Effects of extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) on F-actin in senescent microvascular endothelial cells

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**Abstract**

**OBJECTIVE:** To investigate the effects of extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) on the endothelial actin cytoskeleton in senescent human cardiac microvascular endothelial cells (HCMECs), and to propose the possible mechanism underlying the actions.

**METHODS:** Lentiviral mediated RNA interference was applied to a replicative senescent HCMEC model by knocking down heat shock protein 27 (HSP27) gene. Cells were treated with extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) at final concentrations of 50, 100, and 200 mg/L, respectively and with 10 μM resveratrol for 48 h. Untreated cells were used as controls. Senescence was detected by senescence β-galactosidase staining and cell proliferation was analyzed by cell counting kit-8 assays. Secreted nitric oxide levels were detected by nitrate reductase. Morphological changes of F-actin were observed by laser scanning confocal microscopy. Protein and gene expression of F-actin and HSP27 was detected by western blotting.

**RESULTS:** Compared with the control group, the proportion of senescent HSP27 shRNA cells treated with the extracts was decreased and their proliferation was increased. In the extract intervention group, F-actin around the cell periphery became irregular and jagged fractures formed gradually and then dissipated. Moreover, some dynamic actin stress fiber filaments appeared. The G-actin stretched to the cell periphery and punctate staining was scattered in the cytoplasm. In addition, the mean optical density value of F/G-actin was decreased significantly and the protein expression of F-actin was downregulated.
CONCLUSION: The extracts delayed microvascular endothelial cell senescence by downregulating the expression of F-actin through HSP27.

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Keywords: Endothelial cells; Aging; Actin cytoskeleton; HSP27 heat-shock proteins; Renshen (Radix Ginseng); Sanqi (Radix Notoginseng); Chuanxiong (Rhizoma Chuanxiong)

INTRODUCTION

Estimates of vascular aging (VA) have been found to be closely related to chronological age or as Thomas Sydenham stated, “A man is as old as his arteries”. VA accelerates the body’s aging process and is associated with the occurrence of various diseases including atherosclerosis. It is characterized by functional and structural changes of the endothelium and smooth muscle cells that form the vascular wall. The main aspects of VA include increased arterial stiffness, dilation of central elastic arteries, impaired endothelial dysfunction, and vasodilatation. Aging is considered as an independent factor associated with endothelial cell dysfunction, even in the absence of other cardiovascular risk factors. In addition, biological changes in endothelial cells are considered as the foundation of human aging and disease, and endothelial cell dysfunction is the major characteristic of VA.

Cellular senescence, which is defined as permanent proliferative cell cycle arrest that is resistant to growth factors and other signals that induce cell proliferation, contributes to the physiological processes of normal organismal aging. Replicative senescence was first reported as a state of long-term growth inhibition in viable diploid human fibroblasts in vitro. In 1965, Hayflick discovered that cultured normal human cells had a limited capacity to divide, after which they stop growing and become enlarged. Cellular senescence has been used a cellular model to understand the mechanisms underlying the aging process. The characteristics of aging cells are characterized by loss of their proliferative capacity, an increased volume, flat cell body, and the proportion of senescent cells increases significantly.

Microfilaments, microtubules, and intermediate filament proteins are essential components of the vascular endothelial cell cytoskeleton. Among these proteins, microfilaments, including monomeric actin (globular or G-actin) and filamentous actin (F-actin) have the most prominent roles in regulating the endothelial cell structure and function. Endothelial cell senescence is characterized by dysfunction of the endothelial cell junction, apoptosis, changes in cell permeability, intracellular accumulation of oxidation products, and inflammation. The actin cytoskeleton has an important role in the maintenance of endothelial cell morphology and permeability, endothelium-dependent contraction and relaxation, and apoptosis. In addition, it has a crucial role in mediating cell responses to both internal and external signals. These findings suggest that the actin cytoskeleton has an important role in endothelial cell senescence.

