FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 59, No. 2, 2021
pp. 134–143

ORIGINAL PAPER

VIA MEDICA

Golgi α-mannosidase II mediates the formation of vascular smooth muscle foam cells under inflammatory stress

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Abstract

Introduction. Vascular smooth muscle cells (VSMCs)-based foam cell formation is a crucial factor in the atherosclerosis process. We aimed to explore the mechanism of Golgi α-mannosidase II (GMII) effects on the VSMCs-based foam cell formation.

Material and methods. VSMCs were exposed to different concentrations of low-density lipoproteins (LDLs), lipopolysaccharide (LPS), and/or GMII inhibitor (swainsonine). The qRT-PCR and western blot were used for expression analysis. Oil Red O staining was used to verify changes of lipid droplets in VSMCs. The translocation of the SCAP from the endoplasmic reticulum (ER) to Golgi was detected by immunofluorescence (IF).

Results. LPS disrupted the LDLs-mediated regulation of LDL receptor (LDLr) and increased intracellular cholesterol ester, which was inversely inhibited by swainsonine. The activity of α-mannosidase II and GMII expression were decreased by LDLs but increased by the addition of LPS. Conversely, LPS-induced enhancement was reversed by swainsonine. Additionally, swainsonine reversed the LPS-induced increase of intracellular lipid droplets in the presence of LDLs. Expression analysis demonstrated that LDLr, SCAP, and SREBP2 were up-regulated by LPS, but reversed by swainsonine in LDLs-treated cells. IF staining revealed that swainsonine inhibited the translocation of SCAP to Golgi under inflammatory stress.

Conclusions. Collectively, swainsonine restrained LDLr expression to suppress the formation of VSMCs-based foam cells by reducing SREBP2 and SCAP under inflammatory stress conditions, suggesting that GMII contributes to the formation of VSMCs-based foam cells under inflammatory stress. *(Folia Histochemica et Cytobiologica 2021, Vol. 59, No. 2, 134–143)*

Key words: vascular smooth muscle cells; foam cells; LDLr; Golgi α-mannosidase

Introduction

Atherosclerosis is a chronic progressive inflammatory disease with high incidence and mortality, which is characterized by intimal accumulation of lipids (mainly cholesterol and cholesterol esters), the proliferation of vascular smooth muscle cells (VSMCs), and the infiltration of inflammatory cells in the wall of arteries [1, 2]. Particularly, VSMCs play a key role in atherogenesis by proliferating and migrating excessively in response to repeated injuries [3]. Foam cells, a special cell type located mainly in the intima of arteries [4], occur when lipid accumulation exceeds the cells' control mechanisms and is related to chronic inflammation in metabolic, infectious, and cardiovascular diseases (atherosclerosis, deep vein thrombosis, *etc.*) [5]. Foam cells play a significant role at all stages

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Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0015
ISSN 0239-8508, e-ISSN 1897-5631

www.journals.viamedica.pl/folia_histochemica_cytobiologica

of atherosclerotic lesion development, from initial lesions to advanced plaques [6]. In an early atherosclerotic lesion, the fatty streak appears on the arterial wall. With the progress of atherosclerosis, foam cells begin to release matrix-degrading enzymes, causing plaque rupture and blood vessel occlusion [7]. In pathological conditions, foam cells are derived from different cell types, including VSMCs and macrophages that originate from circulating monocytes [8].

Previous studies have focused on the accumulation of lipids in macrophages and vascular endothelial cells to form foam cells that deposit in the arterial wall. However, a limited number of studies focused on the role of VSMCs-based foam cells in the development of atherosclerosis. Recently, VSMCs were demonstrated to form foam cells after exposure to cholesterol or lipoproteins [9]. Notably, VSMCs-based foam cells account for about 45–90% of all foam cells [6]. Nevertheless, the mechanisms underlying the formation of VSMCs-based foam cells have not been explained.

It is widely accepted that the formation of foam cells is related to the dysregulation of intracellular cholesterol metabolism. Golgi α-mannosidase II (GMII), sterol regulatory element-binding proteins (SREBPs), and SREBP cleavage-activating protein (SCAP) are important components of the intracellular cholesterol regulation system. GMII is a key glycosyl-hydrolase in the N-linked glycosylation pathway [10], which is required for SCAP glycosylation [11]. Additionally, SREBP2 is a transcription factor for the low-density lipoprotein (LDL) receptor (LDLr) gene, which is involved in cholesterol uptake [12]. SCAP can regulate the activity of SREBPs by adjusting their subcellular localization [13]. This feedback mechanism consisting of SCAP and SREBP2 can timely regulate intracellular cholesterol concentration under physiological conditions [14].

Inflammation participates in the pathophysiological process of atherosclerosis in many ways. Innate immune cells can secrete inflammatory mediators and promote the early stage of the atherosclerosis process [15]. Studies have shown that based on the pathophysiology of atherosclerosis, anti-inflammatory treatments are highly likely to be used to prevent atherosclerotic thrombosis [16].

