Quyu Shengxin Capsule (QSC) Inhibits Ang-II-Induced Abnormal Proliferation of VSMCs by Down-Regulating TGF-β, VEGF, mTOR and JAK-STAT Pathways

Jinjin Yu
Xi'an Jiaotong University
Weifeng Li
Xi'an Jiaotong University
Lintao Zhao
Xi'an Jiaotong University
Yuan Qiao
Shaanxi Provincial Institute of Archaeology
Xin Xiao
Xi'an Jiaotong University
Dong Guo (787422146@qq.com)
Shaanxi Provincial Institute of Archaeology

Research

Keywords: Angiotensin Ⅱ, Quyu Shengxin capsule, Inflammation, Vascular smooth muscle cells

Posted Date: September 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-65412/v1

Abstract

Background: Quyu Shengxin capsule (QSC) are an herbal compound commonly used to treat blood stasis syndrome in China, and blood stasis syndrome is considered to be the root of cardiovascular diseases (CVD) in traditional Chinese medicine. However, the potential molecular mechanism of QSC is still unknown.

Methods: The rat thoracic aorta vascular smooth muscle cells (VSMCs) were cultured in vitro, and then stimulated with Angiotensin Ⅱ (Ang-II) (10^{-7} mol/L) for 24 h to establish a cardiovascular cell model. The cells were then treated with different concentrations of QSC drug-containing serum or normal goat serum. MTT assay was used to detect the viability of VSMCs and abnormal cell proliferation. In order to analyze the possible signal transduction pathways, the content of various factors in the supernatant of VSMCs was screened and determined by means of the Luminex liquid suspension chip detection platform, and the phosphoprotein profile in VSMCs was screened by Phospho Explorer antibody array.

Results: Compared with the model group, serum cell viability and inflammatory factor levels with QSC were significantly decreased ($P < 0.001$). In addition, the expression levels of TGF-β, VEGF, mTOR and JAK-STAT in the QSC-containing serum treatment group were significantly lower than those in the model group. QSC can regulate the pathological process of CVD by reducing the levels of inflammatory mediators and cytokines, and protecting VSMCs from the abnormal proliferation induced by Ang-II.

Conclusion: QSC inhibits Ang-II-induced abnormal proliferation of VSMCs, which is related to the down-regulation of TGF-β, VEGF, mTOR and JAK-STAT pathways.

Background

The pandemic of Coronavirus Disease 2019 (COVID-19) has developed rapidly in the world. According to earlier reports, people over 65 years of age with cardiovascular diseases (CVD) basis such as coronary heart disease or high blood pressure are more likely to be infected and have more severe symptoms[1]. In recent years, researches on CVDs caused by blood stasis have been increasing. Studies believe that blood stasis is not only an important pathological product of this disease, but also a pathogenic factor[2, 3]. CVD is a systemic disease in which multiple genetic inheritance interacts with the environment and multiple risk factors. It is the most common and a major public health problem worldwide[4]. The main pathological feature of CVD is vascular remodeling (VR), and the pathological changes are manifested by increased hypertrophy and stiffness of the arterial wall[5]. The thickening of the arterial wall is mainly caused by the active proliferation of vascular smooth muscle cells (VSMCs). In this pathological process, the proliferation and migration of VSMCs are affected and regulated by many factors, such as vasoactive substances, growth factors, and extracellular matrix[6]. Risk factors for CVD cause local inflammation in blood vessels. A large number of inflammatory factors can be released to damage vascular endothelial function, which leads to the synthesis and release of some cytokines, especially growth factors, acting on VSMC membrane receptors and activating intracellular signaling pathways[7, 8]. Eventually it leads to the

expression of genes in the nucleus to promote the excessive proliferation of VSMCs and aggravate the pathological process of VR[9].

Angiotensin Ⅱ (Ang-Ⅱ) is a bioactive peptide produced by the renin-angiotensin-aldosterone system and acts on vascular smooth muscle[10]. As an important active substance for regulating cardiovascular activity, it has a specific role in the occurrence of CVDs such as hypertension, myocardial hypertrophy, and heart failure[11]. Existing studies have found that Ang-Ⅱ can induce VSMCs to release a large amount of inflammatory mediators, resulting in abnormal proliferation and formation of arterial plaques[12]. As an initiator of the inflammatory response, interleukin-6 (IL-6) can induce B cells to differentiate and produce antibodies. In addition, it also induces T cell activation, proliferation, and differentiation, thereby participating in the body's immune response[13]. Studies have shown that Chaihu-Shugan-San reduces the local inflammation of blood vessels by inhibiting the level of IL-6 in serum, thereby reducing the area of hyperplastic arterial plaque[14]. Therefore, normalizing the body's inflammatory mediator levels may provide new insights for the prevention and treatment of vascular proliferative diseases. Intercellular cell adhesion molecule-1 (ICAM-1) as an important member of the immunoglobulin superfamily (IGSF) in adhesion molecules, is actively expressed in proliferating vascular endothelial cells. At the same time, it participates in physiological and pathological processes such as signal transduction and activation of cells, immune response, inflammatory response and angiogenesis[15]. Vascular endothelial growth factor (VEGF) is the most critical factor in the process of angiogenesis[16]. Studies have found that it mainly acts in the early stage of VR in combination with other growth factors to promote vascular hyperplasia greatly, thereby participating in the process of VR[17]. More importantly, Studies have shown that VEGF and ICAM-1 are positively correlated with the degree of proliferation and migration of vascular cells, which can be used as indicators to judge abnormal proliferation of blood vessels[18, 19]. Transforming growth factor-β (TGF-β) can regulate the phenotypic transformation of VSMCs. It also affects the process of vascular wall remodeling by regulating the proliferation, migration and matrix deposition functions of VSMCs[20]. Mammalian target of rapamycin (mTOR) is an important serine-threonine protein kinase downstream of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt). It can regulate the proliferation, survival, invasion and metastasis of tumor cells by activating ribosomal kinase[21]. The study found that TGF-β, VEGF and PI3K/Akt/mTOR signaling pathways are closely related to cell proliferation and affect normal cardiovascular function[16, 20, 22]. Therefore, it is very important to deeply explore their related mechanisms. At present, the treatment methods for most CVDs are mainly divided into surgical intervention and drug intervention, but they all have certain side effects or problems of low patient compliance[23]. Therefore, it is urgent to find a safe and effective medicine with low side effects.

