Advanced glycation end products (AGEs) increase human mesangial foam cell formation by increasing Golgi SCAP glycosylation in vitro

Yang Yuan,1,2 Lei Zhao,2,3 Yaxi Chen,2 John F. Moorhead,3 Zac Varghese,3 Stephen H. Powis,3 Shane Minogue,4 Zilin Sun,1 and Xiong Z. Ruan2,3

1Department of Endocrinology, Zhongda Hospital, Southeast University, Nanjing; 2Centre for Lipid Research, Key Laboratory of Molecular Biology on Infectious Diseases, Ministry of Education, Chongqing Medical University, Chongqing, China; 3John Moorhead Research Laboratory, Centre for Nephrology and 4Centre for Molecular Cell Biology, University College London (UCL) Medical School, Royal Free Campus, London, United Kingdom

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Advanced glycation end products (AGEs) increase human mesangial foam cell formation by increasing Golgi SCAP glycosylation in vitro. Am J Physiol Renal Physiol 301: F236–F243, 2011. First published April 20, 2011; doi:10.1152/ajprenal.00646.2010.—Advanced glycation end products (AGEs) is one of the causative factors of diabetic nephropathy, which is associated with lipid accumulation in glomeruli. This study was designed to investigate whether Nε-(carboxymethyl) lysine (CML; a member of the AGEs family) increases lipid accumulation by impairing the function of sterol-regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) in human mesangial cells (HMCs). Intracellular cholesterol content was assessed by Oil Red O staining and quantitative assay. The expression of molecules controlling cholesterol homeostasis was examined using real-time quantitative RT-PCR and Western blotting. The activity of Golgi-processing enzymes was determined using enzyme-based methods, and the translocation of SCAP from the endoplasmic reticulum (ER) to the Golgi was detected by confocal microscopy. CML increased cholesterol accumulation in HMCs. Exposure to CML increased expression and abnormal translocation of SCAP from the ER to the Golgi even in the presence of a high concentration of LDL. The increased SCAP translocation carried more SREBP-2 to the Golgi for activation by proteolytic cleavages, enhancing transcription of 3-hydroxy-3-methylbutyl-CoA reductase and the LDL receptor. CML increased Golgi mannosidase activity, which may enhance glycosylation of SCAP. This prolonged the half-life and enhanced recycling of SCAP between the ER and the Golgi. The effects of CML were blocked by inhibitors of Golgi mannosidases. AGEs (CML) increased lipid synthesis and uptake, thereby causing foam cell formation via increasing transcription and protein glycosylation of SCAP in HMCs. These data imply that inhibitors of Golgi-processing enzymes might have a potential renoprotective role in prevention of mesangial foam cell formation.

Nε-(carboxymethyl) lysine (CML); LDL receptor; SREBP cleavage-activating protein; diabetic nephropathy

TYPE 2 DIABETES, WHICH AFFECTS 8–10% of the population worldwide, is a group of metabolic disorders characterized by insulin resistance, chronic hyperglycemia, hypertension, dyslipidemia, proteinuria, and inflammation. It has been shown that 30% of patients with type 2 diabetes may develop microalbuminuria and diabetic nephropathy. Although multiple risk factors are involved, hyperglycemia seems to have an important role in the pathogenesis of complications which affect several organs, such as blood vessels and the kidney (1). Hyperglycemia is accompanied by an accelerated rate of advanced glycation end products (AGEs) formation, which is formed by the nonenzymatic reaction between reducing sugars and free amino groups of proteins (33). Nε-(carboxymethyl) lysine (CML) is one of the major AGEs in vivo (24), and its levels increase in tissues of diabetic patients (6, 21). The increased circulating CML and accumulation of CML in tissues have been recognized as a critical step in the pathogenesis of insulin resistance, dyslipidaemia, and diabetic nephropathy by unknown mechanisms (19, 36).