Therefore, the present study aimed to investigate whether GMII affecting the VSMCs-based foam cells formation through the SCAP and SREBP2 pathway, so as to provide a new direction for the diagnosis and treatment of atherosclerosis. As consequence, the study preliminarily demonstrated that under *in vitro* conditions GMII contributes to the formation of VSMCs-based foam cells under inflammatory stress by promoting the translocation of SCAP complex from ER to Golgi, producing N-SREBP2 and eventually activating LDLr and lipid accumulation in VSMCs.

Materials and methods

Cell culture and treatments. Human VSMCs from the coronary artery (TCS Cellworks, Buckingham, UK) were a gift from Professor X.Z. Ruan, University College London, London, England, UK. Human VSMCs were cultured in DMEM/F-12 (1:1) medium (Hyclone, Beijing, China), supplemented with 20% fetal bovine serum (FBS), 2 mmol/l glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin under a humidified atmosphere at 37°C and 5% CO_2.

LDLs were isolated from the plasma of healthy human volunteers by sequential ultracentrifugation in the presence of 3 mmol/l EDTA, as previously described [17]. The plasma of healthy human volunteers was purchased from Luzhou Blood Center (Sichuan, China). After preparation of LDLs, cells were exposed to different concentrations of LDLs (25, 50, 100 and 200 μg/ml), lipopolysaccharide (LPS; 50, 100, 200, 400 ng/ml) and/or swainsonine (2.5, 5, and 7.5 μg/ml) according to the study design. LPS (from *Escherichia coli*, Cat. No. L4391) and swainsonine (Cat. No. 72741-87-8) were purchased from Sigma-Aldrich Corp. (Saint Louis, MO, USA). Cells exposed to DMEM/F-12 (1:1) medium were used as the control.

Total RNA isolation and real-time quantitative polymerase chain reaction (qRT-PCR). Total RNA was isolated from VSMCs using RNAiso kit (Takara, Dalian, China). Total RNA (1 mg), was used as a template for reverse transcription using Takara prime Script qRT-PCR Kit (Takara). qRT-PCR was performed in an ABI 7000 Sequence Detection System (Applied Biosystems, Waltham, MA, USA) using SYBR Green PCR Kit (Invitrogen, Carlsbad, CA, USA). GAPDH was used as an endogenous control to calculate the mRNA relative expression [18]. All the PCR primers were designed by Primer Express Software V2.0. PCR primer sequences were available in Table 1. The relative amount of mRNA was calculated using the comparative threshold cycle method.

Measurement of intracellular cholesterol. Treated cells were washed twice in phosphate-buffered saline (PBS); intracellular lipids were extracted in a chloroform/methanol (2:1) mix and dried under a vacuum. The total cholesterol, free cholesterol, and cholesterol ester content were measured by an enzymatic assay, as described previously [19], and normalized by total cell proteins determined by the Lowry assay (KeyGEN, Nanjing, China).

Activity assay of α-mannosidase. The activity of α-mannosidase was determined using an Alpha-Mannosidase Assay Kit (Cat: ab272519, Abcam, USA) according to the

Table 1. Primers used for qRT-PCR analysis

Gene	Primer sequence (Forward)	Primer sequence (Reverse)
LDLr	5'-GTGTCACAGCGGCGAATG-3'	5'-CGCACTCTTTGATGGGTTCA-3'
SREBP2	5'-CCGCCTGTTCCGATGTACAC-3'	5'-TGCACATTCAGCCAGGTTCA-3'
SCAP	5'-GGGAACTTCTGGCAGAATGACT-3'	5'-CTGGTGGATGGTCCCAATG-3'
β-actin	5'-CCTGGCACCCAGCACAAT-3'	5'-GCCGATCCACACACGGAGTACT-3'

qRT-PCR — quantitative Reverse Transcription-Polymerase Chain Reaction

manufacturer's instruction. Briefly, the supernatant was firstly prepared from the homogenate of treated cells. Then, standards (100 μl) and samples (10 μl) were added into appropriate wells; subsequently, after adding the α-mannosidase substrate, reaction mixes were incubated for 10 min at 25°C. The reaction was terminated with a stop reagent. The absorbance was detected at 405 nm. The α-mannosidase activity was calculated using the formula: α-mannosidase activity = $(OD_{sample} - OD_{blank})$/Slope (linear regression fit of the standard data points) × Dilution factor.

Morphological examination by Oil Red O (ORO) staining. Treated cells were firstly washed three times with PBS and then fixed with 5% formalin solution in PBS for 30 min. Subsequently, the fixed-cells were stained with ORO for 20 min and counterstained with hematoxylin for another 5 min. Finally, cell morphology was visualized under Olympus BX43 light microscope (Olympus, Tokyo, Japan).