Quyu Shengxin capsule (QSC) is the result of this subject starting from the theory of etiology and pathogenesis of traditional Chinese medicine, based on the experience of clinical practice, and combining modern medicine's understanding of CVD. It is made into hard capsules by the combination of astragalus, guizhi, trigonum, zedoary, tulipa, and zucchini. QSC has been used in the treatment of clinical blood stasis syndrome in China for many years. The blood stasis syndrome is closely related to the etiology of CVDs such as coronary heart disease, hypertension and atherosclerosis. The chemical

markers of QSC include baicalin, astragaloside, cinnamaldehyde, curcumin, and brucine, which are reported to improve inflammation and abnormal cell proliferation[24–27]. A variety of active ingredients, including astragaloside, in its main component, astragalus (traditional Chinese medicine), have a certain effect on metabolic diseases such as diabetes and atherosclerosis[28, 29]. In recent years, the gene chip has become a new field of pharmacological research. It has been used to elucidate the interaction between multiple components and multiple pathways of active ingredients of Chinese herbal medicine. With the rapid development of bioinformatics and systems biology, more and more studies rely on gene chips to fully explore the potential mechanisms and potential targets of complex systems[30]. Therefore, this study used gene chip detection to study the potential targets of QSC. To reveal whether QSC has an interventional treatment effect on CVDs such as abnormal vascular hyperplasia, the effect of QSC on inflammation and proliferation in VSMCs induced by Ang-II was studied, and the mechanism of related signaling pathways was further explored.

Methods And Materials

Preparation of QSC

The qualified Chinese herbal medicine QSC (Supplementary Table 1) was provided by the Shaanxi Provincial Hospital of Traditional Chinese Medicine (Shaanxi, China) (approval number: Shaanxi Pharmaceutical Z20150050). Briefly, *Radix Astragali seu Hedysa*, *Cinnamomum cassia Presl*, *Rhizoma Sparganii*, *Rhizoma Curcumae*, *Tubeimoside*, *Semen Strychni Praeparata* and other raw herbs were mixed and extracted twice with water (decocted). Then, this extract was collected and concentrated to the required density (1 mL of extract contains 1 g crude drug), which was ready for use.

Animals and treatment

Male SD rats (weight 200 ± 20 g) were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Shaanxi, China). The rats were placed in a temperature-controlled (20–26 °C), pathogen-free environment, with a light/dark cycle of 12 h, and free access to food and water. The Animal Research Ethics Committee of Xi'an Jiaotong University approved the animal research protocol and was consistent with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH).

Preparation of drug-containing serum

Male SD rats were randomly divided into 4 groups: control group, QSC high, medium and low dose groups (0.48 g/kg, 0.24 g/kg and 0.12 g/kg). The animals in the QSC group were given the same volume (1 mL/100 g) of drugs by intragastric administration, while the control group was given the same volume of saline. Dosing for 7 consecutive days (1 time/1 day). One hour after the last administration, blood samples were collected from the abdominal aorta, and then allowed to stand for 2 h (room temperature). Serum samples were centrifuged at 3000 × g for 15 min, collected and aliquoted, and stored at -80 ℃.

Cell culture

VSMCs were purchased by Beijing Beina Innovation Biotechnology Institute (Beijing, China). VSMCs were cultured in DMEM containing 10% FBS in a humidified incubator containing 95% air and 5% CO_2 at 37 °C. In order to ensure the normal state of cells, all the experimental cells were between the third and eighth generations. VSMCs grown to 90% fusion were passaged by trypsin-EDTA digestion.

MTT assay

In order to find the safe experimental proportion of QSC drug-containing serum and verify its effect on cell proliferation at a later stage, we used the MTT assay to determine. First, inoculate VSMCs in 96-well plates and incubate overnight in DMEM containing 10% normal FBS. When the cells were confluent to 80%, serum starvation (incubation with blank DMEM) was performed for 24 h. Then inoculate a series of different proportions of DMEM containing QSC drug-containing serum and incubate for 24 h. (In the cell proliferation experiment, Ang-Ⅱ was used to stimulate 24 h after incubation with QSC drug-containing serum.) This cell supernatant was removed and MTT was added and incubated at 37 °C for 4 h. The formazan crystals were then dissolved in DMSO and shaken for 10 min (120 r/min). Finally, the absorbance was measured on a microplate reader at a wavelength of 490 nm.

Cytokine composition analysis and bioinformatics analysis

The Luminex 200 system (Luminex Corporation, Austin, TX, USA) using the Luminex liquid suspension chip detection platform analyzed the composition of cytokines in the supernatant of VSMCs. The Wayen Biotechnologies (Shanghai, China), Inc. provided contract services.

VSMCs were evenly seeded in 24-well plates and incubated in DMEM containing 10% common FBS. After the cells were grown and fused to 80%, the culture solution was discarded and the serum was starved for 24 h. After incubation with QSC drug-containing serum, Ang-Ⅱ was used to stimulate for 24 h. Finally, the cell culture solution was centrifuged at 8000 × g for 15 min, and the supernatant was collected for chip detection.The operation of chip detection is as follows:

a) According to the instructions, use the Rat Premixed Multi-Analyte Kit (article number: LXSARM-10) to incubate the sample in a shaker for 2 h. b) Then discard the sample and incubate the detection antibody (Biotin Antibody Cocktai) for 1 h. c) Color development: Biotin Antibody Cocktail is discarded, and Streptavidin-PE diluted according to the instructions is incubated for 30 min, and finally sent to the calibrated Luminex 200 machine for reading.

After the samples and standards tested in this experiment are detected by the Luminex 200 detector, the obtained fluorescence is automatically calculated and optimized by the software to form a file output.

Analysis of cellular pathways

The protein gene chip CSP100 plus (Full Moon Bio systems,Inc.) was used to analyze the related pathways involved in VSMCs protein samples. Contract services are provided by Wayen Biotechnologies (Shanghai, China), Inc.

Sample collection and processing: VSMCs were evenly inoculated in 60 mm dishes and incubated in DMEM containing 10% common FBS. After the cells were grown and fused to 80%, the culture solution was discarded and serum starved for 24 h. After incubation with QSC drug-containing serum, Ang-Ⅱ was used to stimulate for 24 h. Finally, the cell culture solution was discarded, and chip lysis solution (Full Moon Bio systems,Inc.) was added to lyse and collect protein samples. The experimental protein samples used Full Moon's chips and chip kits, according to standard operating procedures, the specific steps are as follows: a) sample protein extraction, b) lysis solution/labeling buffer replacement, c) protein quantification, d) sample mark.

Protein chip detection operation: follow the standard chip detection process provided by Full Moon Bio systems. a) Chip sealing, b) Chip incubation, c) Agilent SureScan Dx Microarray Scanner chip scanner, scan the chip at 532 nm, Power (100%).

Statistical analysis

The data were expressed as the mean ± standard error of mean (SEM). The statistical analysis was performed using GraphPad Prism 5.01 (California, USA). Significant differences between groups were compared using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls test. Significance was accepted at the level of $p < 0.05$.

Results

Cytotoxicity assay

The MTT assay was used to determine the safe concentration of QSC drug-containing serum and the effective stimulation concentration of Ang-II. As shown in Fig. 1A, the MTT results showed that after QSC drug-containing serum (addition ratio/concentration 0.5%, 1%) was administered, the normal growth of VSMCs was inhibited. However, after administration of 2.5% QSC drug-containing serum, the survival rate of VSMCs was greater than 97%. Compared with the control group, when the concentration of QSC drug-containing serum was 5% and 10%, it had a significant effect on promoting the proliferation of VSMCs ($P < 0.001$). Therefore, in the follow-up experiment, the concentration of QSC drug-containing serum is 2.5% within the safe concentration range.

