Smilacis Glabrae Rhizoma Reduces Oxidative Stress Caused by Hyperuricemia via Upregulation of Catalase

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Key Words
Hyperuricemia • Bioinformatic analysis • Smilacis Glabrae Rhizoma • Oxidative stress • Catalase

Abstract
Background/Aims: Reports have suggested that the traditional Chinese medicine Smilacis Glabrae Rhizoma attenuates hyperuricemia, but its mechanism is unclear. Our previous study demonstrated that uric acid could induce the generation of reactive oxygen species (ROS), which subsequently cause endothelial dysfunction. Therefore, we focused on the oxidative stress process. In this study, we would use LC-MS and bioinformatic analysis to investigate the underlying mechanism. Methods: We utilized LC-MS to reveal the differential protein expression in the kidneys of rats in the hyperuricemia group and the Smilacis Glabrae Rhizoma treatment group and then subjected the differentially expressed proteins to bioinformatic analysis. We also determined the serum ROS level of the two groups. According to the above results, we built our hypothesis and performed in vitro experiments to validate this hypothesis. Results: We found that catalase was upregulated in the group treated with Smilacis Glabrae Rhizoma, and the level of reactive oxygen species was higher in the hyperuricemia group. Thus, we speculated that Smilacis Glabrae Rhizoma could alleviate oxidative stress by upregulating catalase. In vitro experiments, we found that high concentrations of uric acid reduced catalase expression in endothelial cells, which was alleviated by Smilacis Glabrae Rhizoma and resulted in a reduction of reactive oxygen species. Knockdown of catalase led to an increase in reactive oxygen species. Conclusion: We demonstrated that Smilacis Glabrae Rhizoma could alleviate the oxidative stress caused by hyperuricemia by upregulating catalase expression. This finding could represent a new application for Smilacis Glabrae Rhizoma in the treatment of hyperuricemia.
Introduction

Hyperuricemia is a common disease. Numerous studies have confirmed that hyperuricemia is an independent risk factor for chronic kidney disease (CKD) and cardiovascular disease (CVD) [1-4]. Therefore, investigating ways to control serum uric acid levels has become a topic of great interest.

Interest in the use of traditional Chinese medicine (TCM) for the treatment of hyperuricemia has grown in recent years [5, 6]. Many TCM remedies contain Smilacis Glabrae Rhizoma (SGR), which belongs to the Smilacaceae family and the Smilax genus. SGR has been found to be effective for the treatment of xeranthem, for detoxification, and for easing joint movement in the TCM literature, including the Compendium of Materia Medica and the State Pharmacopoeia of the People’s Republic of China. Hu et al reported that a Simiao pill could reduce elevated serum uric acid (UA) levels and alleviate renal injury caused by hyperuricemia [7]. However, the mechanism of its protective effects on renal function under hyperuricemic conditions is not known. It has been demonstrated that the production of reactive oxygen species (ROS) increased significantly under hyperuricemic conditions [8], and our previous studies [9, 10] and other research [11-14] have confirmed that high concentrations of uric acid could cause endothelial dysfunction by generating reactive oxidative species (ROS), which induce calcium overload and inflammation. It has also been reported that high concentrations of uric acid could induce renal failure by damaging endothelial cells [15], but the underlying mechanism remains unclear. Given the above research, we hypothesized that, to some extent, hyperuricemia causes renal injury by increasing the ROS that damage endothelial cells. SGR could play a protective role for the kidneys by diminishing ROS production, thereby ameliorating endothelial damage. In this study, we used label-free quantitative liquid chromatography mass spectrometry (Label-free LC-MS/MS) to analyze protein expression in the kidneys of control rats and hyperuricemic rats that were treated with SGR. Of the differentially expressed proteins, catalase is noteworthy because it is responsible for the degradation of hydrogen peroxide [16], and it is a protective enzyme that is present in nearly all animal cells [17-19]. In this study, we also demonstrated that catalase could be upregulated by SGR and that it reduced the generation of ROS that were induced by high concentrations of uric acid in vitro.