Heat shock protein (HSP) 27 is a member of the small HSP family with a molecular weight of approximately 27 kDa. The main functions of HSP27 include F-actin modulation and polymerization inhibition. Expression of HSP27 decreases the intracellular levels of reactive oxygen species (ROS), abolishes the burst of intracellular ROS induced by transforming growth factor-α (TNF-α), and protects cells against angiotensin 2 induced inflammation. Aging has been associated with characteristic changes in gene expression, particularly HSP expression. Recently, HSPs have been used as biomarkers for aging. Nevertheless, the exact role and effects of HSP27 on the actin cytoskeleton in senescent cells remain unclear.

It is believed that some Chinese herbs have anti-aging properties. Such herbs in traditional Chinese medicine may boost vital energy of the human body. They may cure disease, relieve symptoms, and modulate multiple pathological aspects by a holistic approach. Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong), which are herbal medicines, balance Qi and activate blood circulation. Their active constituents are closely associated with anti-aging effects.

In this study, we investigated the effects of extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) on the actin cytoskeleton in senescent HCMECs and provide the possible mechanism underlying the actions.

METHODS

The Minimum Standards of Reporting Checklist contains the details of the experimental design, statistics, and resources used in this study. The ratio of Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) was 2: 3: 4. After the original herbal medicine was mixed, the preparation process of the dry paste powder included 70% alcohol extraction, concentration to a density of 1.20-1.30, and vacuum drying under reduced pressure. A total of 1 g dry paste powder was derived from 4.286 g crude herbal medicine. The main constituents of the dry paste powder are: Ferulic Acid 1.00 mg/g, Notoginsenoside R1 8.45 mg/g, Ginsenoside Rg1 55.84 mg/g, Ginsenoside Re 6.47 mg/g, Ginsenoside Rb1 44.57 mg/g. They were dissolved in 0.9% phosphate buffered saline (PBS) at 25 mg/mL as a stock solution and diluted with cell culture medium before use. Resveratrol was purchased from the China National Institute for Food.
and Drug Control (No. 111535-200502). Materials used in the experiments included Endothelial Cell Medium (ScienCell, CA, USA), antibodies against HSP27 and F-actin (abcam, UK), DNAase I Alexa Fluor 488, Rhodamine 594 Phalloidin (Molecular Probes, MA, USA), and a HRP-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch, WP, USA).

**Cell culture and treatments**

HCMECs and human embryonic kidney 293 cells were purchased from ScienCell and the American Type Culture Collection, respectively. HCMECs were cultured in Endothelial Cell Medium supplemented with 5% fetal bovine serum, 1% endothelial cell growth supplement, and 1% penicillin/streptomycin solution in a humidified atmosphere containing 5% CO2 at 37 °C. Through passaging, primary cells exhibited replicative senescence.

Cells were divided into groups with and without knockdown. The groups without knockdown included a passage 8 cells, resveratrol group, herbal treated low dose group (50 mg/L), herbal treated intermediate dose group (100 mg/L), and herbal treated high dose group (200 mg/L). The knockdown group included a control group, HSP27 shRNA group (passage 8 cells), HSP27 shRNA + resveratrol group, HSP27 shRNA + herbal treated low dose group, HSP27 shRNA + herbal treated intermediate dose group, and HSP27 shRNA + herbal treated high dose group. The treatments were conducted for 48 h.

**shRNA constructs, lentiviral infection, and siRNA transfection**

HSP27 shRNA-specific interference sequences were designed by primer 5.0 software (Primer-E Ltd., Plymouth, UK) (Table 1). The oligoDTs of interference sequences were synthesized by Sangon Biotech (Shanghai, China). Single stranded oligonucleotides were annealed at 60 °C. After ligation, the interference sequence was ligated into the Lenti-KDP vector. Lentiviral particles were produced by cotransfecting psPAX2 and pMD2 plasmids. After puromycin selection, the interfering sequence was ligated into the Lenti-KDP vector. Lentivirus was used to infect cells and a large number of cells that HSP27 gene knockdown were prepared.