Compared with the control group, different concentrations of Ang-II (10^{-5}, 10^{-6}, 10^{-7} mol/L) showed significant inhibitory effects on the growth of VSMCs (Fig. 1B) ($P < 0.001$). According to the needs of the experiment, we chose the most cost-effective concentration, namely 10^{-7} mol/L.

QSC drug-containing serum inhibits Ang-II-induced inflammatory factors in VSMCs

According to the results of the chip test, the treatment with QSC drug-containing serum significantly reduced the production of IL-6 induced by Ang-II ($P < 0.001$). Compared with the control group cell level (57.26 pg/mL) (Fig. 2A), after Ang-II stimulation, the IL-6 level (196.51 pg/mL) in the VSMCs supernatant

was greatly increased. The incubation of 2.5% QSC-containing serum with different doses (0.48 g/kg, 0.24 g/kg and 0.12 g/kg) after treatment significantly reduced the level of IL-6 (112.40 pg/mL, 71.02 pg/mL, 68.14 pg/mL) ($P < 0.001$), almost the same as control cell performance.,

QSC drug-containing serum inhibits the expression of ICAM-1 and VEGF induced by Ang-II in VSMCs

The chip detection data showed that compared with the control group, Ang-II induction caused a sudden increase in the expression level of ICAM-1 in the supernatant of VSMCs ($P < 0.001$). After the intervention of 2.5% QSC drug-containing serum, QSC of different doses showed an inhibitory effect on ICAM-1 (Fig. 2B), especially the high-dose QSC drug-containing serum performed best, which can sharply down-regulate the expression level of ICAM-1($P < 0.001$). Surprisingly, after Ang-II induction, the expression of VEGF in VSMCs also increased significantly (Fig. 2C) ($P < 0.001$). Interestingly, different doses of QSC-containing serum can reduce the expression level of VEGF in cells and have a certain inhibitory effect on the abnormal growth of blood vessels.

QSC drug-containing serum regulated TGF-β, VEGF, JAK-STAT and mTOR pathways

In this study, we tested the expression of multiple target-related proteins. The phosphorylation ratio and protein expression ratio in the four pathways of TGF-β, VEGF, mTOR and JAK-STAT were analyzed by heat map (High dose group/control group) (Fig. 3). In the Ang-II model group, compared with the control group, TGF-β, VEGF and other angiogenesis and cell adhesion pathway protein expression levels were significantly up-regulated. However, after intervention with 2.5% QSC-containing serum, the expression of related proteins in the TGF-β and VEGF pathways generally showed a downward trend, thereby participating in the inhibition of angiogenesis and cell adhesion. The study also found that the stimulation of VSMCs by Ang-II activated the inflammation and proliferation pathways of JAK-STAT and mTOR. Interestingly, after incubating with 2.5% QSC-containing serum, the expression levels of related proteins in the JAK-STAT and mTOR pathways were inhibited to varying degrees, which may be related to their involvement in inhibiting inflammation and proliferation.

QSC drug-containing serum inhibits Ang-II-induced abnormal proliferation of VSMCs

In the same way as the detection of cytotoxicity, we used the MTT assay to test whether the QSC drug-containing serum had an inhibitory effect on the abnormal proliferation of VSMCs induced by Ang-II. Surprisingly, compared with the control group, Ang-II induction caused VSMCs to exhibit severe abnormal proliferation (Fig. 4) ($P < 0.001$). The QSC drug-containing serum reversed this situation, and the abnormal proliferation level of VSMCs gradually returned to the normal cell level. This shows that QSC drug-containing serum can inhibit abnormal cell proliferation.

Discussion

More and more data show that traditional compound Chinese medicine has a specific effect on the treatment of chronic diseases such as atherosclerosis, hypertension and high cholesterol, which are characterized by VR[12, 14, 31]. VR refers to changes in the structure and function of blood vessels. It is not only an important pathological feature of CVD, but also the basis of disease deterioration and target organ damage[32]. Therefore, reversing VR is of great significance for the prevention and prognosis of CVDs. VSMCs are located in the middle layer of blood vessels and are the only cells in the media of blood vessels. Under normal conditions, VSMCs regulate vascular tone through contraction and relaxation, while secreting and releasing vascular regulatory factors to maintain normal blood vessel function. In a disease state, external stimulus signals cause VSMCs to proliferate excessively, causing changes in the structure of blood vessel walls[33, 34]. The migration of VSMCs from the vascular media to the intima is the most characteristic cytological event among these pathological changes. Under normal circumstances, VSMCs have no migration characteristics, but when VSMCs are converted from contractile to synthetic, they can migrate to the vascular intima and secrete a large amount of collagen matrix to the surroundings, thereby promoting the formation of neointimal[35].

As a clinical practice prescription of traditional Chinese medicine for the treatment of blood stasis syndrome, QSC's concept of "removing blood stasis and stopping bleeding, replenishing qi and replenishing qi" is consistent with the modern treatment of VR. Baicalin, the effective chemical component in QSC, also has the effect of inhibiting abnormal cell proliferation, which can significantly shorten the pathological process of CVD[36]. Studies have shown that when VSMCs develop pathological changes including proliferation, migration and inflammation, they will accelerate the progression of CVD[6]. According to reports, Ang-II can significantly change the phenotype of VSMCs and promote cell proliferation and migration, so we chose Ang-II at the appropriate concentration as the stimulant in this study to carry out the experiment[11].

In this study, the MTT assay indirectly reflects the proliferation of cells by showing the number of living cells. The light absorption value of the blank control group and a series of QSC drug-containing serum groups is significantly lower than the model group, indicating that QSC has the effect of inhibiting the proliferation of VSMCs. It revealed that QSC may improve the VR of hypertension by inhibiting the proliferation of VSMCs. In addition, it is reported that CVD and inflammation are inseparable, in which atherosclerosis is a chronic inflammatory process produced after various damages to the blood vessel wall. The injured endothelial cells and VSMCs will release the inflammatory factor IL-6. IL-6 will accelerate the abnormal proliferation and migration of cells, resulting in the over-expression of cytokines VEGF and ICAM-1, and destroy the stability of arterial plaque. Interestingly, our results show that after intervention with different concentrations of QSC drug-containing serum, compared with the control group, it can significantly inhibit the production of IL-6, VEGF and ICAM-1 induced by Ang-II stimulation. The experimental results show that QSC can effectively improve the Ang-II-induced inflammation of VSMCs and inhibit the abnormal cell proliferation.

Previous reports indicate that the activation of inflammation and angiogenesis-related pathways in VSMCs is closely related to abnormal cell proliferation and accelerated development of CVD[37, 38]. For example, patients with severe COVID-19 have been shown to have elevated serum IL-6 that plays a key role in activating the JAK-STAT signaling pathway, thereby affecting oxidative stress and normal cell growth[39]. The results of our phosphorylated antibody chip study showed that TGF-β, VEGF, mTOR and JAK-STAT pathways were actively expressed in VSMCs induced by Ang-II (Fig. 5). What is surprising is that QSC drug-containing serum administration can significantly inhibit the overexpression of multiple related proteins of TGF-β, VEGF, mTOR and JAK-STAT pathway in VSMCs. Studies have also shown that the mTOR and JAK-STAT pathways are key signaling pathways that regulate abnormal proliferation of vascular cells[40, 41]. Therefore, the protective effect of QSC on the proliferation of VSMCs induced by Ang-II may be related to the reduction of inflammatory cytokines and the inhibition of the activation of proliferation-related pathways mTOR and JAK-STAT.