Materials and Methods

Materials and reagents

The dried rhizome of SGR was purchased from the Beijing Tong Ren Tang Pharmacy (Beijing, China). Ethyl alcohol and methanol (analytical grade) were purchased from the Beijing Chemical Reagent Company. Allopurinol (its purity exceeded 99.2%, as determined by HPLC), uric acid and oxonic acid were purchased from Sigma–Aldrich (St Louis MO, USA). Assay kits for uric acid ROS were obtained from Jiancheng Biotech (Nanjing, China).

Establishment of hyperuricemic rat models

Hyperuricemic rat models were established as described by Yang et al. [20, 21], with slight modifications. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Chinese PLA General Hospital. Sprague Dawley (SD) rats, obtained from the Experimental Animal Center of Academy of Military Medical Sciences (China), were housed in temperature-controlled cages on a 12 h light-dark cycle and were given free access to water and normal chow. After 1 week of breeding for adaptation, the rats were grouped into control (n=8) and hyperuricemic (n=8) groups. The rats were injected intraperitoneally with 250 mg/kg-d of oxonic acid potassium salt (Sigma) and 250 mg/kg-d of uric acid (Sigma). Fourteen days after modeling, the levels of UA in the blood were evaluated. Then, the rats were orally administered a solution with or without 1 mL of SGR (1 g/mL) every day for the subsequent four weeks.
LC-MS and data processing

LC-MS was performed as described previously [22, 23]. Continuum LC-MS data were processed and searched using Protein Lynx Global Server software, version 2.4 (Waters Corp.), with Expression software, version 2 (Waters Corp.). During the data preparation, the low- and elevated-energy thresholds were set at 100 and 50, respectively. In the workflow, fixed modification was set as carbamidomethyl (C) and variable modification as oxidation (M). The default settings were used for all the other parameters. The NCBI rat database (released March 2012) was used for protein identification. Quantitative analyses were performed using the protein expression module in PLGS software, version 2.5. The entire data set of differentially expressed proteins was further filtered by considering only those identified from the alternate scanning LC-MSE data, with the identified peptides exhibiting a good replication rate (two out of three injections). The threshold for statistical significance of a regulation level was set at a 2-fold change with a P-value <0.05. The data analysis was completed by http://www.uniprot.org and http://www.capitalbio.com/zh-hans/support/MAS3.

Pathway analysis

Pathway analysis was used to determine the significant pathways that involved the differential genes, according to KEGG and Genemap [24, 25]. Fisher’s exact test and the χ² test were used to classify the GO category, and the false discovery rate (FDR) was calculated to correct the P-value. A smaller FDR indicated that more functional descriptions in the experiment. Within the significant category, the enrichment, Re, was given by: Re = (nf / n) / (Nf / N), where nf is the number of differential proteins within the particular category, n is the total number of proteins within the same category, Nf is the number of differential proteins in the entire microarray, and N is the total number of proteins in the LC-MS.

Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from ATCC (No. CRL-1730) and were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified incubator in an atmosphere containing 5% CO₂.

Western blot assay

RIPA lysis buffer (containing 50 mmol/L Tris-HCl at pH 7.5, 150 mmol/L NaCl, 0.5% deoxycholate, 1% Nonidet P-40, 0.1% SDS, 1 mM PMSF, and a variety of protease inhibition agents at 1 μg/mL) was used to extract the proteins. The tissue lysate was taken to determine the concentrations of proteins using a BCA kit. Approximately 80 μg of protein was used for 10% SDS-PAGE and then was transferred to a PVDF membrane, which was kept overnight in a 5% no-milk solution at 4°C after Ponceau S staining. The membrane was incubated in primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The blots were developed with an ECL reagent (Santa Cruz Biotechnology) according to the manufacturer’s instructions and were exposed to X-ray film. The protein bands were quantified using Quantity One software (Bio-rad).

Small interfering RNA (siRNA) transfection

Catalase siRNA (100 nM, sc-29204, Santa Cruz Biotechnology) was transfected into HUVECs using the RNAi MAX transfection reagent, according to the manufacturer’s instructions (Invitrogen). A non-silencing siRNA was used as the negative control. Forty-eight hours after transfection, the cells were starved for 16 h and then were treated with or without UA.

