-C cells

The cardioprotective effect of YXS against HF in vitro was evaluated on ET-1-induced dysfunctional hiPS-C cells. The morphologic change of the dysfunctional hiPS-C cells
was induced by ET-1, while both YXS and positive control bosentan can partially reverse these changes (Supplementary Fig. S1). After treatment by ET-1, the BNP level of hiPS-C cells were increased compared to that of the vehicle group, and YXS could inhibit the BNP content of hiPS-C cells compared to that of the ET-1 group ($P < 0.05$). All the results indicated that YXS can improve ET-1–induced dysfunctional hiPS-C cells, thus exerting a good cardioprotective effect in vitro.

**Dynamic changes of proteins in ET-1–induced dysfunctional hiPS-C cells**

Dynamic changes of proteins in hiPS-C cells during cardiac dysfunction were investigated by collecting cells after exposure to ET-1 for 12, 24, 48, and 72 h for high-throughput proteomic profiling. An accurate iBAQ-based approach was used (24, 31, 37), and the iBAQ value of each protein was normalized to the total iBAQ value for all the identified proteins to avoid possible experimental variations (24, 31). Three individual samples were analyzed in each group, and dynamic changes were precisely identified by statistical analyses of the measured protein amounts from each individual sample (24). In total, 4015 proteins were identified as a result (Supplemental Table S1). Among them, 362 proteins in the 12-h group had significantly up-regulated expression levels ($P < 0.05$) compared to the naive group (Supplemental Fig. S2). Their biologic process or molecular function was investigated (43), which was found to function in various cellular protein processes, such as cell aging, response to calcium ion, regulation of actin cytoskeleton organization, and platelet degranulation (Fig. 2). A total of 438 proteins in the 24-h group had significantly up-regulated expression levels ($P < 0.05$) compared to control, which function in the regulation of macroautophagy, actin filament organization, microtubule-based movement, and others (Fig. 2B). A total of 283 proteins in the 48-h group related to cytoskeleton organization and negative regulation of cell growth, and other processes had significantly up-regulated expression levels ($P < 0.05$) compared to control (Fig. 2B). Finally, after 72 h of exposure to ET-1, 354 proteins had significantly up-regulated expression levels ($P < 0.05$) compared to control, which function in actomyosin structure organization, negative regulation of cell death, response to stress, and others. In total, 902 proteins in hiPS-C cells were noticeably up-regulated during cardiac dysfunction.

Moreover, all ET-1–interacting proteins and those that could be identified in the proteome were analyzed. On the basis of the STRING 10 database, 3 ET-1 receptors may interact with the 116 proteins listed in Supplemental Table S2, whereas 52 proteins can be identified by proteomic profiling (Fig. 2D and Supplemental Table S2). Considering that the numbers of cells used for the proteomic analysis is only $10^6$, a good depth of coverage for ET-1–interacting protein detection by the proteomic approach could also be obtained, which helped make the investigation of the signature targets of YXS convincing.

**Prediction of signature biomarkers associated with efficacy of YXS against HF**

Next, by using network pharmacology strategies, signature biomarkers related to the efficacy of YXS against HF
were computationally predicted. All ET-1-interacting proteins, potential targets of YXS predicted by BATMAN-TCM, and 902 proteins with significantly up-regulated expression levels during cardiac dysfunction were used to construct the putative targets network. In total, 92 major nodes were identified, among which were 12 major differentially expressed proteins (Supplemental Fig. S3). These 12 differentially expressed proteins were thus considered putative signature biomarkers associated with the efficacy of YXS against HF.

**Figure 2.** Proteomic investigation of dynamic changes of proteins in hiPS-C cells during cardiac dysfunction after exposure to ET-1 for 12, 24, 48, and 72 h (model 12, 24, 48, and 72 h). A) Hierarchical clustering of quantitative information from identified proteins. Normalized iBAQ value of each protein is shown as heat map of green to red that represents down-regulation and up-regulation, respectively. Z score transformation was used. B) Biologic process or molecular function of proteins of model 12, 24, 48, and 72 h with up-regulated expression levels (P < 0.05) compared to control (naive). C) Venn diagram of identified proteins between time points (12, 24, 48, and 72 h). D) All ET-1-interacting proteins and those that can be identified in proteome.
**Proteomic analysis of signatures regulated by YXS against HF**