Diabetic nephropathy, characterized by glomerulosclerosis, proteinuria, and decline in glomerular filtration rate is the major complication of type 2 diabetes and also the major cause of end-stage kidney disease in Western countries. However, the mechanisms by which hyperglycemia causes glomerulosclerosis remain unclear. Type 2 diabetes often exhibits an atherogenic lipid profile (high triglycerides and low HDL cholesterol) (35). Recent clinical evidence suggests that some type 2 diabetes patients with higher cholesterol have a greater risk of developing secondary complications (22, 23). Giancoli et al. (7) also observed significantly higher levels of hypercholesterolemia in type 2 diabetic patients with complications compared with the patients without complications, suggesting that cholesterol may play a very important role in development of the complications of type 2 diabetes. In recent years, a number of studies have shown that the histological features of glomerulosclerosis are similar to the changes observed in atherosclerosis, and the term “glomerular atherosclerosis” has been proposed (4, 34). Thus diabetic glomerulosclerosis and foam cell formation might therefore result from an increase in intracellular cholesterol accumulation by unknown mechanisms (10, 15, 17, 34).

Multiple enzymes, carrier proteins, and lipoprotein receptors are involved in cholesterol homeostasis. The key molecule in maintaining intracellular cholesterol levels is sterol-regulatory element binding protein (SREBP) cleavage-activating protein (SCAP). SCAP regulates cholesterol homeostasis through its interactions with SREBP-1 and -2. SREBP-2 is more selective than SREBP-1 in controlling gene expression of 3-hydroxy-3-methylbutyl (HMG)-CoA reductase (HMG-CoAR) and the LDL receptor (LDLR). SCAP has been identified as a cholesterol sensor and chaperone of SREBP-2. When the cellular demand for cholesterol rises, SCAP shuttles SREBPs from the endoplasmic reticulum (ER) to the Golgi, where they are cleaved by two proteases (site 1 and site 2 proteases). The cleaved N-terminal fragment (nSREBP) enters the nucleus,
binds to the sterol-regulatory elements in the HMG-CoAR and LDLr promoters, and increases these gene transcriptions (3, 9). Meanwhile, SCAP is glycosylated by the sequential action of Golgi enzymes α-mannosidase I, α-mannosidase II, and GlcNAc transferase I (5, 32) before returning to the ER. The modification of SCAP plays an important role in the cycling of SCAP between the ER and the Golgi (25). However, a high intracellular concentration of cholesterol prevents transport of the SCAP-SREBP complex from the ER to the Golgi and downregulates HMG-CoAR and LDLr expression. This feedback regulation mediated by SCAP prevents intracellular cholesterol overloading under physiological conditions.

Since CML can alter enzymatic activity, decrease ligand binding, modify protein half-life, and alter immunogenicity (33), the present studies were designed to investigate whether CML could disrupt the SCAP-mediated feedback regulation for HMG-CoAR and LDLr by increasing SCAP gene transcription and protein glycosylation by Golgi enzymes, thereby enhancing the cycling of SCAP between the ER and the Golgi. This increases lipid accumulation by activating HMG-CoAR and LDLr in human mesangial cells.

MATERIALS AND METHODS

Cell culture. An established stable human mesangial cell line (HMCs; kindly donated by Dr. J. D. Sraer, Hopital Tenon, Paris, France) were cultured in RPMI-1640 medium with 10% fetal calf serum, 2 mmol/l glucose, 100 U/ml penicillin, and 100 µg/ml streptomycin. All experiments were carried out in serum-free RPMI-1640 medium containing 0.2% BSA, 2 mmol/l glucose, 100 U/ml penicillin, and 100 µg/ml streptomycin. All reagents for cell culture were obtained from Sigma (Poole, Dorset, UK). CML, which is produced by organic synthesis (no material of animal or human origin is used), was obtained from NeoMPS (Strasbourg, France).

LDL preparation. The plasma containing antioxidants (100 µmol/l BHT and 20 µmol/l EDTA) was adjusted to a density of 1.019 g/ml by adding sodium bromide (NaBr), and centrifuged at 60,000 rpm for 22 h at 4°C using a 70 Ti Rotor to remove VLDL and IDL. Next, the density was adjusted to 1.063 g/ml and centrifuged at 60,000 rpm for 22 h at 4°C. The LDL fraction on the top layer was aspirated and desalted by a PD-10 column. The LDL protein concentration was measured by a Lowry assay (28).

