Original Paper

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.

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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 xeransis, 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 χ^2 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 a smaller error in judging the P-value. The FDR was defined as $FDR = 1 - \frac{N_k}{T}$, where N_k refers to the number of Fisher's test P-values that are less than the χ^2 test P-values. We computed P-values for the pathways of all of the differentially expressed proteins. Enrichment provided a measure of the significance of the function: as the enrichment increased, the corresponding function was more specific, which helped us to find those GOs that had more concrete functional descriptions in the experiment. Within the significant category, the enrichment, Re, was given by: $Re = (n_f / n) / (N_f / N)$, where n_f is the number of differential proteins within the particular category, n is the total number of proteins within the same category, N_f 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 Biotechnologies) 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 $\pm$ 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 SGR treatment group was lower than that in the hyperuricemia group (Fig. 2B). Additionally, catalase expression

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

Uniprot ID	Symbol	Protein names	SGR/model Ratio
Downregulated			
O35078	B0BNJ4	Ethylmalonic encephalopathy 1	0.0309
D8NU12	AK1A1	Alcohol dehydrogenase [NADP(+)]	0.0364
Q6AXY0	Q6SA19	Triosephosphate isomerase	0.0369
B3RLY8	ACTG	Actin, cytoplasmic 2 (Gamma-actin)	0.0485
Q63041	Q5PQU1	Kininogen 1	0.0669
B7SE64	ACTH	Actin, gamma-enteric smooth muscle (Alpha-actin-3)	0.0728
P42123	HAOX2	Hydroxyacid oxidase 2 (HAOX2)	0.0758
Q6AYS7	Q8R4I6	Actinin alpha 3, isoform CRA_a (Protein Actn3)	0.0837
Q38RV9	MYL9	Myosin regulatory light polypeptide 9	0.0860
Q63707	Q6AY18	Protein Sar1a	0.0916
B0BNJ4	ACTB	Actin, cytoplasmic 1 (Beta-actin)	0.0993
Q07936	MYL6	Myosin light polypeptide 6	0.1023
P07340	CO3	Complement C3	0.1044
P29411	GSTA3	Glutathione S-transferase alpha-3	0.1054
P51647	KAD3	GTP:AMP phosphotransferase AK3, mitochondrial	0.1097
P51635	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	0.1249
O88989	B0FTC1VI	Cytochrome c oxidase subunit 2	0.1262
Q07523	MYH10	Myosin-10 (Cellular myosin heavy chain, type B)	0.1557
Q9WU21	VIME	Vimentin	0.2230
Q9JJI1	TPIS	Triosephosphate isomerase (TIM)	0.2276
B7SE69	ACTN4	Alpha-actinin-4 (F-actin cross-linking protein)	0.2299
E7C9H5	ANXA2	Annexin A2 (Annexin II)	0.2865
Q498M9	OXDA	D-amino-acid oxidase (DAAO)	0.2894
E7C9K1	AT1B1	Sodium/potassium-transporting ATPase subunit beta-1	0.2894