Measurement of total intracellular ROS

The changes in intracellular ROS were detected with CM-H₂DCFDA oxidant-sensing fluorescent probes, at a final concentration of 10 μmol/L. Fluorescence images were obtained with a laser confocal system mounted on an inverted microscope and equipped with an argon-krypton laser. For studies using CM-H₂DCFDA, an excitation wavelength of 488 nm (argon laser) and an emission wavelength of 515 nm were used.
Statistical analysis
The results are presented as the means ± SDs. The data were analyzed using one-way analysis of variance plus Bonferroni’s correction (Student-Newman-Keuls). P-values less than 0.05 were considered statistically significant.

Results

LC-MS/MS data
We obtained 102 different proteins from our software analysis, 72 of which were significantly different between the SGR treatment group and the group with high concentrations of uric acid (HUA). Table 1 displays all of the differentially expressed proteins (P<0.05). In total, 40 proteins were upregulated and 32 were downregulated (Table 1).

GO (Gene ontology) analysis
GO analysis was applied to analyze the main functions of the differentially expressed proteins, using the UniProt workstation, according to Gene Ontology, the key functional classification of NCBI. This analysis revealed that there were 68 proteins that participated in biological processes, 69 proteins that belonged to the cellular component domain and 71 proteins that participated in molecular function (Fig. 1A). The subclassification of the proteins in the biological process domain included cellular processes (65 proteins), metabolic processes (53), single-organism processes (52), biological regulation (34) and others. (Fig. 1B). The subclassification of the proteins in the cellular components domain included cell parts (67), organelles (60), organelle parts (42), membranes (33) and others (Fig. 1C). The subclassification of the proteins in the molecular function domain included binding (66), catalytic (47), oxidoreductase (25), transporter (18) and other functions (Fig. 1D).

Pathway analysis
Pathway analysis indicated that the KEGG pathway included proteins related to leukocyte transendothelial migration, actin cytoskeleton regulation, focal adhesion, adherent junctions, tight junctions and others. The GenMAPP pathway included proteins related to striated muscle contraction (physiological processes), motor activity (molecular function), oxidoreductase activity (molecular function), extracellular space (cellular component), and enzyme inhibitor activity (molecular function). The top 10 significant pathways for KEGG and GenMAPP are listed in Tables 2 and 3, and the pathway maps are shown in http://www.rayfile.com/en/files/6615d5eb-5830-11e4-bfe0-0015c55db73d/ca8106d7/.

Analyses of catalase protein
Because our previous study demonstrated that uric acid could induce ROS generation that subsequently caused endothelial dysfunction [9], we focused on the oxidative stress process in this study. Based on our gene-pathway and GO-protein networks (supporting information), we noticed that catalase (CAT) was related to oxidative stress and was upregulated approximately 6.4-fold more in the SGR treatment group than in the HUA group. GO analysis indicated that catalase contained 30 significant GO terms, such as a response to reactive oxygen species, aminoacylase activity and others. All of the GO terms are listed in Table 4. There were 8 significant pathways related to catalase, including tryptophan metabolism, oxidative stress, methane metabolism, iron ion binding, amyotrophic lateral sclerosis (ALS), tryptophan metabolism, oxidoreductase activity, and electron transport.

SGR could induce catalase upregulation and decrease ROS generation
We confirmed that catalase expression was higher in the SGR treatment group than in the hyperuricemia group using western blotting (Fig. 2A). Serum ROS in the SRG treatment group was lower than that in the hyperuricemia group (Fig. 2B). Additionally, catalase expression...
was decreased when the HUVECs were incubated with 600 μM uric acid. However, if the cells were incubated with a medium containing SGR and uric acid, catalase expression was