**SA-β-gal staining**

HCMECs were fixed and stained for senescence-associated β-gal (SA-β-gal) activity, according to a previously described approach.[23] Briefly, cells were washed in phosphate buffered saline (PBS), fixed for 5 min at room temperature in 4% formaldehyde, washed twice, incubated for 12 h at 37 °C with fresh SA-β-Gal (Genmed, Shanghai, China) stain solution, and then observed under an inverted microscope.

**Cell proliferation assays**

A total of 6-8 × 104 cells in 100 μL medium/well were cultured in 96-well plates at 37 °C for 24 h. At 70% confluence, 10 μL of cell counting kit-8 (CCK-8) solution (Dojindo, Japan) was added to each well, followed by incubation for an additional 2 h. The absorbance at 450 nm was determined using a microplate reader.

**Measurement of nitric oxide**

Nitric oxide (NO) content in endothelial cells was measured using a nitrate reductase kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), following the manufacturer’s instructions. The reaction mixtures were incubated at room temperature for 10 min. A magenta color was developed and the absorbance was measured in a plate reader with a 550 nm filter (Bio-Rad) within 30 min.

**Immunofluorescence confocal microscopy**

The morphological changes of F/G-actin in endothelial cells were measured as described previously.[22,31] To visualize the distributions of F/G-actin, the cells were cultured until 70% confluence in black 96-well plates. After washing with PBS for 5 min three times, fixed cells were permeabilized with 0.1% Triton X-100 (Solarbio, Beijing, China) in PBS for 5 min at room temperature and then washed three times with PBS. Cells were then double stained with DNAase I AlexaFluor 488 and Rhodamine 594 Phalloidin to colocalize monomeric

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**Table 1** HSP27 shRNA-specific interference sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>HSP-1F</td>
<td>CCGG CAGTCCACAGGAGTACCATCT CTCGAG GATGTTGATCTCGTTGACCTG TTTTTG</td>
</tr>
<tr>
<td>HSP-1R</td>
<td>AAATCCAATAGAGTTCCAGGATGACCATCACCCAT CTCGAG GATGTTGATCTCGTTGACCTG</td>
</tr>
<tr>
<td>HSP-2F</td>
<td>CCGG GATCCACATCCACGTCACCCTT CTCGAG AGGGTACTGGGATGTTGATC ATTTTG</td>
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<td>AAATCCAATAGAGTTCCAGGATGACCATCACCCAT CTCGAG AGGGTACTGGGATGTTGATC ATTTTG</td>
</tr>
<tr>
<td>HSP-3F</td>
<td>CCGG CCGGAGGAGCCTGGACGCTGCAAA CTCGAG TTGACCGCTACGCTGTCCGGG TTTTTG</td>
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<tr>
<td>HSP-3R</td>
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<tr>
<td>HSP-4R</td>
<td>AAATCCAATAGAGTTCCAGGATGACCATCACCCAT CTCGAG TTGACCGCTACGCTGTCCGGG TTTTTG</td>
</tr>
</tbody>
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Notes: HSP: heat shock protein; shRNA: short hairpin Ribonucleic Acid.
globular actin (green, G-actin) and polymerized filamentous actin (red, F-actin), respectively. The unbound fluorescent probe was rinsed with PBS and the cells were visualized and photographed under a Leica fluorescence microscope.

**Western blotting**
Standard procedures for Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed according to previously described methods. Equal amounts of proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, blocked with 5% albumin from bovine serum (BSA) in TBS-Tween 20 (0.5%) for 1 h, and then incubated with primary antibodies against HSP27 and F-actin in 5% BSA in TBS-Tween 20 at 4 °C overnight. The membranes were then washed in PBS with 0.1% Tween 20 and then incubated with an HRP-conjugated goat anti-rabbit secondary antibody, followed by further washing. The membrane was then developed with ECL reagent (Millipore, MA, USA) and exposed on film.