Dynamic changes of proteins in ET-1–induced dysfunctional hiPS-C cells after perturbation with YXS were further investigated by proteomic profiling of the cells when treated with YXS for 12, 24, 48, and 72 h. The same protein quantification approach was used. A total of 4404 proteins were identified (Supplemental Table S3). Molecular function investigation (43) indicated that after treatment with YXS for 12 h, some proteins had significantly up-regulated expression levels ($P < 0.05$) compared to the model (Fig. 3), which function in the positive regulation of cardiac muscle hypertrophy, voltage-gated calcium channel activity, cardiac muscle cell growth, and others. With the prolonged administration time, the proteins involved in ventricular cardiac muscle tissue development, vascular smooth muscle contraction, actin filament reorganization, negative regulation of relaxation of cardiac muscle, and others were markedly up-regulated compared to the model (Fig. 3B).

After a comprehensive consideration of their suitability as easy-to-use biomarkers (23, 24) and preceding computational prediction information, 8 differentially expressed proteins were selected as a novel candidate panel associated with the efficacy of YXS against HF; their dynamic changes are shown in Fig. 4. Among them, natriuretic peptide precursor A, troponin T type 2 (cardiac), fatty acid-binding protein (heart), CKAP5 and ATP–citrate synthase were found to indicate cardiac dysfunction significantly earlier than others after 12 h of treatment. All the protein changes resulting from the malfunction could be attenuated by YXS. Thus, these signature candidates are thought to correspond to the specific efficacy of YXS against HF.

**ELISA-based verification of the signatures on hiPS-C cells**

Candidate signatures regulated by YXS against HF were further verified by ELISA-based assays measuring the dynamic changes of candidate signatures on hiPS-C cells. As shown in Fig. 5, the expression level of BNP, TNNI3, GAL-3, HSP 70, and FABP3 increased after 12 h exposure to ET-1, and the dysfunction of BNP, TNNI3, GAL-3, and FABP3 continued with the prolongation of the expression time, while YXS lowered its elevations in hiPS-C cells. Even when the expression level of HSP 70 naturally decreased as time went on, YXS could still reduce its content. After treatment with YXS for 48 h, the upward tendency of CKAP5 was inhibited. Overall, except ATP–citrate synthase, dynamic changes of the signatures measured by ELISA were consistent with the ones obtained from the proteomic analysis.

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**Figure 3.** Dynamic changes of proteins in ET-1–induced dysfunctional hiPS-C cells after perturbation with YXS. A) Hierarchical clustering of quantitative information from identified proteins. Normalized iBAQ value of each protein is shown as heat map of green to red that represents down-regulation and up-regulation, respectively. Z score transformation was used. B) Biologic process or molecular function of proteins of models after perturbation with YXS with up-regulated expression levels ($P < 0.05$) compared to models. Model 12, 24, 48, and 72 h: cells after exposure to ET-1 for 12, 24, 48, and 72 h. YXS 12, 24, 48, and 72 h: model after treatment with YXS for 12, 24, 48, and 72 h. Naive indicates control.
**In vivo efficacy evaluation of YXS against HF in ischemia-induced HF rats**

The pharmacologic effect of YXS against HF in vivo was evaluated in ischemia-induced HF rats. Dynamic changes in the EF from individual rats indicated that after permanent occlusion of the coronary artery, substantial reduction in EF was observed compared to sham-treated rats, which means that progressive heart dysfunction occurred (Supplemental Fig. S4). Compared to high-dose

**Figure 4.** Proteomic quantitative analysis of candidate signatures regulated by YXS against HF in hiPS-C cells. Three individual samples were analyzed in each group. Normalized iBAQ value of each protein from each individual sample was used for 1-way ANOVA (24). Model 12, 24, 48, and 72 h: cells after exposure to ET-1 for 12, 24, 48, and 72 h. YXS 12, 24, 48, and 72 h: model after treatment with YXS for 12, 24, 48, and 72 h. Naive indicates control; ns, not significant.

**Figure 5.** ELISA-based verification of candidate signatures regulated by YXS against HF in hiPS-C cells. Three individual samples were analyzed in each group. Results were analyzed by 1-way ANOVA. Green bar indicates control; red bar, model (ET-1 treated); gray bar, YXS (ET-1 treated plus YXS); and 12, 24, 48, and 72 h, duration time.
YXS, medium-dose YXS and valsartan treatment (positive control) all increased the EF of HF rats and improved their cardiac function, while high-dosage YXS also showed good efficacy after a shorter administration time (Fig. 6A). Increased left ventricle diameter and progressive left ventricle dilation can also be reversed by YXS (Fig. 6B). Thus, these results revealed that YXS can exert a good cardioprotective effect against HF in vivo.