Table 1. 72 proteins showed significant differences between SGR treatment group and HUA group

| UniProt ID | Symbol | Protein names | SGR/AR ratio |
|------------|--------|---------------|--------------|
| O35079     | BBN1   | Dihydropyridine-endothelial 1 | 0.0309       |
| D9NU12     | AK1A1  | Alcohol dehydrogenase [NADP(+)] | 0.0064       |
| Q4AX70     | Q6SA19 | Trisphosphate isomerase | 0.0369       |
| E9RY19     | ACTG   | Actin, cytoplasmic 2 (Gamma-actin) | 0.0405       |
| D3041      | Q8QP1L1| Kininogen 1 | 0.0669       |
| B755E4     | ACTB   | Actin, gamma-enteric smooth muscle (Alpha-actin-3) | 0.0728       |
| P41223     | HMRE2  | Hydroxycacid oxidase 2 (HAXOX2) | 0.0758       |
| Q4AY57     | Q8R4H6 | Actin alpha 3, isoform CRA_a (Actin Actn3) | 0.0873       |
| Q38Y9      | MYL6   | Myosin regulatory light polypeptide 9 | 0.0860       |
| Q6V707     | Q6AY1B | Protease SerA a | 0.0916       |
| B60N4      | ACT10  | Actin, cytoplasmic 1 (Beta-actin) | 0.0993       |
| Q07936     | MYL6A  | Myosin light polypeptide 6 | 0.1023       |
| P07340     | C3     | Complement C3 | 0.1044       |
| P29411     | GSTA3  | Glutathione-S-transferase alpha 3 | 0.1054       |
| P51487     | KAD3   | GTP-AMP phosphotransferase AUS, mitochondrial | 0.1097       |
| P51025     | ITIH1  | Inter-alpha-trypsin inhibitor heavy chain 3 | 0.1249       |
| D98909     | H07CF1V| Cytochrome c oxidase subunit 2 | 0.1262       |
| Q07723     | MYH10  | Myosin-10 (Cellular myosin heavy chain, type B) | 0.1557       |
| Q9W021     | VIM    | Vimentin | 0.2208       |
| Q9JH1      | TP15   | Triosephosphate isomerase (TIM) | 0.2276       |
| B755E5     | ACTN4  | Alpha-actin-4 (F-actin-cross-linking protein) | 0.2299       |
| E7C085     | ANX2   | Annexin A2 (Annexin II) | 0.2965       |
| Q40832     | OXDA   | D-amino-acid oxidase (DAMO) | 0.2984       |
| E7C091     | AT1B   | Sodium/potassium-transporting ATPase subunit beta-1 | 0.2984       |
| P15171     | D8603 | Protein RGD1307063 (Putative uncharacterized protein RGD1307063_predicted) | 0.3296       |
| E7C06D     | AL1A1  | Retinal dehydrogenase 1 (RALDI1) | 0.4148       |
| P10266     | B766CRT| Cytochrome c oxidase subunit 2 | 0.4449       |
| E7C041     | B0B9T9 | RCG47746, isoform CRA_a (Smooth muscle alpha-actin) | 0.4630       |
| P12346     | ETO9D4 | Cytochrome c oxidase subunit 2 | 0.5169       |
| Q9B2Z5     | TERA   | Transitional endoplasmic reticulum ATPase | 0.5399       |
| R7887      | ACTN1  | Alpha-actin-1 (Cellular myosin heavy chain, type B) | 0.7172       |
| E7C092     | ALB1   | Serum albumin | 0.6950       |

Table 1. 72 proteins showed significant differences between SGR treatment group and HUA group