**Data analysis**
Quantitative data were expressed as the mean±standard deviation ( ±s). The t-test, Spearman’s correlation, Fisher’s exact test, and χ² test were conducted with SPSS 18.0 (SPSS Inc., Released 2009. SPSS Statistics for Windows, Version 18.0. Chicago, IL, USA). P < 0.05 was considered as statistically significant.

**RESULTS**

**Establishment of a replicative senescence model in HCMECs**

Compared with passage 5 cells, passage 8 cells were sparse on the plate and grew slowly. The morphology of these cells was mainly characterized by cellular enlargement and flattening with a concomitant increase in the size of the nucleus and nucleoli. In addition to the morphological changes, a higher number of multinucleated cells were observed among passage 8 cells (Figure 2A). In addition, the rates of SA-β-gal-positive cells among passage 8 cells were significantly higher compared with passage 5 cells (mean SI: passage 5 cells, 10.2 ± 2.0; passage 8 cells, 80.5 ± 2.8; P < 0.01, t-test) (Figure 2B, 2C). Moreover, significantly reduced cell proliferation was observed in passage 8 cells compared with passage 5 cells [mean staining index (SI): passage 5 cells, 0.90±0.09; passage 8 cells, 0.71 ± 0.05; P < 0.01, t-test] (Figure 2D). Therefore, passage 8 cells were selected as replicative senescence model cells and used to further explore the mechanism of senescence.

**Effects of the extracts on SA-β-gal expression, cell proliferation, and NO production in senescent cells**

The extracts in our study may induce cell growth and exert anti-aging effects on aging cells. To evaluate the effects of the extracts on HCMECs, we performed SA-β-gal staining and cell viability assays. The extracts decreased the proportion of SA-β-gal-positive cells (P < 0.01, t-test) and promoted cell proliferation after 48 hours (P < 0.01, t-test) (Figure 3C, 3D). Next, we investigated changes of actin in the regulation of endothelial cell functions by examining the levels of

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**Figure 1** Expression levels of HSP27 protein in cells when infected with lentiviruses carrying various gene sequences. The expression level of HSP27 protein in cells was the lowest when infected with the lentivirus carrying the HSP2 gene sequence.

**Figure 2** Change of morphology, senescence staining, and proliferation changes during cell passaging. A1-A2: cell morphology (×200); A1: passage 5 cells; A2: passage 8 cells. Compared with passage 5 cells, passage 8 cells grew slowly and were sparse. The morphology of passage 8 cells was mainly characterized by cellular enlargement and flattening with a concomitant increase in the size of the nucleus and nucleoli. B1-B2: the senescence staining (×100); B1: passage 5 cells; B2: passage 8 cells. C: the rates of SA-β-gal-positive cells among passage 8 cells were significantly higher compared those among passage 5 cells. P < 0.01 vs passage 5 cells. D: cell proliferation between different groups. Proliferation of passage 8 cells was significantly reduced compared with that of passage 5 cells. *P < 0.01 vs passage 5 cells.
endothelium-dependent vasodilator factors. NO is the main vasodilator produced by the endothelium that has a protective role in the vessel wall. Endothelial cell dysfunction associated with aging affects the ability of the vascular endothelium to release NO.34 We found a decrease of NO levels in passage 8 cells compared with those in passage 5 cells [(21 ± 3) vs (68 ± 6) μmol/L; \( P < 0.01, t\)-test] However, after treating the cells with the extracts, an increase of NO levels was observed after 48 h (\( P < 0.05 \)). These results suggested that the extracts reduced the proportion of SA-β-gal-positive cells, promoted cell proliferation, and stimulated NO production in aging cells (Figure 3E, 3F).