Conclusion

This study shows that QSC can inhibit the activation of TGF-β, VEGF, mTOR and JAK-STAT pathways, thereby reducing the inflammatory response of VSMCs induced by Ang□, and inhibiting the abnormal proliferation of aortic VSMCs. Our results provide further evidence for QSC as a valuable therapeutic strategy for the treatment of cardiovascular diseases.

Abbreviations

QSC: Quyu Shengxin capsule; CVDs: Cardiovascular diseases; VSMCs: Vascular smooth muscle cells; Ang-II: Angiotensin □; COVID-19: Coronavirus Disease 2019; VR: Vascular remodeling; ICAM-1: Intercellular cell adhesion molecule-1; IL-6: Interleukin-6; IGSF: Immunoglobulin superfamily; VEGF: Vascular endothelial growth factor; TGF-β: Transforming growth factor-β; mTOR: Mammalian target of rapamycin; PI3K /Akt: Phosphatidylinositol 3-kinase /protein kinase B; NIH: National Institutes of Health; SEM: standard error of mean.

Declarations

Acknowledgements

We are particularly grateful to Weifeng Li for his valuable comments on the data analysis.

Author contributions

JJY and WFL contributed to the concept and design of this research. YJJ is responsible for all data collection and manuscript writing. WFL and DG provided equal support for concept, design and project funding. LTZ and YQ have done a lot of work for data analysis. XX contributed to key revisions. All authors approved the final manuscript.

Funding

This work were supported by Project of Shaanxi Key Research and Development support (No. 2018ZDXM-SF-005) and Key Laboratory Construction Project of Shaanxi Traditional Chinese Medicine Administration (No. 2018-24) (Shaanxi Province, PR China).

Data availability statement

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

Author details

[a] School of Pharmacy, Xi'an Jiaotong University, Xi'an, P.R. China.

[b] Institute of traditional Chinese Medicine, Shaanxi Academy of traditional Chinese Medicine, Xi'an, P.R. China.

*Corresponding author.

Dong Guo, Institute of traditional Chinese Medicine, Shaanxi Academy of traditional Chinese Medicine, No. 4 Xihuamen, Xi'an City, Shaanxi Province 710003, P.R China. E-mail: 787422146@qq.com

Weifeng Li, School of Pharmacy, Xi'an Jiaotong University, No. 76 Western Yanta Road, Xi'an City, Shaanxi Province 710061, P.R China. E-mail:liwf@mail.xjtu.edu.cn

References

1. Li G, Hu R, Gu XF. A close-up on COVID-19 and cardiovascular diseases. Nutrition Metabolism Cardiovascular Diseases. 2020;30(7):1057–60. http://dx.doi.org/10.1016/j.numecd.2020.04.001.
2. Shihan W, Jie W, Ji L. Relationship between the Gensini Score of Blood-Stasis Syndrome in Coronary Heart Disease and VEGF. World Science Technology. 2010;12(3):355–7. http://dx.doi.org/10.1016/s1876-3553(11)60015-x.

3. Bai D, Song JN. Plasma metabolic biomarkers for syndrome of phlegm and blood stasis in hyperlipidemia and atherosclerosis. J Tradit Chin Med, 2012, 32(4): 578–83.http://dx.doi.org/Doi 10.1016/S0254-6272(13)60074-6.
4. Buja LM, Ottaviani G, Mitchell RN. Pathobiology of cardiovascular diseases: an update. Cardiovasc Pathol. 2019;42:44–53. http://dx.doi.org/10.1016/j.carpath.2019.06.002.
5. Garcia-Redondo AB, Aguado A, Briones AM, Salaices M. NADPH oxidases and vascular remodeling in cardiovascular diseases. Pharmacol Res. 2016;114:110–20. http://dx.doi.org/10.1016/j.phrs.2016.10.015.
6. Li W, Zhi W, Zhao J, Yao Q, Liu F, Niu X. Cinnamaldehyde protects VSMCs against ox-LDL-induced proliferation and migration through S arrest and inhibition of p38, JNK/MAPKs and NF-kappaB. Vascul Pharmacol. 2018;108:57–66. http://dx.doi.org/10.1016/j.vph.2018.05.005.
7. Deng YC, Song L, Nie XQ, Shou WN, Li XH. Prenatal inflammation exposure-programmed cardiovascular diseases and potential prevention. Pharmacol Ther. 2018;190:159–72. http://dx.doi.org/10.1016/j.pharmthera.2018.05.009.
8. Satoh K, Satoh T, Kikuchi N, Omura J, Kurosawa R, Suzuki K, et al. Basigin mediates pulmonary hypertension by promoting inflammation and vascular smooth muscle cell proliferation. Circ Res. 2014;115(8):738–50. http://dx.doi.org/10.1161/CIRCRESAHA.115.304563.
9. Lu QB, Wang HP, Tang ZH, Cheng H, Du Q, Wang YB, et al. Nesfatin-1 functions as a switch for phenotype transformation and proliferation of VSMCs in hypertensive vascular remodeling. Biochimica Et Biophysica Acta-Molecular Basis of Disease. 2018;1864(6):2154–68. http://dx.doi.org/10.1016/j.bbadis.2018.04.002.
10. te Riet L, van Esch JHM, Roks AJM, van den Meiracker AH, Danser AHJ. Hypertension Renin-Angiotensin-Aldosterone System Alterations. Circ Res. 2015;116(6):960–75. http://dx.doi.org/10.1161/Circresaha.116.303587.
11. Daugherty A, Cassis L. Angiotensin II-mediated development of vascular diseases. Trends Cardiovasc Med. 2004;14(3):117–20. http://dx.doi.org/PII. S1050-1738(04)00004 – 0.
12. DOI. 10.1016/j.tcm.2004.01.002.
13. 10.1016/j.jep.2020.112767
Yu N, Shen A, Chu J, Huang Y, Zhang L, Lin S, *et al.* Qingda granule inhibits angiotensin â□□ induced VSMCs proliferation through MAPK and PI3K/AKT pathways. *Journal of Ethnopharmacology*, 2020, **258**.http://dx.doi.org/10.1016/j.jep.2020.112767.
14. Mendieta D, De la Cruz-Aguilera DL, Barrera-Villalpando MI, Becerril-Villanueva E, Arreola R, Hernandez-Ferreira E, et al. IL-8 and IL-6 primarily mediate the inflammatory response in fibromyalgia patients. J Neuroimmunol. 2016;290:22–5. http://dx.doi.org/10.1016/j.jneuroim.2015.11.011.
15. Li L, Yu AL, Wang ZL, Chen K, Zheng W, Zhou JJ, *et al.* Chaihu-Shugan-San and absorbed meranzin hydrate induce anti-atherosclerosis and behavioral improvements in high-fat diet ApoE(-/-)mice via anti-inflammatory and BDNF-TrkB pathway. *Biomedicine & Pharmacotherapy*, 2019, **115**.http://dx.doi.org/ARTN 108893.