Protein isolation and Western blots analysis. The cells were washed twice with PBS, placed at 4°C, and lysed for 10 min in lysis buffer. The concentration of protein was measured by bicinchoninic acid (BCA) (Solarbio, Beijing, China). After denaturation, the protein was electrophoresed on 8% SDS polyacrylamide gel in a Bio-Rad micro protein device (Bio-Rad, Hercules, CA, USA), then the membrane was transferred (100 V, 350 mA). After blocking with 5% skim milk, the following antibodies were diluted with antibody dilution buffer, and the protein strips were incubated at 4°C: rabbit anti-GMII antibody (Cat. No. ab224611, Abcam, Cambridge, UK), rabbit anti-LDLr antibody (1:1500, Cat. No. A00076-2, Boster, Wuhan, China), goat anti-SREBP2 antibody (1:1500, R&D Systems, Minneapolis, MN, USA), mouse anti-SCAP antibody (1:2000, Cat. No. sc-13553, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and mouse anti-β-actin antibody (1:5000, Cat. No. A1978, SigmaAldrich). Rabbit anti-mouse (1:5000, Cat. No. sc-358914, Santa Cruz Biotechnology) or goat anti-rabbit HRP-labeled antibodies (1:5000, Cat. No. sc-2357, Santa Cruz Biotechnology) were used as the secondary antibodies correspondingly. Finally, detection procedures were performed using an ECL Advance TM western blotting detection kit, and autoradiography was performed on Hyperfilm TM ECL (Amersham Bioscience, Bucks, UK).

Immunofluorescence (IF) staining. The treated cells were fixed with 4% paraformaldehyde for 10 min and washed with PBS with 0.05% Tween-20 (SigmaAldrich) for 5 min. Then cells were permeabilized with 0.02% TritonX-100 (Solarbio) and blocked with goat serum (Solarbio) for 1 h. All the cells were incubated with rabbit anti-human SCAP antibody (1:100, Cat. No. ab190103, Abcam) and mouse anti-Golgi antibody (1:100, Cat. No. A-21270, Invitrogen) for 12 h at 4°C. After washing three times using PBS/Tween-20 over 30 min, cells were dual-stained with goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 488 (green) for SCAP (1:100, Cat. No. A-11034, Invitrogen) and goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 594 (red) for Golgin (1:100, Cat. No. A-11032, Invitrogen) for 1 h at room temperature. Finally, cells were observed under a Leica TCS SP8 confocal microscope (Leica, Wetzlar, Germany).

Statistical analysis. Statistical analysis was performed with IBM SPSS statistics 17.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as means ± standard deviation. Comparison between groups was performed with repeated-measures analysis of variance (ANOVA) followed by least-significant difference (LSD) *post-hoc* test. $p < 0.05$ was considered statistically significant.

Results

GMII inhibitor reverses the LPS-induced damage of LDLs-related LDLr negative feedback regulation

We investigated the impact of LPS-simulated inflammation on the negative feedback regulation of LDLr and choose the suitable concentration for the following experiments. We found that LDLs concentration at 25, 50, 100, 200 μg/ml significantly inhibited the expression of LDLr at the mRNA level (Fig. 1A, $p < 0.05$). LDLs at 25 μg/ml were sufficient to trigger negative feedback regulation, so the LDLs concentration used in this experiment was 25 μg/ml (Fig. 1A). Then, LPS was used to activate the inflammatory response. In the presence of LDLs (25 μg/ml), LDLr mRNA level was obviously up-regulated by LPS at 400 ng/ml ($p < 0.01$, Fig. 1B).