P11517	D4A8S2	Protein RGD1307603 (Putative uncharacterized protein RGD1307603_predicted)	0.3296
E7C9D6	AL1A1	Retinal dehydrogenase 1 (RALDH 1) (RALDH1)	0.4148
P01026	B7SE64RT	Cytochrome c oxidase subunit 2	0.4449
E7C9L4	B0BMT0	RCG47746, isoform CRA_a (Smooth muscle alpha-actin)	0.4630
P12346	E7C9D6LE	Cytochrome c oxidase subunit 2	0.5169
Q8SEZ5	TERA	Transitional endoplasmic reticulum ATPase	0.5599
B7SE77	ACTN1	Alpha-actinin-1 (Alpha-actinin cytoskeletal isoform)	0.5712
E7C9G2	ALBU	Serum albumin	0.6050
Upregulated			
Q6P9U5	Q9WU21	Glutathione S-transferase Yb4 (EC 2.5.1.18)	1.7333
Q6IE52	Q9JJI1	Alpha-2u globulin	1.7438
Q6B345	Q8SEZ5	Cytochrome c oxidase subunit 2	1.8932
Q5BJZ3	PYRD	Dihydroorotate dehydrogenase (quinone), mitochondrial (DHODEHASE)	2.0340
P46462	HS90B	Heat shock protein HSP 90-beta	2.0544
Q8C1W8	SPA3N	Serine protease inhibitor A3N	2.0544
P19112	FIBG	Fibrinogen gamma chain	2.1383
P46418	HBB2	Hemoglobin subunit beta-2 (Beta-2-globin)	2.2255
P34058	GSTA6	Glutathione S-transferase A6	2.2933
P08932	COX2	Cytochrome c oxidase subunit 2 (Cytochrome c oxidase polypeptide II)	2.3164
Q5KI38	Q5BJZ3	Nicotinamide nucleotide transhydrogenase (Protein Nnt)	2.3396
Q5U300	Q498M9	Similar to glyceraldehyde-3-phosphate dehydrogenase	2.3632
Q64122	Q6P9U5	Ribosomal protein L9	2.3869
Q64119	Q6IN37	GM2 ganglioside activator	2.4109
P85972	MUG2	Murine globulin-2	2.4843
D4A8S2	ACY1A	Aminoacylase-1A (N-acyl-L-amino-acid amidohydrolase)	2.6645
P04904	CH60	60 kDa heat shock protein, mitochondrial	2.6645
P48500	HSP7C	Heat shock cognate 71 kDa protein	2.7183
Q6SA19	S10AB	Protein S100-A11 (Calgizarin)	2.7183
D25W96	ACTS	Actin, alpha skeletal muscle (Alpha-actin-1)	2.7183
P17751	F16P1	Fructose-1,6-bisphosphatase 1 (FBPase 1)	2.7456
Q5PQU1	Q38RV9EX	Cytochrome c oxidase subunit 2	2.8577
P18666	FIBB	Fibrinogen beta chain	2.9447
P63039	LDHB	L-lactate dehydrogenase B chain (LDH-B)	2.9743
P63259	LEG1	Galectin-1 (Gal-1) (14 kDa lectin)	3.3872
P60711	KNT2	T-kininogen 2 (Alpha-1-MAP) (Major acute phase protein)	3.4212
P68136	MRLCA	Myosin regulatory light chain RLC-A (Myosin RLC-A)	3.7810
P68035	ML12B	Myosin regulatory light chain 12B (Myosin RLC-B)	3.9749
B0BMT0	A1M	Alpha-1-macroglobulin (Alpha-1-M)	4.2207
P63269	MDHC	Malate dehydrogenase, cytoplasmic	4.3929
P04762	CATA	Catalase	6.7499
Q6RUV5	RAC1	Ras-related C3 botulinum toxin substrate 1 (p21-Rac1)	8.6320
Q63416	Q5XI38	Lymphocyte cytosolic protein 1 (Protein Lcp1)	8.9352
P11762	D8NU12	Chromosome partition protein Smc	9.6794
Q9Z1P2	VINC	Vinculin (Metavinculin)	9.7767
P31000	GSTA5	Glutathione S-transferase alpha-5	9.8749
Q9JLT0	TRFE	Serotransferrin (Transferrin)	10.2779
P09006	D2SW96	L-lactate dehydrogenase (EC 1.1.1.27)	11.8224
Q9QXQ0	UBA1	Ubiquitin-like modifier-activating enzyme 1	12.0613
Q8R4I6	SUCA	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	12.1825