16. 1016/j.biopha.2019.108893.
17. Bhowmick T, Berk E, Cui XM, Muzykantov VR, Muro S. Effect of flow on endothelial endocytosis of nanocarriers targeted to ICAM-1. J Controlled Release. 2012;157(3):485–92. http://dx.doi.org/10.1016/j.jconrel.2011.09.067.
18. Canavese M, Altruda F, Ruzicka T, Schauber J. Vascular endothelial growth factor (VEGF) in the pathogenesis of psoriasis–a possible target for novel therapies? J Dermatol Sci. 2010;58(3):171–6. http://dx.doi.org/10.1016/j.jdermsci.2010.03.023.
19. Krum JM, Mani N, Rosenstein JM. Roles of the endogenous VEGF receptors flt-1 and flk-1 in astroglial and vascular remodeling after brain injury. Exp Neurol. 2008;212(1):108–17. http://dx.doi.org/10.1016/j.expneurol.2008.03.019.
20. Feng J, Zhang YJ, Xing D. Low-power laser irradiation (LPLI) promotes VEGF expression and vascular endothelial cell proliferation through the activation of ERK/Sp1 pathway. Cell Signal. 2012;24(6):1116–25. http://dx.doi.org/10.1016/j.cellsig.2012.01.013.
21. Habas K, Shang LJ. Alterations in intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in human endothelial cells. Tissue Cell. 2018;54:139–43. http://dx.doi.org/10.1016/j.tice.2018.09.002.
22. DiRenzo DM, Chaudhary MA, Shi X, Franco SR, Zent J, Wang K, et al. A crosstalk between TGF-beta/Smad3 and Wnt/beta-catenin pathways promotes vascular smooth muscle cell proliferation. Cell Signal. 2016;28(5):498–505. http://dx.doi.org/10.1016/j.cellsig.2016.02.011.
23. Wang YE, Xu K, Yue WH, Xu QM, You BG, Zhang MY, et al. Hederacolchiside A1 suppresses proliferation of tumor cells by inducing apoptosis through modulating PI3K/Akt/mTOR signaling pathway. Chinese Herbal Medicines. 2018;10(2):215–22. http://dx.doi.org/10.1016/j.chmed.2018.03.007.
24. Zhang M, Zhu RR, Zhang LB. Triclosan stimulates human vascular endothelial cell injury via repression of the PI3K/Akt/mTOR axis. *Chemosphere*, 2020, **241**.http://dx.doi.org/ARTN 125077.
25. 1016/j.chemosphere.2019.125077.
26. 10.1016/j.athx.2019.100003
Catapano AL, Barrios V, Cicero AFG, Pirro M. Lifestyle interventions and nutraceuticals: Guideline-based approach to cardiovascular disease prevention. *Atherosclerosis: X*, 2019, **1**.http://dx.doi.org/10.1016/j.athx.2019.100003.
27. Liao P, Li Y, Li M, Chen X, Yuan D, Tang M, et al. Baicalin alleviates deoxynivalenol-induced intestinal inflammation and oxidative stress damage by inhibiting NF-kappaB and increasing mTOR signaling pathways in piglets. Food Chem Toxicol. 2020;140:111326. http://dx.doi.org/10.1016/j.fct.2020.111326.
28. Yan L, Song F, Li H, Li Y, Li J, He QY, et al. Submicron emulsion of cinnamaldehyde ameliorates bleomycin-induced idiopathic pulmonary fibrosis via inhibition of inflammation, oxidative stress and epithelial-mesenchymal transition. Biomed Pharmacother. 2018;102:765–71. http://dx.doi.org/10.1016/j.biopha.2018.03.145.

29. Zhang L, Wang X, Zhang L, Virgous C, Si H. Combination of curcumin and luteolin synergistically inhibits TNF-alpha-induced vascular inflammation in human vascular cells and mice. J Nutr Biochem. 2019;73:108222. http://dx.doi.org/10.1016/j.jnutbio.2019.108222.
30. Agrawal SS, Saraswati S, Mathur R, Pandey M. Brucine, a plant derived alkaloid inhibits inflammatory angiogenesis in a murine sponge model. Biomedicine Preventive Nutrition. 2011;1(3):180–5. http://dx.doi.org/10.1016/j.bionut.2011.06.014.
31. Tian HP, Lu J, He HR, Zhang L, Dong YL, Yao HP, et al. The effect of Astragalus as an adjuvant treatment in type 2 diabetes mellitus: A (preliminary) meta-analysis. J Ethnopharmacol. 2016;191:206–15. http://dx.doi.org/10.1016/j.jep.2016.05.062.
32. Sarikurkcu C, Sahinler SS, Tepe B, Astragalus gymnolobus A leporinus var. hirsutus, and A. onobrychis: Phytochemical analysis and biological activity. *Industrial Crops and Products*, 2020, **150**.http://dx.doi.org/ARTN 112366.
33. 1016/j.indcrop.2020.112366.
34. Choke E, Cockerill GW, Laing K, Dawson J, Willson WRW, Loftus IM, et al. Whole Genome-expression Profiling Reveals a Role for Immune and Inflammatory Response in Abdominal Aortic Aneurysm Rupture. Eur J Vasc Endovasc Surg. 2009;37(3):305–10. http://dx.doi.org/10.1016/j.ejvs.2008.11.017.
35. Qiao L, Chen WQ. Atheroprotective effects and molecular targets of bioactive compounds from traditional Chinese medicine. Pharmacol Res. 2018;135:212–29. http://dx.doi.org/10.1016/j.phrs.2018.07.012.
36. de Vries MR, Seghers L, van Bergen J, Peters HAB, de Jong RCM, Hamming JF, et al. C57BL/6 NK cell gene complex is crucially involved in vascular remodeling. J Mol Cell Cardiol. 2013;64:51–8. http://dx.doi.org/10.1016/j.yjmcc.2013.08.009.
37. Hall CL, Taubman MB, Nemerson Y, Turitto VT. Factor Xa generation at the surface of cultured rat vascular smooth muscle cells in an in vitro flow system. Journal of Biomechanical Engineering-Transactions of the Asme, 1998, 120(4): 484–90.http://dx.doi.org/Doi 10.1115/1.2798018.
38. Liu P, Liu L, Tang YP, Duan JA, Yang NY. A new cerebroside and its anti-proliferation effect on VSMCs from the radix of Cyperus rotundus L. Chin Chem Lett. 2010;21(5):606–9. http://dx.doi.org/10.1016/j.cclet.2010.01.012.
39. Wang N, Xu F, Lu SE, Zhang NJ, Sun YX. Septin4 as an autophagy modulator regulates Angiotensin-II mediated VSMCs proliferation and migration. Biochem Biophys Res Commun. 2020;525(2):272–9. http://dx.doi.org/10.1016/j.bbrc.2020.02.064.
40. Ju MZ, Liu YQ, Li MY, Cheng MJ, Zhang Y, Deng GZ, *et al.* Baicalin improves intestinal microecology and abnormal metabolism induced by high-fat diet. *European Journal of Pharmacology*, 2019, **857**.http://dx.doi.org/UNSP 172457.
41. 1016/j.ejphar.2019.172457.
42. Crossland H, Pereira SL, Smith K, Phillips BE, Atherton PJ. Gene-based analysis of angiogenesis, mitochondrial and insulin-related pathways in skeletal muscle of older individuals following

nutraceutical supplementation. Journal of Functional Foods. 2019;56:216–23. http://dx.doi.org/10.1016/j.jff.2019.03.022.