Morphological examination. HMCs were plated in chamber slides (Becton Dickinson, Oxford, UK) and incubated in serum-free experimental medium in the absence or presence of 50 µg/ml CML alone, CML plus 200 µg/ml LDL, and LDL alone. After 24-h incubation, the cells were washed three times in PBS, fixed with 5% formalin solution in PBS, stained with Oil Red O for 30 min, and counterstained with hematoxylin for another 5 min. Finally, the cells were examined by light microscopy.

Measurement of intracellular cholesterol. HMCs in six-well plates were cultured for 24 h in serum-free experimental medium in the absence or presence of 50 µg/ml CML alone, CML plus 200 µg/ml LDL, and LDL alone. Cells were then washed twice in PBS, intracellular lipids were extracted in a chloroform/methanol (2:1) mix and dried under a vacuum, and the total cholesterol (TC), free cholesterol (FC), and cholesterol ester content were measured by an enzymatic assay normalized by total cell proteins determined by the Lowry assay as described previously (cholesterol ester = total cholesterol – free cholesterol) (20).

Total RNA isolation and real-time quantitative PCR. Total RNA was isolated from cultured HMCs using TRIzol (Ambion, Huntingdon, UK). Total RNA (1 µg) was used as a template for RT with a High Capacity cDNA RT Kit from ABI (Applied Biosystems, Warrington, UK). Real-time RT-PCR was performed in an ABI 7000 Sequence Detection System using SYBR Green dye according to the manufacturer’s protocol (Applied Biosystems). All the PCR primers were designed by Primer Express Software V2.0. (Table 1).

**Protein isolation and Western blot analysis.** Identical amounts of protein from whole-cell extract or nuclear extract were denatured and then subjected to electrophoresis on an 8% SDS polyacrylamide gels in a Bio-Rad miniprotein apparatus (Bio-Rad Laboratories, Hemel Hempstead, UK). Electrophoretic transfer to nitrocellulose was accomplished at 100 V, 350 mA for 1–2 h. The membrane was then blocked with 5% skim milk for 1 h at room temperature and probed with the following antibodies: chicken anti-human LDLr polyclonal antibody (Abcam, Cambridge, UK), goat anti-human nSREBP-2 polyclonal antibody (Santa Cruz Biotechnology, Wiltshire, UK), goat anti-human SCAP polyclonal antibody (Millipore, Watford, UK), and rabbit anti-human β-actin polyclonal antibody (Sigma) in antibody dilution buffer (1% BSA in PBST). A goat anti-chicken or rabbit anti-goat or goat anti-rabbit horseradish peroxidase-labeled antibody (Abcam) was diluted in antibody dilution buffer. Finally, detection procedures were performed using an ECL Advance Western blotting detection kit and autoradiography was performed on Hyperfilm ECL (Amersham Bioscience, Buckinghamshire, UK).

**Protein degradation.** For a protein stability assay, HMCs were treated with cycloheximide (CHX; 50 µmol/l) in the presence or absence of CML for different times. Total proteins prepared for SCAP degradation were resuspended in Complete Lysis Buffer (10 mmol/l DTT, lysis buffer, protease inhibitor cocktail). DTT (1:1,000) and a protease inhibitor cocktail (1:100) were added fresh to the buffer just before use. An equal amount of protein was subjected to Western blotting.

**Plasmid constructions.** A green fluorescent protein (GFP)-SCAP expression construct was made by ligating human SCAP cDNA into the BstXI-XbaI sites of the pEGFP-C1 vector.

**Confocal microscopy.** HMCs were plated on chamber slides and incubated in growth medium for 24 h. The cells were subsequently transfected with pEGFP-SCAP using Effectene Transfection Reagent (Qiagen, West Sussex, UK) according to the manufacturer’s protocol. The transfected cells were incubated with or without 200 µg/ml LDL in the absence or presence of CML (50 µg/ml). After 24 h, the cells were washed with PBS, fixed in 5% formalin solution for 30 min, permeabilized with 0.25% of Triton X-100 for 15 min, and stained with mouse anti-Golgin-97 antibody (Invitrogen, Paisley, UK) for 1 h at room temperature. After washing, the cells were further stained by a secondary fluorescent antibody (goat anti-mouse Alexa Fluor 594) for 1 h. Cells were then imaged by confocal microscopy using a Zeiss LSM 510 Meta (Carl Zeiss, Hertfordshire, UK). The Golgi area was