Final verification of signatures in HF rat blood

Specific signatures regulated by YXS against HF were ultimately verified by measuring their changes in HF rat blood. Multiple classic HF biomarkers, including BNP, related NT-proBNP, and ANP, were also measured. Similar to the results from the preceding in vitro tests, the amounts of these signatures in HF rat blood increased compared to that of the sham-treated group, and YXS can inhibit their elevations compared to that of the model group (Fig. 7). More importantly, YXS exhibits an advantage in contrast to valsartan, and only YXS can lower the amounts of FABP3 and CKAP5 (Fig. 7). As a result, the biomarker panel can be utilized as a specific indicator for personalized management of HF by YXS.

Interaction between chemical components of YXS and its specific signatures against HF

A network of interactions among these signatures and their relationship with the chemical components of YXS was constructed and is shown in Fig. 8 and Supplemental Fig. S5, respectively. Among 6 specific signatures, BNP, TNNI3, GAL-3, FABP3, and CKAP5 all have broad interactions with the proteins frequently involved in cardiac muscle contraction, regulation of actin cytoskeleton, dilated cardiomyopathy, hypertrophic cardiomyopathy, cAMP signaling pathways, and others (Fig. 8). Furthermore, 79 chemical components of YXS had interactions with these signatures through their corresponding putative targets (Supplemental Fig. S5), suggesting that these signatures are closely associated with the therapeutic effects of YXS on HF.

DISCUSSION

Because HF is an eventual syndrome triggered by a variety of factors, such as ventricular dysfunction, volume overload, or pressure overload, and because HF is a significant source of mortality in patients (3), safe and effective treatment is the key to improving quality of life for HF patients. Chinese herbs—traditional medicine used in Asian countries for many years—are demonstrating advantages in safety and efficacy in the management of HF (11–15). The YXS used in this study contains 7 types of herbs, including radix ginseng, radix ophiopogonis, fructus schisandrae chinensis, Salvia miltiorrhiza, astragalus membranaceus, ligusticum wallichii, and fructus crataegi, which all can be safely used in both food and medicine. Most importantly, not only on the in vitro dysfunctional hiPS-C cells but also in the in vivo ischemia-induced HF rats, YXS can exert a cardioprotective effect against HF. Furthermore, investigations of the molecular function of proteins perturbed by YXS also indicate that proteins that function

![Figure 6. In vivo efficacy evaluation of YXS against HF in ischemia-induced HF rats. A) Increased EF (Δ%EF) after administration of YXS. B) Doppler echocardiography of rat hearts. Increased left ventricle diameter and progressive left ventricle dilation can be reversed by YXS (arrows).](https://www.fasebj.org)
in the positive regulation of myocardial function have significantly up-regulated expression levels.

However, as with many other TCMs, one of the great challenges in its extensive application is the unclear potential mechanisms and ambiguous clinical locations. The characteristics of multiconstituents and multitargets also increase the complexity of the analysis of its mechanism. The present study can address these problems. First, a signature-oriented investigation can effectively provide reliable clinical clues, resolve precise localization problems, and avoid the confusion of complex mechanism process. Second, the developed integrated approach in our study can be satisfied to realize this goal. hiPS-C cells provide a unique and simple human system compared to

**Figure 7.** Verification of candidate signatures regulated by YXS against HF in HF rat blood. Each group contains 10 rats, and results from each rat were analyzed by 1-way ANOVA. Ns, not significant.

**Figure 8.** Network of interactions among signatures regulated by YXS against HF, identified protein targets, and HF-related pathways involved by these genes. Red round nodes refer to signatures regulated by YXS against HF. Blue round nodes refer to identified protein targets. Green nodes refer to HF-related pathways involving these genes.
animal cells. By utilizing convenient real-time cellular analysis and high-content analysis, the effects of drugs on cardiac dysfunction are easily evaluated (35). Investigations of the molecular function of the changed proteins in ET-1-induced dysfunctional hiPS-C cells also indicate that this is an excellent in vitro model for the precise investigation of the efficacy of anti-HF drugs. Moreover, with the aid of proteomics approaches with powerful depth coverage and the ability to perform quantitative analysis, we can utilize fewer hiPS-C cells (low to 10^4 cells) to obtain more information about the changes of a variety of proteins when perturbed with drugs, and a broad dynamic range for the detection of low concentrations of biomarkers (e.g., BNP, only picograms per milliliter in blood) can also be obtained, thus facilitating the investigation of the signature targets of multicomponent drugs.