43. Wang BB, Zhang LJ, Liu JY, Ma L, Wang HW, Zheng NB, et al. Chlamydia pneumoniae infection promotes vascular endothelial cell angiogenesis through an IQGAP1-related signaling pathway. Int J Med Microbiol. 2017;307(4–5):276–86. http://dx.doi.org/10.1016/j.ijmm.2017.03.001.
44. Luo W, Li YX, Jiang LJ, Chen Q, Wang T, Ye DW. Targeting JAK-STAT Signaling to Control Cytokine Release Syndrome in COVID-19. Trends Pharmacol Sci. 2020. http://dx.doi.org/10.1016/j.tips.2020.06.007.
45. Peng K, Fan X, Li QN, Wang YY, Chen XL, Xiao PX, et al. IRF-1 mediates the suppressive effects of mTOR inhibition on arterial endothelium. J Mol Cell Cardiol. 2020;140:30–41. http://dx.doi.org/10.1016/j.yjmcc.2020.02.006.
46. Zhang L, Shao J, Zhou Y, Chen H, Qi H, Wang Y, et al. Inhibition of PDGF-BB-induced proliferation and migration in VSMCs by proanthocyanidin A2: Involvement of KDR and Jak-2/STAT-3/cPLA2 signaling pathways. Biomed Pharmacother. 2018;98:847–55. http://dx.doi.org/10.1016/j.biopha.2018.01.010.

Tables

A3

Table 1

The putative major ingredientsand major targets of 6 herbs.

Chinese name	Latin name	Part used	Major ingredients
□□	Radix Astragali seu Hedysa	Dried root	Astragaloside IV, Astragalus polysaccharide
□□	Cinnamomum cassia Presl	Dried root	Coumarin, Cinnamic acid, Cinnamaldehyde
□□	Rhizoma Sparganii	Dried root	Benzeneethanol, 3,4- dihydro-8-Hydroxy-3-methyl-1H-2-benzopyran-4-one, Dehydrocostuslactone
□□	Curcuma zedoaria (Christm.) Rosc	Dried root	Curcumin, Curcumol, Curdione
□□□	Rhizoma Bolbostematis	Dried tuber	Tubeimoside
□□□□	Semen Strychni Praeparata	Dried seed	Strychnine

Figures

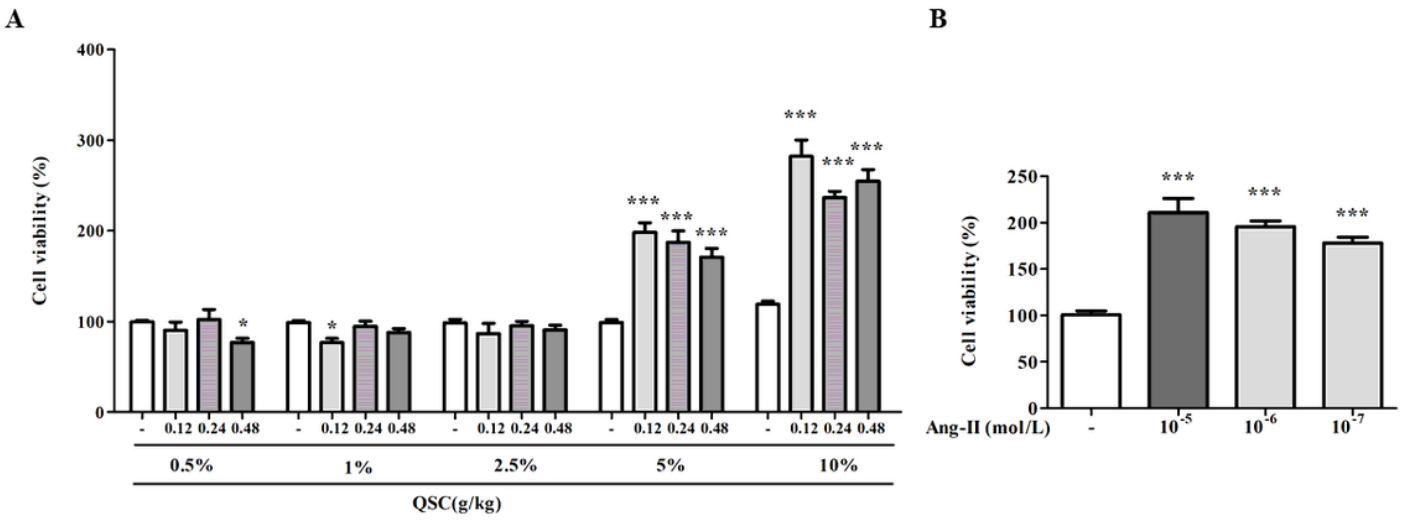

Figure 1

The safe concentration range of QSC drug-containing serum for normal growth of VSMCs and the Ang-II experimental concentration were screened. (A) After VSMCs were incubated with different proportions of QSC drug-containing serum (0.5%, 1%, 2.5%, 5% and 10%). The cell viability was detected by MTT assay. (B) After VSMCs were incubated with different concentrations of Ang-II (10-5, 10-6, 10-7 mol/L). The cell viability was detected by MTT assay. All the values are represented by the means ± S.E.M. *P < 0.01, **P < 0.01 or ***P < 0.001 compared with the control group.