**F/G-actin morphological changes**

Some studies have indicated that changes in the dynamic state of actin influence cell longevity.55 To observe actin morphological changes in aging endothelial cells, the cells were double stained with DNAse I Alexa fluor 488 and rhodamine phalloidin to colocalize G-actin (green) and polymerized F-actin (red), respectively. In passage 5 cells, F-actin was predominantly localized along the cell periphery, showing the outline of a typical cobblestone, while G-actin was mainly distributed in the central region of the cell, showing a mellow clear boundary as a dense oval shape with a slender tail. In passage 8 cells, F-actin became irregular, had saw-tooth-like fractures, and tended to disintegrate. The distribution of G-actin was transferred to the surrounding area, the cell staining was weakened in the central region, and some staining intensity was recovered in senescent cells, although the distance between the cells was increased significantly (Figure 4A). The optical density ratio of F/G-actin was decreased in these cells (mean SI: passage 5 cells, 1.79 ± 0.48; passage 8 cells, 0.79 ± 0.06; \( P < 0.01, t\)-test) (Figure 4B). In addition, after 48 h of pretreatment with the extracts, stress fibers disappeared and G-actin was centrally located and clearly visible (Figure 4A). Compared with passage 8 cells, the optical density ratio of F/G-actin was also re-

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**Figure 3** Effects of the extracts on aging, proliferation, and NO production in the replicative senescence model of microvascular endothelial cells.

A: the viability of HMECs treated with the extracts. HMECs were incubated with the extracts (25-1000 μg/L) for 48 h. \( P < 0.05 \) and \( P < 0.01 \) vs control group; B: the viability of HMECs treated with the resveratrol. HMECs were incubated with resveratrol (0.1-100 μmol/L) for 48 h. \( P < 0.01 \) vs control group. C1-C6: the senescence staining (x100); C1: passage 5 cells; C2: passage 8 cells; C3: resveratrol group (10 μmol/L); C4: low dose extract group (50 μmol/L); C5: intermediate dose extract group (100 μmol/L); C6: high dose extract group (200 μmol/L). The treatment time was 48 h. D: the rates of SA-β-gal-positive cells among groups. Compared with passage 8 cells, extracts decreased the proportion of SA-β-gal-positive cells. \( P < 0.01 \) vs passage 8 cells. E: cell proliferation between different groups. Compared with passage 8 cells, extracts promoted cell proliferation after 48 h. \( P < 0.01 \) vs passage 8 cells. F: NO content in cell culture medium. Compared with passage 8 cells, Extracts stimulated NO production after 48 h. \( P < 0.05 \) and \( P < 0.01 \) vs passage 8 cells.

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duced significantly after pretreatment with the intermediate/high dose extracts for 48 h (P < 0.05) (Figure 4B).

**Effects of the extracts on protein expression and the integrity of F-actin in HSP27 knockdown cells**

Neuronal expression of HSP27 improves lifespan and increases resistance to oxidative stress in Drosophila. In addition, HSP27 levels in cardiomyocytes may be a determinant of longevity in rodents. However, its effect on vascular aging remains unclear. To assess the effect of HSP27 on the cytoskeleton, we investigated whether changes in HSP27 expression altered actin levels in HCMECs. Passage 8 cells were transduced by lentiviral infection. The levels of F-actin were significantly decreased in passage 8 HSP27 knockdown cells (P < 0.01) (Figure 5A, 5B). We next determined the effect of the extracts on F-actin expression in these cells and found significant reduction in protein expression compared with control groups after 48 h of treatment (P < 0.01) (Figure 5A-5C). In addition, compared with control groups, F-actin around the cell periphery became irregular. The jagged fracturing occurred gradually and tended to dissipate. Moreover, some dynamic actin stress fiber filaments appeared in these cells. The G-actin-surrounding area of cells stretched to the cell periphery and punctate staining was scattered in the cytoplasm (Figure 6A). The mean optical density value of F/G-actin was decreased (P < 0.05) (Figure 6B). These data suggested that the decreased protein expression level of actin caused by the extracts may, at least in part, contribute to actin remodeling by HSP27.