Another distinction of our integrated approach is network pharmacology analysis. By this approach, not only can we narrow down the candidates based on experimental data and predict the putative signatures but we can also use it to depict the network of interactions among these signatures and their relationships to the chemical components of the drug. In fact, network pharmacology-based analysis may be an indispensable approach in the study of TCM because there are too many constituents and their interaction can be quite complex, whereas we can smooth this relationship as much as possible using this computational approach.

By using the integrated approach, specific signatures regulated by YXS against HF are confirmed, such as BNP (including relevant ANP, NT-proBNP), TNNI3, GAL-3, HSP 70, FABP3, and CKAP5. Among them, BNP and NT-proBNP are already recognized as prominent standards in the diagnosis of acute HF and are useful in guiding HF management (44). Troponin involves cardiac muscle contraction and is released into the plasma because of myocardial injury, necrosis, or apoptosis (45). It can be a prognostic marker used in therapeutic guidance. GAL-3 is related to the inflammatory cascade after cardiac injury, and it plays an important role in the development and progression of HF (46). It is also a prognostic marker in patients with chronic ambulatory HF (47). The HSP 70 level was reported to be elevated in chronic HF patients, particularly in those with cardiac cachexia, and is related to disease severity (48). A survival analysis also indicated that higher HSP 70 levels are associated with significantly increased mortality in patients with HF (49). Research findings showed that FABP3 has a greater predictive capacity for myocardial damage events than troponin T in patients with chronic HF (50). Elevation of FABP3 could be a prognostic marker that indicates latent and ongoing cardiomyocyte damage and identifies patients at high risk for future cardiac events in congestive HF (51). For CKAP5, although it is not yet an accepted marker in HF, it regulates microtubule dynamics and organization (52) and may reflect the deterioration of cardiac function (53). Overall, YXS depends on its diverse chemical composition and effectively acts on multiple biomarkers that reflect different underlying mechanisms/pathways of HF, thus exerting good cardioprotective effects against HF. Given these facts, YXS still shows distinctive treatment features against HF. On the basis of our findings, YXS may be particularly suitable for HF patients with elevations of FABP3 and CKAP5, besides other classic HF biomarkers. This deduction is supported not only by the experimental results in this study but also by the network analysis of interactions among these signatures and chemical components of YXS. Many components of YXS contribute to its action against HF. Moreover, this finding is based on the experimental results from humanized cells. Therefore, it has a better prospect of clinical application in the personalized treatment of HF.

CONCLUSIONS

In this study, an integrated approach was developed to address challenges in the clinical application of YXS in personalized medical care for HF. The pharmacologic effect of YXS against HF was evaluated in vitro on ET-1–induced dysfunctional hiPS-C cells and in vivo in ischemia-induced HF rats. Then, with the aid of proteomics and a network pharmacology approach, specific signatures regulated by YXS against HF were confirmed. YXS shows distinctive treatment features against HF. This study provides the first precise investigation of the efficacy of multicomponent drugs against HF; in addition, it offers a practicable and low-cost approach for personalized management of HF.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the National Science and Technology Major Project (2014ZX09201021-009 to H.Y.), the National Program on Key Basic Research Project (2015CB554406 to H.Y.), and the National Natural Science Foundation of China (81330086 to H.Y., 81603422, and 81703951 to F.G.). All data related to this study have been made publicly available on iProX (http://www.iprox.org/) with ID IPX0001125000/PXD008489. The authors declare no conflicts of interests.

AUTHOR CONTRIBUTIONS

J. Wei conceived the study and wrote the report; F. Guo performed the network pharmacology analysis; M. Zhang, T. Wang, J. Gao, H. Wu, Y. Zhang, and D. Li performed the in vitro and animal studies; L. Song conducted the proteomics experiments; M. Xian conducted the ELISA experiments; H. Yang and L. Huang conceived the study; and all authors reviewed and approved the final manuscript.

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Received for publication April 8, 2018.
Accepted for publication August 27, 2018.