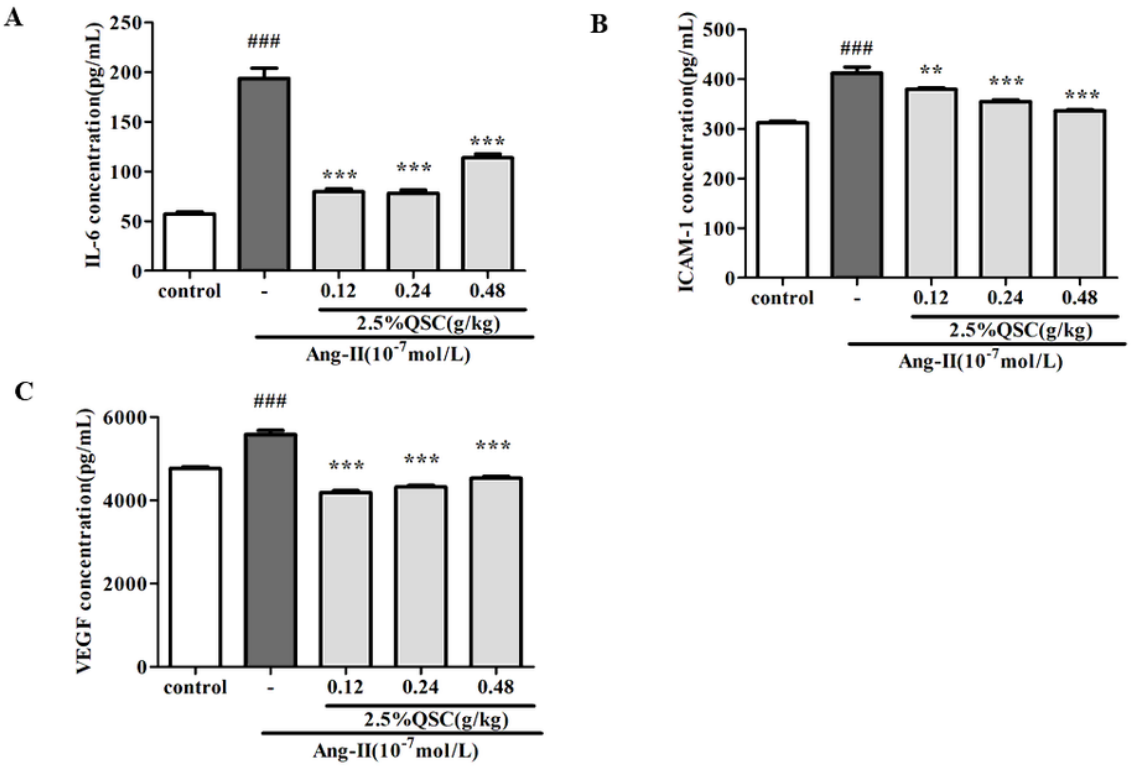

Figure 2

QSC drug-containing serum inhibited the production of IL-6, VEGF and ICAM-1 in VSMCs treated with Ang-II. VSMCs were pretreated with 2.5% QSC drug-containing serum (0.48 g/kg, 0.24 g/kg and 0.12 g/kg) for 24 h, and then stimulated with Ang-II (10-7 mol/L) for 24 h. (A)The level of IL-6 was determined by Luminex liquid suspension chip detection platform. (B) The level of VEGF was determined by Luminex liquid suspension chip detection platform. (C) The level of ICAM-1 was determined by Luminex liquid suspension chip detection platform. All the values are represented by the means ± S.E.M. #P < 0.05, ##P < 0.01 or ###P < 0.001 compared with the control group. *P < 0.01, **P < 0.01 or ***P < 0.001 compared with the Ang-II-treated group.

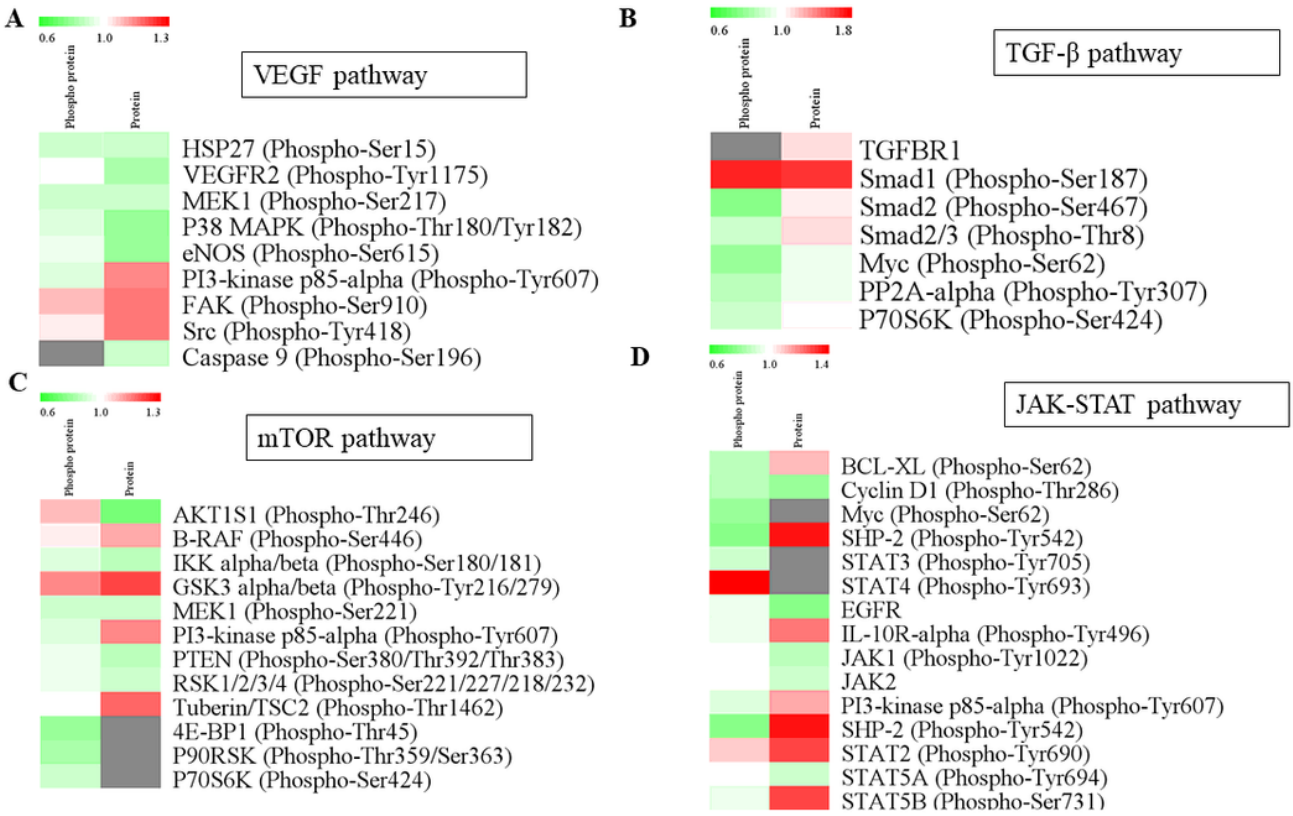

Figure 3

Bioinformatics analysis of QSC to Ang-II-induced VSMCs protein supernatant. A heatmap has been drawn for the proteins whose phosphorylation ratio and protein expression ratio in the four pathways of VEGF (A), TGF-β (B), mTOR (C), and JAK-STAT (D) are highly modulated. From the graphic level, the chip detects the comparison of the modulation of the phosphorylation ratio and the protein expression ratio of the protein intuitively, so as to find the protein with regular expression and perform more in-depth protein function analysis. In the heatmap, the red color block represents a large ratio value, the green color block represents a small ratio value, and white color represents a ratio of 1. The stronger the red or green degree, the greater the modulation. This time, using Mev software, the phosphorylation ratio and protein expression ratio in the four pathways of TGF-β, VEGF, mTOR and JAK-STAT were analyzed by heat map.