**DISCUSSION**

In this study, we investigated the effects of extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) on the actin cytoskeleton in senescent HCMECs to provide the possible mechanism underlying the actions. Our findings were as follows: (a) F-actin aggregated from the edge to the middle and formed stress fibers in aging endothelial cells; (b) monomeric G-actin was transferred to the surrounding area of replication-aged endothelial cells; (c) destruction of the microfilament structure and stress fiber formation were regulated by protein expression of HSP27; (d) the efficacy of the extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) to delay endothelial aging was closely related to intracellular microfilament changes; (e) regulation of the actin cytoskeleton was related to protein expression of HSP27.

VA refers to the pathological changes of the arterial wall during aging. Common pathological changes include thickening of the vessel wall and luminal enlargement, which in turn decrease arterial elasticity and compliance. Vascular aging is a gradual process involving biochemical, enzymatic, and cellular changes of the vasculature, and modification of the signals that modulate them. Increased arterial stiffness, dilation of central elastic arteries, impaired endothelial functions, and vasodilatation are dominant aspects of this premature process. The changes of vascular bed functions and structure during VA mainly involve changes of endothelial cells, smooth muscle cells, and their signal transduction pathways. Vascular dysfunction in turn leads
Figure 5 Protein expression of F-actin as a result of changes in HSP27 protein expression
A, B, C: Western blot analysis of F-actin protein expression in all groups. Cells were divided into groups with and without knockdown. 1-6: the knockdown group in turn included control group, HSP27 shRNA group (passage 8 cells), HSP27 shRNA + resveratrol group, HSP27 shRNA + herbal treated low dose group, HSP27 shRNA + herbal treated intermediate dose group, and HSP27 shRNA + herbal treated high dose group. 7-11: the groups without knockdown in turn included passage 8 cells, resveratrol group, herbal treated low dose group (50 mg/L), herbal treated intermediate dose group (100 mg/L), and herbal treated high dose group (200 mg/L). The treatments were conducted for 48 h. \( P < 0.01 \) vs passage 8 cells without knockdown; \( P < 0.01 \) vs herbal treated low dose group without knockdown; \( P < 0.01 \) vs herbal treated intermediate dose group without knockdown; \( P < 0.01 \) vs herbal treated high dose group without knockdown. \( P < 0.01 \) vs control group when HSP gene knocked down.

Figure 6 Changes in F/G-actin morphology and optical density in passage 8 cells compared with extract pretreatment for 48 h in HSP27 knockdown cells
A, B, C: changes of F/G-actin morphology and optical density in passage 8 cells compared with extract pretreatment for 48 h in HSP27 knockdown cells (×200). Cells were divided into groups with and without knockdown. A1-A5: the groups without knockdown in turn included passage 8 cells, resveratrol group, herbal treated low dose group (50 mg/L), herbal treated intermediate dose group (100 mg/L), and herbal treated high dose group (200 mg/L). A6-A11: the knockdown group in turn included HSP27 shRNA group (passage 8 cells), HSP27 shRNA + resveratrol group, HSP27 shRNA + herbal treated low dose group, HSP27 shRNA + herbal treated intermediate dose group, and HSP27 shRNA + herbal treated high dose group, control group. \( P < 0.05 \) and \( P < 0.01 \) vs control group when HSP27 gene knocked down. \( P < 0.05 \) vs passage 8 cells without knockdown; \( P < 0.01 \) vs herbal treated low dose group without knockdown; \( P < 0.01 \) vs herbal treated intermediate dose group without knockdown; \( P < 0.01 \) vs herbal treated high dose group without knockdown.
to changes in the vascular structure and aging-related diseases. Endothelial cells are an important component of blood vessels, which form a semi-permeable barrier between blood and vascular smooth muscle. The integrity of the structure and functions has an important role in hemodynamics and the regulation of cell proliferation. Changes during endothelial cell senescence include endothelial cell dysfunction, apoptosis, a permeability change, and accumulation of intracellular oxidation products. In addition to the ion barrier, the cytoskeleton is closely related to the permeability of vessels. In the pathological state of ischemia, as with other cytokines and inflammatory mediators, the fibrous actin skeletal recombination of endothelial cells is a major pathological basis, which causes the increase in endothelial permeability and decrease of endothelial cell functions. Endothelial cell aging causes endolium-induced vasoconstriction and diastolic dysfunction. Such vasoconstriction and diastolic dysfunction are mainly characterized by a progressive reduction of the bioavailability of NO and an increase in the production of cyclooxygenase-derived vasoconstrictor factors. Endothelial nitric oxide synthase (eNOS) is associated with actin. Its polymerization state has an important role in the regulation of eNOS activity. In fact, changes in the cytoskeleton are not only the result of apoptosis, but can also affect the process of apoptosis. Both microfilaments and microtubules are involved in the process of apoptosis initiation. The prevention of F-actin rearrangement by Rho-kinase inhibition or cytochalasin D treatment attenuates I/R-induced endothelial cell apoptosis by maintaining PI3-kinase and Akt activities. In this study, we found that stress fibers were decreased, F/G-actin was increased, and NO secretion, SA-β-gal, and cell proliferation were observed. These results suggested that microfilaments have an important role in the maintenance of endothelial cell functions and the effect may be associated with changes in the NO level.