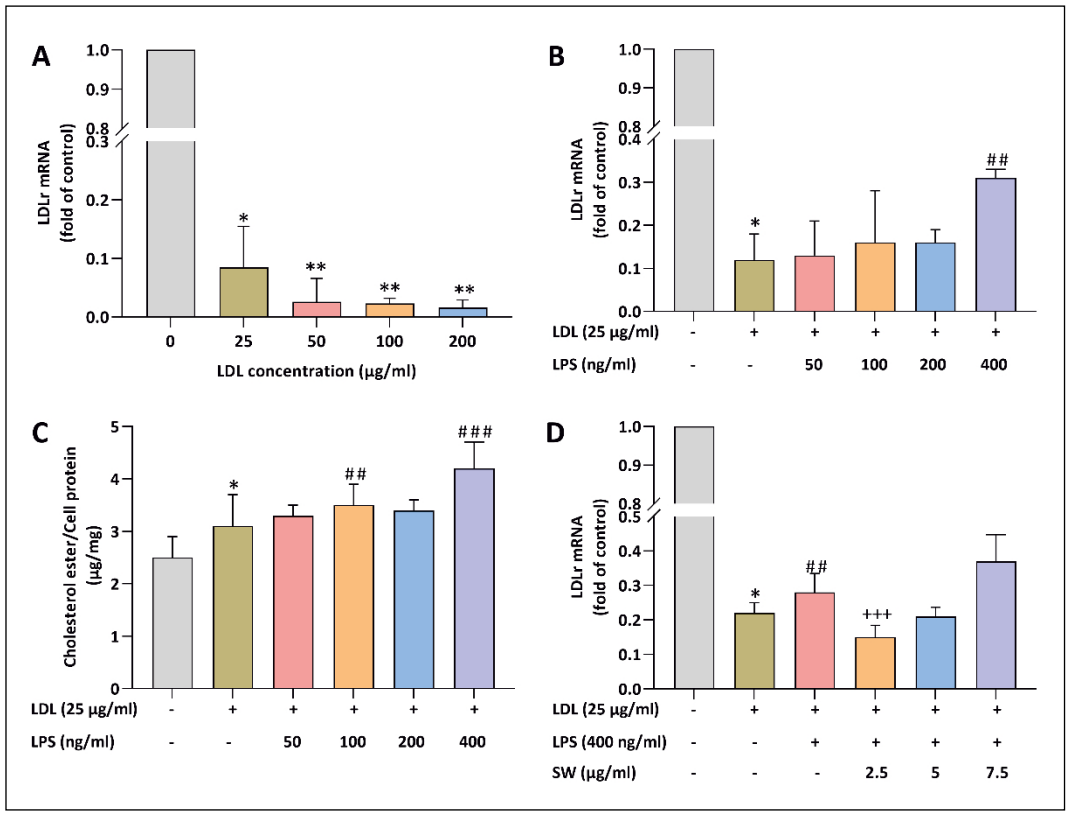

Figure 1. GMII inhibitor swainsonine reverses the LPS-induced damage of LDLs-related LDLr negative feedback regulation in cultured Vascular Smooth Muscle Cells (VSMCs). **A.** The optimal concentration of LDL triggering negative feedback regulation of LDLr. **B.** The optimal concentration of LPS that affected LDLr mRNA expression. **C.** The cholesterol ester content at optimal LDL concentration was affected by different concentrations of LPS. **D.** The optimal concentration of swainsonine that influenced LDLr mRNA expression. Data are expressed as means ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ *vs*. control; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs*. LDL, $^{+++}p < 0.001$ *vs*. LDL + LPS.

Then, we further verified the cholesterol ester content in VSMCs. Cholesterol ester content was increased by LPS at 400 ng/ml ($p < 0.001$), as shown in Figure 1C. Furthermore, in the presence of LDLs (25 μg/ml), swainsonine at a concentration of 2.5 μg/ml effectively reversed the up-regulation of LDLr mRNA mediated by LPS ($p < 0.001$, Fig. 1D). Taken together, based on the negative feedback regulation of LDLr, swainsonine could decrease the expression of LDLr under inflammatory stress conditions, indicating that GMII may be involved in foam cell formation. Moreover, the concentrations of LDLs, LPS, and swainsonine selected in the following experiments were 25 μg/ml, 400 ng/ml, and 2.5 μg/ml, respectively.

Swainsonine inhibits the GMII up-regulation under inflammatory stress conditions

To explore whether GMII participates in the regulation of LDLr, we explored the expression of GMII under inflammatory agent, LPS, stimulation. As expected, LPS up-regulated the protein expression of GMII in the presence of LDLs ($p < 0.01$, Fig. 2A). Moreover, LDLs decreased α-mannosidase activity ($p < 0.05$), but LPS reversed this effect and increased the α-mannosidase activity ($p < 0.01$). Meanwhile, the swainsonine decreased the activity of α-mannosidase ($p < 0.001$, Fig. 2B). Taken together, inflammation stress induced the up-regulation of GMII protein and GMII activity which was involved in the negative feedback regulation of LDLr expression.

GMII inhibitor reduces cholesterol ester up-regulation under inflammatory stress conditions

Swainsonine, as an inhibitor of GMII, was used to further verify the relationship between the GMII and intracellular cholesterol ester. As shown in Figure 3A, compared with the control group, LDLs increased the number of lipid droplets in the VSMCs, which was further enhanced by LPS treatment. Moreover, swainsonine inhibited LPS-induced lipid droplet accumulation in VSCMs (Fig. 3A). Figure 3B showed that LDLs increased the cholesterol ester content