| UniProt ID | Symbol | Protein names | SGR/AR ratio |
|------------|--------|---------------|--------------|
| Q40832     | OXDA   | D-amino-acid oxidase (DAMO) | 0.2984       |
| Q44275     | S6P5    | Ribosomal protein L9 | 0.3869       |
| Q44119     | N3N8Z    | GM2 ganglioside activator | 2.1099       |
| P90572     | MUG2 | Mannosidase B | 2.4843       |
| Q40832 | OXDA | D-amino-acid oxidase (DAMO) | 0.2984       |
| D4A5H5     | ACYLA   | Aminocyclase 1A (Novel 2-aminocyclohexoamide) | 2.7123       |
| P50960     | CIB1    | 60 kDa heat shock protein, mitochondrial | 2.7183       |
| P45806     | HSP7C   | Heat shock cognate 71 kDa protein | 2.7183       |
| P51025     | S10A8  | Protein S10A-11 (Caldron) | 2.7183       |
| D52096     | ACT5   | Actin, alpha skeletal muscle (Alpha-actin-1) | 2.7183       |
| P17751     | F16F1  | Fructose-1,6-bisphosphatase 1 (FBPase-1) | 2.7456       |
| D5805     | Q8B9P7X | Cytochrome c oxidase subunit 2 | 2.8727       |
| P19966     | FIBB   | Fibrinogen beta chain | 2.9477       |
| P60309     | LDHB   | L-lactate dehydrogenase B chain (LDH-B) | 2.9743       |
| P61305     | GLE2   | Galactosyl (Gal-1) (14 kDa lectin) | 3.3872       |
| P69711     | KNT2   | T-kininogen 2 (Alpha-1-MAP) (Major acute phase protein) | 3.4121       |
| P68316     | MRLCA  | Myosin regulatory light chain LCA (Myosin MLC-A) | 3.7101       |
| P60035     | MRLCB  | Myosin regulatory light chain LRB (Myosin MLC-B) | 3.7967       |
| B0B9T0     | ALM    | Alpha-1-macroglubulin (Alpha-1-M) | 4.2207       |
| P63269     | MDHC   | Malate dehydrogenase, cytoplasmic | 4.3692       |
| P61762     | CATA   | Catalase | 6.7491       |
| Q490V5     | RAC1   | Ras-related C3 botulinum toxin substrate 1 (p21-rac1) | 8.6520       |
| Q941L6     | Q4X108 | Lymphoid expressed protein 1 (Protein Lep1) | 8.9352       |
| P11762     | BNU12  | Chromosome partition protein Sec1 | 9.6794       |
| Q7Z2L2     | VINC   | Vinculin (Vinculin) | 9.7767       |
| D31806     | GTS5   | Glutathione S-transferase alpha-5 | 9.8749       |
| P11415     | TRFE   | Serotransferin (Transferrin) | 10.2779      |
| P09066     | D2SW069 | L-lactate dehydrogenase (EC 1.1.1.27) | 11.8224      |
| Q9Q2U0     | USA1   | Ubiquitin-like modifier-activating enzyme 1 | 12.9613      |
| Q98456     | SSGA   | Saccinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial | 12.1425      |
Fig. 1. GO analysis of the differentially expressed proteins. (A) There were 68 proteins that participated in biological processes, 69 proteins belonging to the cellular component domain and 71 proteins that participated in molecular function. (B) The subclassification of the biological processes domain included cellular processes (65), metabolic processes (53), single-organism processes (52), biological regulation (34) and others. (C) The subclassification of the cellular component domain included cell parts (67), organelles (60), organelle parts (42), membrane components (33) and others. (D) The subclassification of the molecular function domain included binding (66), catalytic (47), oxidoreductase (25), transporter (18) and other functions.

increased (Fig. 2C). The total ROS in the cells treated with 600 μM uric acid was significantly higher than in the control cells. Nevertheless, when cells were treated with a medium that
Table 2. Top 10 significant KEGG pathway

| Pathway                                          | Count | p-Value  | q-Value | Gene                  |
|--------------------------------------------------|-------|----------|---------|-----------------------|
| Leukocyte transendothelial migration              | 9     | 3.00E-13 | 1.00E-11| Act1,Actg1,Kv1.5,Mrc2,Akt1,Actr1,Actn3,Rac1 |
| Regulation of actin cytoskeleton                  | 10    | 3.90E-12 | 3.20E-11| Act1,Actg1,Kv1.5,Mrc2,Akt1,Myr10,KvL4,Akt3,Rac1 |
| Focal adhesion                                    | 9     | 5.00E-11 | 3.10E-10| Act1,Actg1,Kv1.5,Mrc2,Akt1,Actn4,Akt3,Rac1 |
| Adherens junction                                  | 7     | 3.60E-10 | 3.10E-10| Act1,Actg1,Kv1.5,Mrc2,Akt1,Actn3,Rac1 |
| Tight junction                                    | 8     | 4.50E-11 | 3.40E-10| Act1,Actg1,Kv1.5,Mrc2,Akt1,Myr10,Akt3,Rac3,Akt5 |
| Glycolysis / Gluconeogenesis                       | 6     | 1.60E-08 | 9.40E-08| Tpl1,LOC509959,Fbp1,LOC303448,Lebh,Akr1a1 |
| Complement and coagulation cascades               | 4     | 5.40E-06 | 0.000013| C3,Fbg,Fgg,Kng1 |
| Fructose and mannose metabolism                   | 3     | 0.000022 | 0.000049| Tpl1,LOC509959,Fbp1 |
| Systemic lupus erythematosus                      | 4     | 0.000006 | 0.000021| C3,Act1,Akt3,Akt5 |
| Glyoxylate and dicarboxylate metabolism           | 2     | 0.00015  | 0.00028 | Mdh1,Hao2 |