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
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<tbody>
<tr>
<td>LDLr</td>
<td>5'-CTTGGGCTCCATAGGTATCT-3' sense</td>
</tr>
<tr>
<td></td>
<td>5'-GGCTGTCAGGGTCATCTCT-3' antisense</td>
</tr>
<tr>
<td>SCAP</td>
<td>5'-CTGCCATCGCTGCGAAGCTT-3' antisense</td>
</tr>
<tr>
<td></td>
<td>5'-CCTTCTGCTCTGCGGAAAGCTT-3' antisense</td>
</tr>
<tr>
<td>SREBP-2</td>
<td>5'-CGATGCCCTTCAGAACAGTT-3' sense</td>
</tr>
<tr>
<td></td>
<td>5'-GCGAACAGGAAATCTGCTG-3' antisense</td>
</tr>
<tr>
<td>HMGC-CoAR</td>
<td>5'-CTGGGATCTACGGGCACTATT-3' sense</td>
</tr>
<tr>
<td>α-Mannosidase I</td>
<td>5'-CTGGTATGGAGAGACGTC3-3' antisense</td>
</tr>
<tr>
<td>α-Mannosidase II</td>
<td>5'-ATGGAGAATGGGCAACCTTT-3' sense</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-CTGGTATGGAGAGACGTC3-3' sense</td>
</tr>
<tr>
<td></td>
<td>5'-GGCAGTTCCACACGGATGACT-3' antisense</td>
</tr>
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LDLr, LDL receptor; SCAP, sterol-regulatory element binding protein (SREBP) cleavage-activating protein; HMGC-CoAR, 3-hydroxy-3-methylglutaryl-CoA reductase.

**Table 1. The primers for real-time PCR**
selected, and the colocalizing pixels in the selected area were measured. The colocalization coefficient was calculated using Carl Zeiss LSM5 software.

Preparation of microsomes. Treated HMCs on two 150-cm² culture flasks were rinsed twice with PBS by incubating with 2 ml of PBS, 2 mmol/l of EDTA for 5–10 min. The cells were pelleted by centrifugation at 500 g for 5 min. The pellet was washed with PBS, recentrifuged, resuspended into 2 ml of low-ionic strength buffer (10 mmol/l Tris·HCl, pH 7.5, 0.5 mmol/l MgCl₂, 1 mmol/l phenylmethylsulfonfyl fluoride, 100 U/ml aprotinin), and then incubated on ice for 15 min. The isotonic homogenate was made by addition of 2 ml of 0.5 mol/l sucrose, 0.3 mol/l KCl, 6 mmol/l /-mercaptoethanol, 40 μmol/l CaCl₂, 10 mmol/l Tris-HCl, pH 7.5, and then centrifuged at 8,000 g for 20 min at 4°C. The supernatant was transferred to a polycarbonate tube containing 0.9 ml of 2.5 mol/l KCl, then centrifuged at 120,000 g for 1 h at 4°C. The pellet was washed twice and then resuspended in 200 μl of buffer (0.25 mol/l sucrose, 0.15 mol/l KCl, 3 mmol/l /-mercaptoethanol, 20 μmol/l CaCl₂, 10 mmol/l Tris-HCl, pH 7.5).

Enzymatic assay of /-mannosidase. /-Mannosidase activity was determined using a previously described enzyme-based method (18) (p-nitrophenyl-/α-d-mannoside + H₂O /α-Mannosidase → β-mannose + p-nitrophenol) and normalized by total cell proteins determined by the Lowry assay.

Data analysis. In all experiments, groups of data were evaluated for significance by one-way ANOVA. Data were considered significant if the P value was <0.05.

RESULTS

Oil Red O staining and intracellular cholesterol assay. We checked lipid accumulation in HMCs in response to CML and LDL. CML increased Oil Red O staining in the absence or presence of LDL in HMCs (Fig. 1A). Intracellular cholesterol levels were also quantified using an enzymatic assay. Intracel-

Fig. 1. Visualization of LDL uptake and lipid droplets in human mesangial cells (HMCs) after N-/carboxymethyl) lysine (CML) treatment. HMCs were incubated for 24 h in experimental medium (control; a) or presence of 50 μg/ml of CML (b) or containing 200 μg/ml LDL (c) or 50 μg/ml CML plus 200 μg/ml LDL (d). The cells were examined for lipid inclusions by Oil Red O (ORO) staining. The results are typical of those observed in 4 separate experiments (×400; A). CE was assayed as described in MATERIALS AND METHODS. Values are means ± SD of duplicate wells from 4 experiments. **P < 0.01 vs. control. ##P < 0.01 vs. LDL (B).