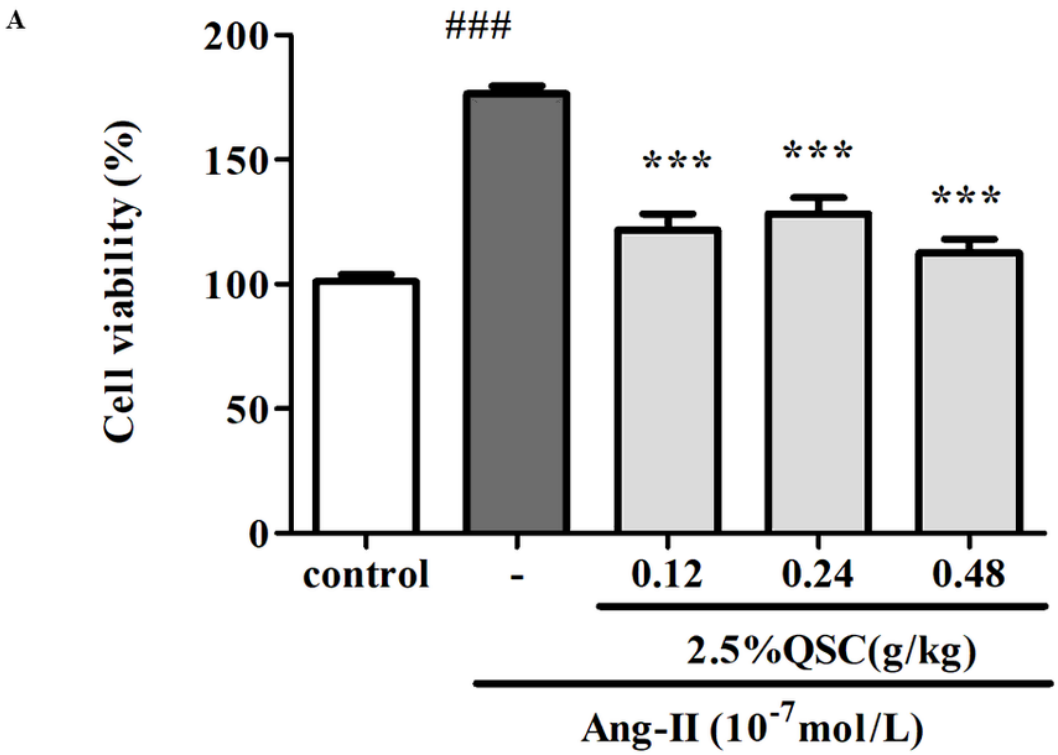

Figure 4

QSC drug-containing serum inhibited Ang-II treated VSMCs proliferation. (A) VSMCs were incubated with 2.5% QSC drug-containing serum (0.48 g/kg, 0.24 g/kg and 0.12 g/kg) before stimulated with 10-7 mol/L Ang-II for 24 h. Cell viability was measured by MTT assay.

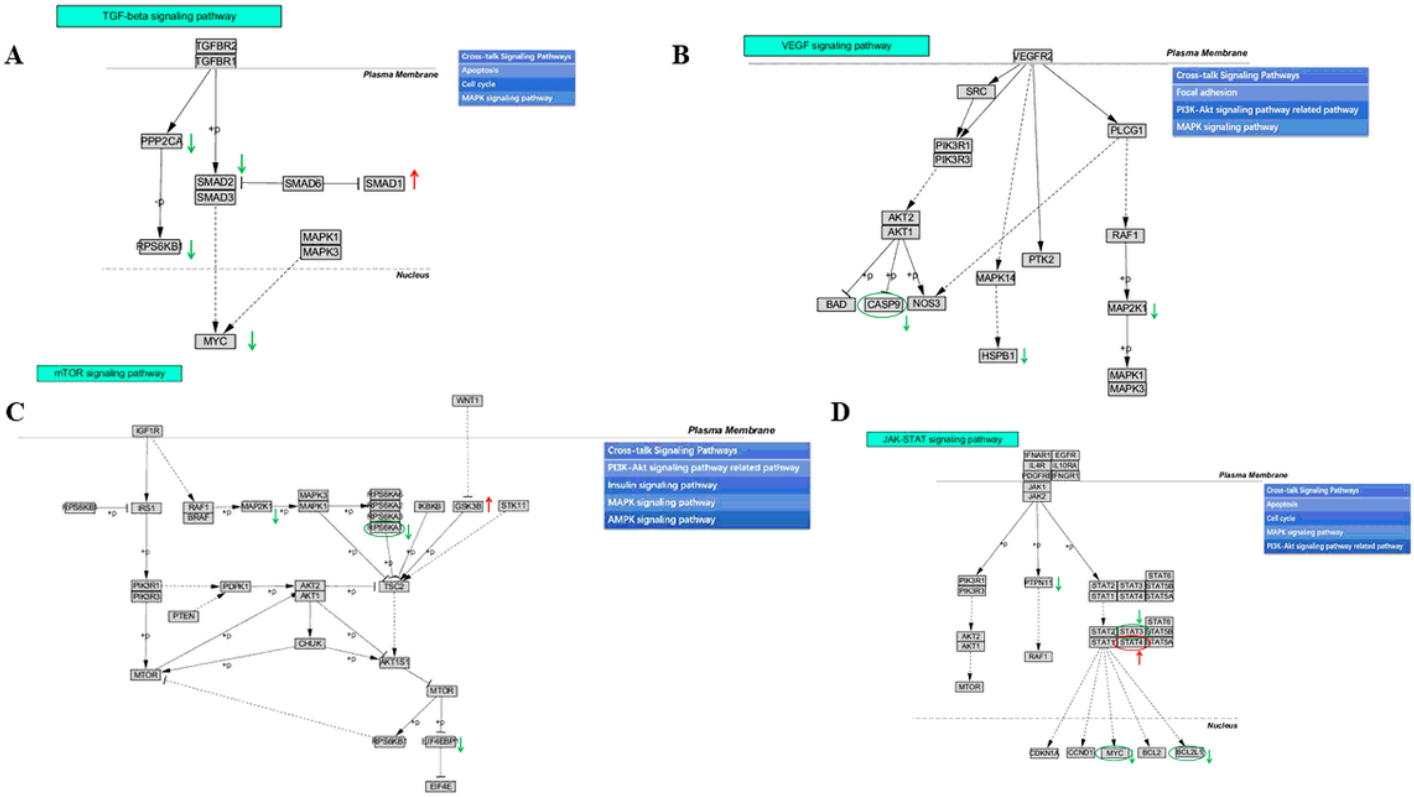

Figure 5

Establish a target network for targets with different phosphorylation ratios and protein expression ratios in the four pathways of TGF-β (A), VEGF (B), mTOR (C) and JAK-STAT (D). The oval node represents the target, and the rectangular node represents the predicted possible target. The red up arrow indicates that the protein expression is increased, and the green down arrow indicates that the protein expression is decreased.