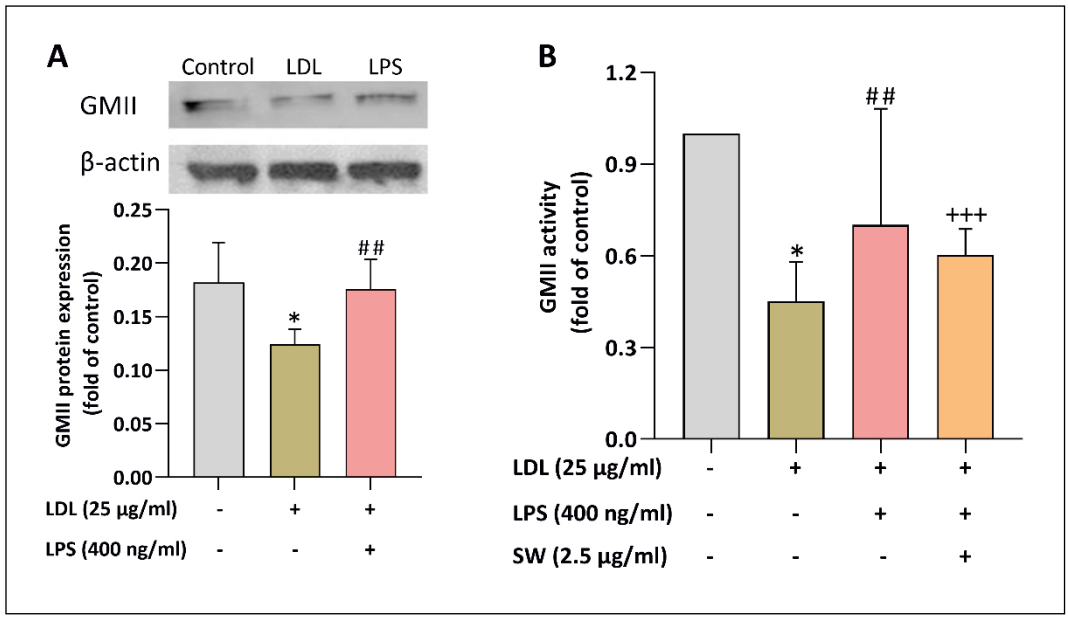

Figure 2. GMII inhibitor swainsonine inhibits the GMII up-regulation under inflammatory stress conditions in VSMCs. **A.** Effects of LDL and LPS on the expression of GMII were measured by Western blot. **B.** Effects of various treatments on GMII activity. Data are expressed as means ± SD. $^{*}p < 0.05$ *vs*. control, $^{\#\#}p < 0.01$ *vs*. LDL, $^{+++}p < 0.001$ *vs.* LDL + LPS.

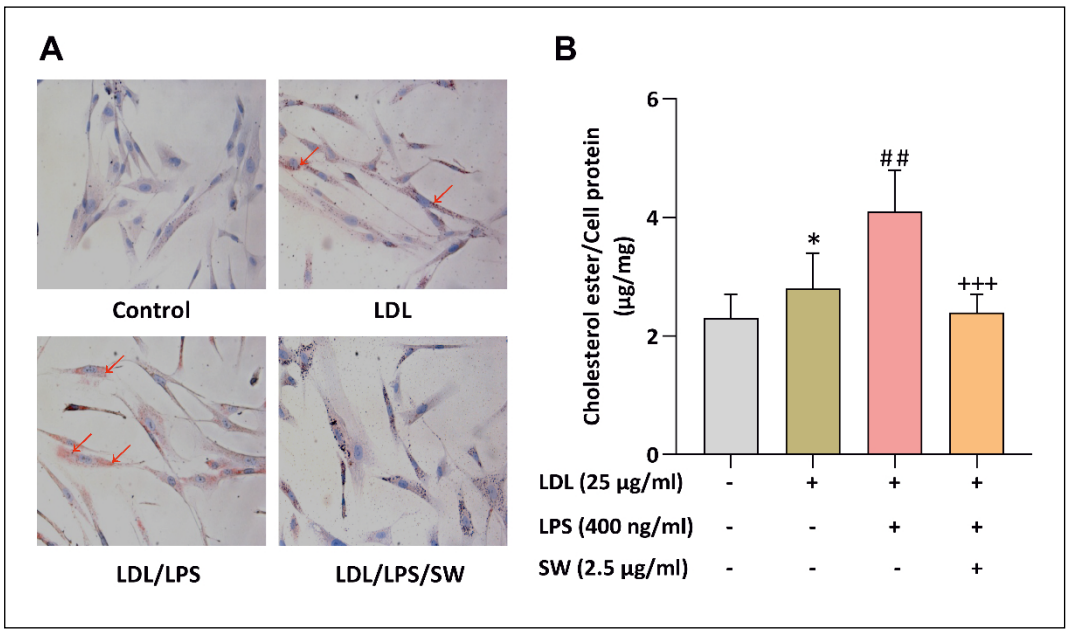

Figure 3. GMII inhibitor swainsonine reduces cholesterol ester up-regulation in the presence of LPS in VSMCs. **A.** Effects of various treatments on lipid droplets content (red arrow) were assessed by Oil Red O staining. **B.** Effects of various treatments on cholesterol ester content in VSMCs. Data are expressed as means ± SD. $^{*}p < 0.05$ *vs*. control, $^{\#\#}p < 0.01$ *vs.* LDL, $^{+++}p < 0.001$ *vs*. LDL + LPS

($p < 0.05$); after LPS treatment, the cholesterol ester content was further increased ($p < 0.01$). After treatment with swainsonine, the content of cholesterol ester of VSMCs was decreased, despite LPS had augmented its level ($p < 0.001$). Thus, swainsonine reduced the level of intracellular cholesterol ester, indicating that GMII was involved in the accumulation of lipids.