Table 3. Top 10 significant GeneMAPP pathway

| Pathway                                          | Count | p-Value  | q-Value | Gene                  |
|--------------------------------------------------|-------|----------|---------|-----------------------|
| physiological_process--                          | 7     | 1.04E-13 | 7.20E-12| Act1,Actg1,Kv1.5,Vim,Akt4,Akt3 |
| Rn_Striated_muscle contraction                    | 8     | 6.00E-12 | 7.80E-11| Act1,Actg1,Kv1.5,Akt1,Myr10,ROD1109537,Myc2,Mhb1 |
| Molecular_function--motor_activity                | 9     | 1.00E-09 | 1.00E-08| Mrt1,Gdhb,Sdhb,Gst,Akr1a1,Akr1a1,Mhb1,Acr2,0ao |
| Molecular_function--oxidoreductase_activity       | 6     | 8.10E-08 | 4.20E-07| Alb,Fbg,Msg1,S100a11,Klg1,Fgg |
| Molecular_function--enzyme inhibitor protein       | 6     | 1.90E-07 | 9.00E-07| Ama2,Fbg1,Klg1,Ihbb,Serpin3,Ah,Pep |
| Molecular_function--cytoskeletal protein binding   | 6     | 2.20E-07 | 9.70E-07| Ama2,Fbg1,Akt1,Akt3,Akt3,Akt3 |
| metabolic_process--                               | 4     | 5.10E-07 | 0.000002| Tpl1,Fbp1,Lebh,Mdb1 |
| Rn_Glycolysis_and_Gluconeogenesis                 | 6     | 5.90E-07 | 0.000002| Act1,Actg1,Myr10,Akt1,Akt1 |
| cellular_process--Rn_Focal_adhesion               | 7     | 6.40E-07 | 0.000002| Tpl1,LOC509959,Gsa1,LOC561166,Gstn3,Akr1a1,Akr1a1 |
| Molecular_function--endothelial_inhibitor activity| 5     | 9.20E-07 | 0.000002| Mgr1,Klg1,Ihbb,Serpin3,Ah,Pep |
| Cellular_component--protease_inhibitor activity    | 5     | 9.00E-07 | 0.000002| Mgr1,Klg1,Ihbb,Serpin3,Ah,Pep |

Table 4. GO analysis of catalase (contains 30 GO terms).

| Molecular Function                                | Biological Process                              | Cellular Component |
|--------------------------------------------------|------------------------------------------------|--------------------|
| GO:0004496 amine oxidase activity                 | GO:0000302 response to reactive oxygen species | GO:0005799 mitrochondrial |
| GO:0004496 catalase activity                      | GO:00006641 triacylglycerol metabolism          | GO:0005750 mitochondrial |
| GO:0006803 growth factor activity                 | GO:0008283 cholesterol metabolism              | intermembrane space |
| GO:0002037 heme binding                          | GO:0008283 cell proliferation                   | GO:0005764 lysosome |
| GO:0002683 protein                               | GO:0009060 aerobic respiration                   | GO:0009777 peroxisome |
| GO:0002683 protein                               | GO:0009560 UV protection                        | GO:0005776 peroxosomal membrane |
| GO:0006872 metal ion binding                      | GO:0016068 positive regulation of phosphoinositide 3- | GO:0005783 endoplasmic reticulum |
| GO:0005061 KDP binding                           | GO:0002927 hemoglobin metabolism                | GO:0005829 cytosol |
| GO:0002683 protein                               | GO:00032080 inhibition of NF-kappal transcription factor |
| GO:0002683 protein                               | GO:00042744 hydrogen peroxide catalase           | GO:0005886 plasma |
| GO:0002683 protein                               | GO:00043066 negative regulation of apoptosis     | GO:0005899 Golgi apparatus |
| GO:00032080 activation of NF-kappal transcription factor |
| GO:0002683 protein                               | GO:00051262 protein tetratransmembrane          | GO:00055114 oxidation reduction |