Fig. 2. Effects of CML on the mRNA and protein expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAR), LDL receptor (LDLr), and cleaved N-terminal fragment of sterol-regulatory element binding protein (nSREBP-2) in HMCs. HMCs were cultured in experimental medium in the absence (control) or presence of 50 μg/ml CML or 200 μg/ml LDL or 50 μg/ml CML plus 200 μg/ml LDL for 24 h. The mRNA levels were determined following the threshold cycle (Ct) protocol for real-time RT-PCR as described in MATERIALS AND METHODS. Actin served as a reference gene. Values are means ± SD from 4 experiments (A). The protein levels were examined by Western blotting (B). The histogram represents means ± SD of the densitometric scans of HMG-CoAR, LDLr, and nSREBP-2 protein bands from 4 experiments, normalized by comparison with actin and expressed as a percentage of control (C). *P < 0.05 vs. control. **P < 0.01 vs. control. *P < 0.05 vs. LDL.
Intracellular cholesterol ester was increased in CML-treated cells in the absence or presence of a high concentration of LDL (Fig. 1B). These data suggest that CML results in intracellular cholesterol accumulation and foam cell formation.

**Effects of CML on HMG-CoAR, LDLr, and SREBP-2 expression.** We investigated effects of CML on the expression of HMG-CoAR, LDLr, and SREBP-2. CML upregulated both mRNA (Fig. 2A) and protein (Fig. 2B and C) expression of HMG-CoAR, LDLr, and SREBP-2 and overrode the suppression of these molecules induced by a high concentration of LDL. These results suggest that CML may increase HMG-CoAR-mediated cholesterol synthesis and LDLr-mediated cholesterol uptake by activating the SREBP pathway.

**Effect of Golgi enzyme inhibitors.** Kifunensine and swainsonine are inhibitors of α-mannosidase I and α-mannosidase II, enzymes which are required for the glycosylation of SCAP.

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Effects of Golgi enzyme inhibitors kifunensine and swainsonine in HMCs. HMCs were exposed to 2.5 μg/ml kifunensine plus 5 μg/ml swainsonine in the presence of 50 μg/ml CML alone or 50 μg/ml CML plus 200 μg/ml LDL for 24 h. Intracellular cholesterol levels [total (TC), free (FC), and ester content (EC)] were assayed as described in MATERIALS AND METHODS. Values are means ± SD of duplicate wells from 4 experiments (A). The mRNA expression of HMG-CoAR, LDLr, and nSREBP-2 was determined following the ΔΔ threshold cycle (Ct) protocol for real-time RT-PCR as described in MATERIALS AND METHODS. Actin served as the reference gene. Values are means ± SD from 4 experiments (B). The protein levels were examined by Western blotting (C). The histogram represents means ± SD of the densitometric scans of HMG-CoAR, LDLr, and nSREBP-2 protein bands from 4 experiments, normalized by comparison with actin and expressed as a percentage of control (D). *P < 0.05 vs. control. **P < 0.01 vs. control. *P < 0.05 vs. LDL. **P < 0.01 vs. LDL.
These inhibitors decreased cholesterol levels induced by CML in the absence or presence of a high concentration of LDL (Fig. 3A) and also reduced HMG-CoAR, LDLr, and SREBP-2 expression induced by CML at both the mRNA and the protein levels (Fig. 3, B–D), suggesting that the Golgi enzyme inhibitors prevent cholesterol accumulation in HMCs induced by CML.

**Effects of CML on SCAP expression and intracellular translocation.** We investigated effects of CML on the expression of SCAP, which is a cholesterol sensor and chaperone of SREBP-2. CML upregulated levels of SCAP mRNA (Fig. 4A) and protein (Fig. 4, B and C) and overrode the suppression of SCAP induced by a high concentration of LDL.