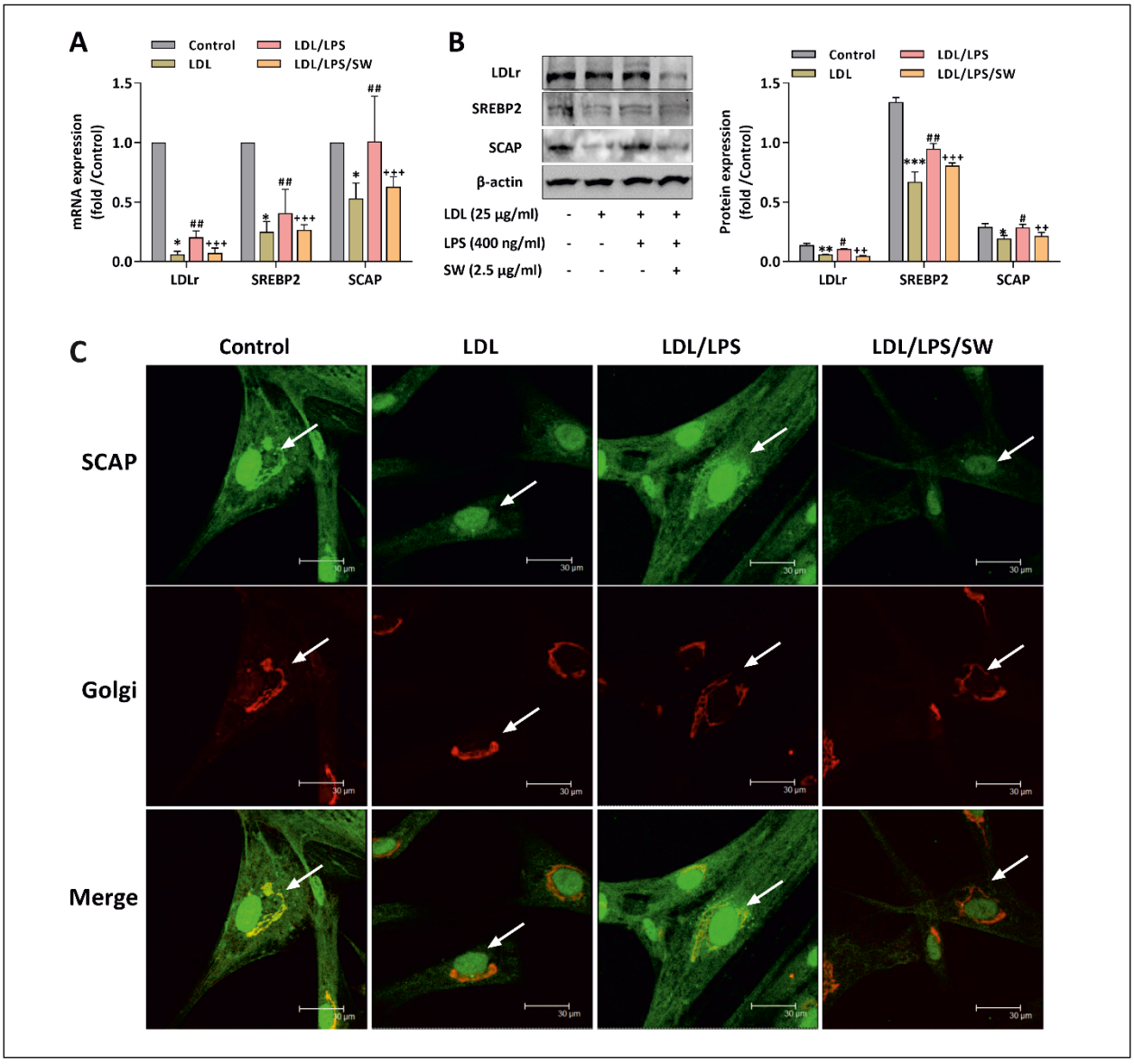

Figure 4. GMII inhibitor swainsonine decreases the expression of LDLr, SREBP2, and SCAP as well as the SCAP translocation under inflammatory stress conditions in VSMCs. **A.** Effects of various treatments on mRNA expression of LDLr, SREBP2, and SCAP were detected by qRT-PCR. **B.** Effects of various treatments on expression of LDLr, SREBP2, and SCAP proteins were verified by Western Blot. **C.** The translocation of the SCAP the endoplasmic reticulum to Golgi was evaluated by immunofluorescent staining. Data are expressed as means ± SD. $p < 0.05$ *vs.* control, $^{\#\#}p < 0.01$ *vs*. LDL, $^{+++}p < 0.001$ *vs*. LDL + LPS