was decreased when the HUVECs were incubated with 600 μ M uric acid. However, if the cells were incubated with a medium containing SGR and uric acid, catalase expression was

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 μ M 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	3.80E-13	1.00E-11	Actb,Actg1,MyI9,Vcl,Mrlc2,Actn1,Actn4,Actn3,Rac1
Regulation of actin cytoskeleton	10	1.90E-12	3.20E-11	Actb,Actg1,MyI9,Vcl,Mrlc2,Actn1,Myh10,Actn4,Actn3,Rac1
Focal adhesion	9	3.00E-11	3.10E-10	Actb,Actg1,MyI9,Vcl,Mrlc2,Actn1,Actn4,Actn3,Rac1
Adherens junction	7	3.60E-11	3.10E-10	Actb,Actg1,Vcl,Actn1,Actn4,Actn3,Rac1
Tight junction	8	4.50E-11	3.40E-10	Actb,Actg1,MyI9,Mrlc2,Actn1,Myh10,Actn4,Actn3
Glycolysis / Gluconeogenesis	6	1.60E-08	9.40E-08	Tpi1,LOC500959,Fbp1,LOC303448,Ldhd,Akr1a1
Complement and coagulation cascades	4	5.4E-06	0.000013	C3,Fgb,Fgg,Kng1
Fructose and mannose metabolism	3	0.000022	0.000049	Tpi1,LOC500959,Fbp1
Systemic lupus erythematosus	4	0.000096	0.00021	C3,Actn1,Actn4,Actn3
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-- Rn_Striated_muscle_contraction	7	1.40E-13	7.20E-12	Actc1,Acta1,Actg1,MyI9,Vim,Actn4,Actn3
Molecular function--motor activity	8	6.00E-12	7.80E-11	Actg2,Actc1,Acta1,Actb,MyI9,RGD1309537,Mrlc2,Myh10
Molecular function--oxidoreductase activity	9	1.60E-09	1.00E-08	Nnt,Ldhd,Dhodh,Cat,Aldh1a1,Akr1a1,Mdh1,Hao2,Dao
Cellular component--extracellular space	6	8.10E-08	4.20E-07	Alb,Fgb,Mug1,S100a11,Kng1l1,Fgg
Molecular function--enzyme inhibitor activity	6	1.90E-07	9.00E-07	Anxa2,Mug1,Kng1l1,Itih3,Serpina3n,Pzp
Molecular function--cytoskeletal protein binding	6	2.20E-07	9.70E-07	Anxa2,Lcp1,Actn1,Myh10,Actn4,Actn3
metabolic_process-- Rn_Glycolysis_and_Gluconeogenesis	4	5.10E-07	0.000002	Tpi1,Fbp1,Ldhd,Mdh1
cellular_process--Rn_Focal_adhesion	6	5.90E-07	0.0000022	Actb,Actg1,MyI6l,Vcl,Actn1,Rac1
Biological Process--metabolism	7	6.40E-07	0.0000022	Tpi1,LOC500959,Gsta3,LOC501110,Gstm3,Aldh1a1,Sucg1
Molecular function--endopeptidase inhibitor activity	5	9.20E-07	0.0000029	Mug1,Kng1l1,Itih3,Serpina3n,Pzp
Cellular component--protease inhibitor activity	5	9.60E-07	0.0000029	Mug1,Kng1l1,Itih3,Serpina3n,Pzp

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

Molecular Function	Biological Process	Cellular Component
GO:0004046 aminoacylase activity	GO:0000302 response to reactive oxygen species	GO:0005739 mitochondrion
GO:0004096 catalase activity	GO:0006641 triacylglycerol metabolism	GO:0005758 mitochondrial
GO:0008083 growth factor activity	GO:0008203 cholesterol metabolism	intermembrane space
GO:0020037 heme binding	GO:0008283 cell proliferation	GO:0005764 lysosome
GO:0042803 protein	GO:0009060 aerobic respiration	GO:0005777 peroxisome
homodimerization activity	GO:0009650 UV protection	GO:0005778 peroxisomal membrane
GO:0046872 metal ion binding	GO:0014068 positive regulation of phosphoinositide 3-kinase cascade	GO:0005783 endoplasmic reticulum
GO:0050661 NADP binding	GO:0020027 hemoglobin metabolism	GO:0005794 Golgi apparatus
	GO:0032088 inhibition of NF-kappaB transcription factor	GO:0005829 cytosol
	GO:0042744 hydrogen peroxide catabolism	GO:0005886 plasma
	GO:0043066 negative regulation of apoptosis	
	GO:0051092 activation of NF-kappaB transcription factor	
	GO:0051262 protein tetramerization	
	GO:0055114 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

Fig. 2. Validation of the mechanism of SGR alleviating the oxidative stress caused by hyperuricemia. (A) The expression level of catalase in the SGR treatment group was higher than in the hyperuricemia group. $*P < 0.05$, hyperuricemia group versus control; $\dagger P < 0.05$, SGR treatment group versus hyperuricemia group. (B) The ROS level in the SGR treatment group was lower than in the hyperuricemia group. $*P < 0.05$, hyperuricemia versus control; $\dagger P < 0.05$, SGR treatment group versus hyperuricemia group. (C) High concentrations of uric acid inhibited the expression of catalase, and SGR alleviated this inhibition. $*P < 0.05$, HUA group versus control; $\dagger P < 0.05$, HUA+SGR group versus HUA group. (D) High concentrations of uric acid increased ROS in HUVEC, and SGR alleviated this increase. $*P < 0.05$, HUA group versus control; $\dagger P < 0.05$, HUA+SGR group versus HUA group. (E) The knockdown of catalase increased the ROS level. $*P < 0.05$, HUA+SGR+siCAT group versus HUA+SGR group; $\dagger P < 0.05$, HUA+SGR+siCon versus HUA+SGR+siCAT.

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.

Acknowledgements

This work is supported by the Beijing NOVA Program (No. Z121107002512078), the National Basic Research Program of China (973 Program) (No. 2013CB530800), the Chinese National Natural Sciences Foundation (No. 81102673, 81330019 and No. 31170810), and the National High Technology Research and Development Program („863“Program) of China (No. 2012AA02A512).

Disclosure Statement

The authors have not declared any conflicts of interest

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