HSP27 belongs to the family of small heat shock proteins in mammals. Its molecular weight is about 27 kDa. As an ATP-dependent chaperone, the expression of HSP27 can prevent cell damage caused by heat shock, oxidative stress, chemotherapeutic drugs, and TNFα. Phosphorylation of HSP27 leads to cytoskeletal rearrangements and changes in cellular functions. Specifically, phosphorylation of HSP27 upregulates protein expression levels of F-actin. Activation of Rho depends on activation of p38 mitogen-activated protein kinase (MAPK) and expression of HSP27. As a ubiquitous and highly conserved molecular chaperone, HSP27 promotes the conformational maturation of many protein kinases, and thus inhibition of HSP27 functions leads to the breakdown of the stable form of protein kinases. Alford et al found that HSP27 was essential for gene expression of proinflammatory cytokines by gene silencing, whereas overexpression of the heat shock protein reduced NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation. Therefore, HSP27 regulates the upstream proteins Rho GTPases, MAPKs, protein kinase c, and inflammatory cytokines that are closely related to the cytoskeleton. HSP27 is a member of the small HSP family with a molecular weight of approximately 27 kDa. Although the regulation of small HSP family about F-actin has been reported in concurrent studies, the regulation of HSP27 about F-actin has been rarely studied. This study found that F-actin around the cell periphery became irregular and jagged fracturing occurred gradually, which showed a tendency to dissipate after expression of HSP27 was knocked down. It is possible that the heat shock protein is the upstream product of F-actin expression.

Recent pharmacological studies on anti-aging drugs have shown that Traditional Chinese Medicine mainly exerts anti-aging effects through anti-lipid peroxidation, scavenging free radicals, regulating glucose and lipid metabolisms, nerve-endocrine functions, the immune system, DNA damage, and prolonging cell life. The active components in anti-aging Traditional Chinese Medicine are glycosides, polysaccharides, polyphenols, alkaloids, amino acids, volatile oil, flavonoids, sterols, organic acids, and trace elements. The herbs used in this study were Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong). They have anti-oxidative and inflammatory properties, regulate the immune system and glucose metabolism, and affect the expression of cell cycle regulators and aging genes. This study mainly explored the anti-aging effect of Chinese medicine from the perspective of the cytoskeleton, which showed the ability to maintain F-actin distribution in the cell periphery and reduce the formation of stress fibers. Moreover, the extracts downregulated the expression of F-actin. The mechanism through which the extracts delayed microvascular endothelial cell aging may involve downregulation of the expression of F-actin by HSP27.

In conclusion, extracts from Renshen (Radix Ginseng), Sanqi (Radix Notoginseng), and Chuanxiong (Rhizoma Chuanxiong) delay microvascular endothelial cell aging and downregulate the expression of F-actin via HSP27.

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