GMII inhibitor decreases the expression of LDLr, SREBP2, and SCAP as well as the SCAP translocation under inflammatory stress conditions

We measured the expression of the LDLr negative feedback modulation-related proteins to explore the molecular mechanism of GMII regulation in VSMCs-based foam cells under inflammatory stress conditions. By the use of qRT-PCR, we found that LDLs inhibited the expression of LDLr, SCAP, and SREBP2 mRNAs ($p < 0.05$, Fig. 4A). Intriguingly, LPS increased mRNA levels of LDLr, SCAP, and SREBP2 in the presence of LDL ($p < 0.01$), overcoming the suppression induced by LDLs (Fig. 4A). Swainsonine reversed the LPS-induced up-regulation of LDLr, SCAP, and SREBP2 mRNA levels ($p < 0.001$), as shown in Fig. 4A. Western blot analysis demonstrated similar results to qRT-PCR measurements since swainsonine reversed the elevated protein levels of LDLr, SCAP, and SREBP2 induced by LPS ($p < 0.01$, Fig. 4B).

Furthermore, IF staining showed that LDLs inhibited the translocation of SCAP from ER to Golgi

in VSCMs; however, in the presence of LDLs, LPS increased the translocation of SCAP from ER to Golgi. It's worth noting that swainsonine reduced LPS-induced SCAP translocation (Fig. 4C). Taken together, swainsonine inhibited the expression of LDLr, SREBP2, and SCAP as well as the SCAP translocation in LDLs- and LPS-treated VSMCs, implying that GMII may contribute to the formation of VSMCs-based foam cells under inflammatory stress conditions, by up-regulating the SCAP expression, promoting SCAP translocation from ER to Golgi, activating SREBP2 and eventually activating LDLr negative feedback.

Discussion

Studies over the past years demonstrated various mechanisms of atherosclerosis [20, 21], particularly the formation of foam cells due to an imbalance in intracellular lipid regulation. Furthermore, macrophage-derived foam cells have attracted extensive scholars' attention while VSMCs-derived foam cells got less noticed. Our study found that with the participation of inflammatory factors, GMII can activate the LDLr and promote lipid accumulation by the SREBP2-SCAP pathway to promote the formation of foam cells.

It has been established that the aggregation of LDLs is a primary cause of atherosclerosis [22]. LDLr expression is usually controlled by strict metabolic regulation in a negative feedback system that is dependent on intracellular cholesterol concentration [23]. When cells lack or contain more cholesterol than physiological needs, the up-regulation or down-regulation of 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGCoAR) and LDLr is respectively mediated through a negative feedback mechanism. It is closely controlled by two other proteins, SREBP2 and SREBP-SCAP, which are important for maintaining the balance of cholesterol in cells [24, 25]. As indicated in our study, the expression of LDLr mRNA was restrained by LDLs, suggesting that LDLs trigger negative feedback regulation of LDLr in VSMCs.

Inflammation is an all-important factor in the atherosclerotic process [26]. Inflammatory mediators, secreted by the immune cells, can lead to the early stage of the atherosclerotic process [27]. Some studies have announced that inflammatory cytokines can modify cholesterol-mediated LDLr regulation in mesangial cells, allowing unmodified LDLs accumulation in cells and causing foam cell formation [28, 29]. In our study, when LDLs were present, LPS disrupted the negative feedback regulation of LDLr. LPS increased the expression of LDLr at the mRNA level and intracellular cholesterol ester content. Previously, studies have shown that intracellular cholesterol accumulation could obstruct mitochondrial function [30] and increase sensitivity to inflammation [31], thus affecting negative feedback regulation of LDLr [32, 33]. Ma *et al*. also reproted that inflammation increased lipid accumulation in VSMCs by disrupting LDLr feedback regulation [34], which further supports our present findings.

GMII, a key glycosyl-hydrolase in the N-linked glycosylation pathway, is an attractive target for inhibition with anti-inflammatory outcomes [10, 36]. It was reported to mediate the glycosylation of SCAP, which regulates cholesterol homeostasis through its interactions with SREBP-1 and -2 [14]. Accordingly, we have investigated whether and how GMII regulates the VSMCs-based foam cell formation by affecting regulation of cholesterol homeostasis. Firstly, we found that LDLs reduced the expression of GMII at the protein level and inhibited the activity of GMII, and that LPS reversed the effect caused by LDLs. Consisting with our results, the exposure of THP-1 macrophages to inflammatory cytokines upregulated the expression of GMII in the presence of LDLs [37]. This evidence implied the participation of GMII in cholesterol metabolism.