contained SGR and uric acid, the total ROS generation in the cells was inhibited (Fig. 2D). However, knockdown of catalase resulted in an increase in ROS (Fig. 2E).

Discussion

From an epidemiologic point of view, hyperuricemia is an increasingly prevalent and pressing clinical problem [26-28]. It has been demonstrated that serum uric acid levels were negatively correlated with the prognosis for metabolic syndrome, diabetes, primary hypertension, atherosclerosis, and chronic kidney disease [29-32]. Smilacis Glabrae Rhizoma (SGR), a component of TCM, has been considered a promising drug for attenuating hyperuricemia and ameliorating nephropathy [33-35], but its mechanism of action is unknown. Our previous study showed that high concentrations of uric acid could alter the expression levels of several kidney proteins [36], many of which might be involved in pathophysiological processes under hyperuricemic conditions. SGR might exert its effects...
by inhibiting these changes. Therefore, we examined the differentially expressed kidney proteins of a group of rats treated with SGR and compared them to those of an untreated hyperuricemic group. The expression of 72 proteins was significantly different between the two groups, and all of these proteins were subjected to bioinformatic analysis. Under hyperuricemic conditions, ROS production increases significantly [8], and excessive ROS can damage endothelial cells, which are important for maintaining kidney function. It was reported that the kidney damage caused by hyperuricemia was primarily due to oxidative stress and inflammation [37], and bioinformatic analysis has shown that the oxidative stress process was altered in the SGR treatment group. Therefore, we speculated that SGR might exert its effects by inhibiting the oxidative stress process induced by hyperuricemia. Based on gene-pathway and GO-protein network results, we found that, among the differentially expressed proteins, catalase was related to the oxidative stress process and was upregulated approximately 6.4-fold more in the SGR treatment group compared with the HUA group. We confirmed this upregulation with western blotting. Catalase can degrade hydrogen peroxide, an important component of ROS, and therefore reduce oxidative stress. It was demonstrated that hyperuricemia could increase ROS generation systemically [38]; therefore, we sought to determine whether SGR could reduce ROS generation systemically. We examined the serum...
ROS of the two groups and found that the serum ROS level of the SGR treatment group was lower than that of the hyperuricemia group. Thus, we speculated that SGR could increase catalase expression, which reduced ROS systemically in hyperuricemic mice, indicating that SGR might reduce the oxidative stress caused by hyperuricemia. To validate this hypothesis, we performed an *in vitro* experiment. Because endothelial cells are among the main targets of oxidative stress caused by hyperuricemia in the kidneys, we used them in our experiment. We determined the expression level of catalase and the total ROS level under high uric acid concentration conditions with and without SGR. The results demonstrated that a high concentration of uric acid reduced the expression level of catalase in endothelial cells, and this decrease could be alleviated by SGR, leading to a reduction in ROS. The knockdown of catalase led to an increase in ROS. These results confirmed our hypothesis. Some studies have found that SGR attenuated hyperuricemia by promoting the excretion of uric acid [39], but it remains unknown whether there is a mechanism that does not rely on increasing the excretion of uric acid. In this study, for the first time, we demonstrated that SGR could alleviate the oxidative stress caused by hyperuricemia by increasing the expression of catalase, and this finding could be the mechanism by which SGR exerts its protective effects on the kidneys.

In conclusion, we demonstrated that SGR could alleviate the oxidative stress caused by hyperuricemia by upregulating catalase expression, and this finding could represent a new application for SGR in the treatment of hyperuricemia.

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Disclosure Statement

The authors have not declared any conflicts of interest

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