Given that foam cell formation is directly related to intracellular cholesterol accumulation [39], swainsonine, a potent inhibitor of GMII, was used for verifying the role of GMII in cholesterol regulation. As expected, swainsonine reversed the LPS-induced increase of intracellular cholesterol content and GMII activity, which suggested that GMII was indeed involved in cholesterol metabolism regulation in some way. In addition, intracellular lipid droplets distribution is an intuitive method to observe lipid accumulation. We found that LDLs increased the number of lipid droplets in VSMCs, which was further enhanced by the LPS treatment. Moreover, swainsonine inhibited LPS-induced lipid droplet accumulation in VSCMs. Therefore, we concluded that GMII may participate in the process of foam cell formation by affecting lipid accumulation.

Subsequently, we investigated the possible mechanisms of GMII regulating foam cell formation by detecting the expression of proteins related to the feedback regulation of cholesterol metabolism. Results of both PCR and western blot showed that swainsonine inhibited the expression of LDLr, SREBP2, and SCAP proteins under inflammatory stress conditions. Mechanically, the feedback regulation of cholesterol metabolism is maintained by the release of SREBPs from cell membranes [42]. As previously reported, the SCAP-SREBP2 complex not only

Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0015
ISSN 0239-8508, e-ISSN 1897-5631

controls cholesterol biosynthesis but also serves as a signaling hub integrating cholesterol metabolism with pro-inflammatory activity of macrophages [43]. When cells are deprived of intracellular cholesterol, SCAP transfers SREBP2 from the ER to the Golgi, where it is cleaved by two membrane-bound proteases [44]. At the same time, SCAP is glycosylated by the continuous action of GMI, GMII, and GlcNAc transferase I, and then recycled to ER [37]. Then, the N-terminal fragment of SREBP2 (N-SREBP2) is released from Golgi to the nucleus, and binds to sterol regulatory elements in HMGCoAR and LDLr promoters, eventually activating the transcription of genes required for lipid synthesis and uptake [37, 42, 44]. In states of intracellular cholesterol overload, the SCAP-SREBP2 complex remains in ER, and SREBP-2 cannot be processed by the protease in Golgi apparatus. Subsequently, LDLr and HMGCoAR expression were down-regulated and the intracellular cholesterol content was reduced [24]. Considering these evidence and results of our study, we propose a following potential functional mechanism for the VSMCs-based foam cell formation. Inflammatory stress can increase the activity of GMII, which promotes the expression of SCAP and SREBP2, and increases glycosylation of SCAP, as well as translocation of the SCAP from the ER to Golgi apparatus, thereby activating the LDLr expression, increasing cholesterol uptake, and leading to the VSMCs-based foam cell formation. As indicated by immunofluorescent staining, under the action of LDLs, LPS promoted the translocation of SCAP from ER to Golgi, whereas swainsonine inhibited the LPS-induced SCAP translocation to Golgi, which further supported the above mechanism. Similar to our findings, Zhou *et al*. recently reported that the increased activity of GMII promoted complex-type conversion of SCAP N-glycans, which in turn mediated SREBP2 activation, and thereby led to intracellular cholesterol accumulation and foam cell formation in cultured VSMCs [45]. SREBP1, which belongs to the SREBP family with two isoforms (SREBP1-a and SREBP1-c), is another important transcriptional regulator of lipogenesis [46]. SREBP1 could regulate fatty acid synthesis, cholesterol synthesis, and cholesterol uptake [47]. SREBP1, as an inactive precursor, also forms a complex with SCAP (SCAP-SREBP1) in the ER membrane [48]. Consisting with the SCAP-SREBP2 complex, the SCAP-SREBP1 complex is also regulated by the concentration of sterols to activate the transcription of lipogenic genes [49]. Overall, GMII may contribute to the formation of foam cells by promoting the SCAP-SREBPs complex formation and activating LDLr in VSMSc. Unfortunately, in the present study, the expression of SREBP1 was not directly assessed; thus, in further studies, this proposed potential mechanism needs to be verified.

In summary, we demonstrated that LPS-simulated inflammation increased the expression of SREBP2 and SCAP by increasing the expression of GMII. GMII further promoted the translocation of the SCAP complex from ER to Golgi, producing more N-SREBP2. Finally, LDLr was activated by N-SREBP2 and promoted lipid accumulation in VSMCs. These processes together mediated the formation of foam cells. Our research provides a new direction and understanding of the molecular mechanism of atherosclerosis and further provides a new entry point for exploring novel therapeutic strategies.

Acknowledgements
None.

Funding
This study was supported by Key Cultivation Project of the Affiliated Hospital of Southwest Medical University (Grant number: 17127).

Author contributions
KZ and QY designed the study, collected and analyzed the data. KZ drafted the manuscript. QY critically reviewed the manuscript. All authors approved the final manuscript.

Data availability statement
The data that support the findings of this study are available from the corresponding author, QY, upon reasonable request.

Experimental ethics
Not applicable.

Conflict of interest
The authors declare that they have no conflict of interest.

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Submitted: 14 May, 2021
Accepted after reviews: 11 June, 2021
Available as AoP: 21 June, 2021