Using immunofluorescent staining, we investigated the effect of CML on the translocation of SCAP-escorting SREBP-2 from the ER to the Golgi in HMCs transiently expressing pEGFP-SCAP. By staining pEGFP-SCAP-transfected HMCs with anti-human Golgin-97 antibody, we demonstrated that LDL reduced SCAP accumulation in the Golgi by inhibiting SCAP translocation from the ER to the Golgi. However, CML increased SCAP accumulation in the Golgi by enhancing translocation of SCAP from the ER to the Golgi even in the presence of a high concentration of LDL (Fig. 5A). The colocalization efficiency of SCAP in the Golgi was increased in the CML-treated cells in the absence or presence of LDL (Fig. 5B). These results suggest that CML may disrupt SCAP-mediated feedback regulation for HMG-CoAR and LDLr by increasing SCAP expression and SCAP translocation from the ER to the Golgi.

**Effect of CML on SCAP stability.** To evaluate the protein stability of SCAP in the absence or presence of CML in HMCs, we used Western blotting to estimate the amount of protein remaining at various time points (0, 2, 4, 8, 24, or 48 h) after HMCs incubated with CHX, a protein synthesis inhibitor (Fig. 6, A and B). We demonstrated that SCAP levels in HMCs in serum-free experimental medium declined in a time-dependent manner without CML. However, the decline of SCAP
protein levels was prevented by CML at all time points, suggesting that CML may increase SCAP protein stability.

Effect of CML on Golgi enzyme mRNA level and activity.

Next, we investigated the effects of CML on the expression of the Golgi enzymes α-mannosidase I and α-mannosidase II, which are required for SCAP glycosylation. CML upregulated the mRNA expression of α-mannosidase I and α-mannosidase II in the absence or presence of LDL (Fig. 7A). We also examined α-mannosidase activity. Consistent with enhanced enzyme mRNA expression, CML also increased enzyme protein activity (Fig. 7B).

DISCUSSION

In the kidney, the formation of glomerular foam cells is encountered in diabetic glomerulosclerosis, for which mesangial lipid accumulation is an important factor (10, 11, 13, 17). We detected intracellular cholesterol content with different concentrations of CML (12.5, 50, and 100 μg/ml) in the absence or presence of LDL. The maximal effect was reached at 50 μg/ml (data not shown) of CML, which was used for all experiments in the study. Using the Oil Red O staining, the present studies show that CML caused massive lipid accumulation in HMCs, which was confirmed by an intracellular cholesterol ester quantitative assay in CML-treated cells. This result is consistent with the observation that there are excessive amounts of lipid deposits in the diabetic kidney, which is associated with increased mesangial expansion, matrix protein accumulation, and tubulointerstitial fibrosis, resulting in diabetic glomerulosclerosis (17, 34).

The generally accepted mechanism of lipid accumulation in cells involves uptake of modified LDL (usually oxidized LDL) via the type A scavenger receptor family (ScrA) (2). Although we have demonstrated that inflammatory cytokines cause lipid accumulation in HMCs by inducing ScrA (30), it seems that the main lipoprotein receptor in both HMCs and tubular cells is LDLr. We have previously demonstrated that inflammatory cytokines increased LDL cholesterol uptake by disrupting LDLr feedback regulation (26, 27, 29–31) and that lipid accumulation in HMCs induced by cytokines could not be inhibited by poly I, a scavenger receptor blocker (16). Instead, this accumulation was blocked by heparin, which removes LDL bound to the cell surface (8), suggesting that the LDLr pathway is involved in cholesterol accumulation in HMCs induced by inflammation. In this study, we demonstrated that CML accelerated lipid accumulation in HMCs by increasing both mRNA and protein expression of HMG-CoAR and LDLr, therefore increasing cholesterol synthesis and uptake and causing mesangial foam cell formation independently of scavenger receptors.

Next, we examined the effects of CML on expression and intracellular translocation of SCAP and SREBP-2, two important molecules regulating HMG-CoAR and LDLr expression. Our result showed that CML increased both mRNA and protein expression of SCAP. It seems that the ER retention factor for SCAP is quantitatively limited in the ER and may be present in insufficient concentrations to retain the increased SCAP levels in the presence of CML (27). Therefore, increased SCAP expression results in SCAP translocation from the ER to the Golgi as shown in the immunofluorescent staining in this study. This abnormal escape of the SCAP-SREBP2 complex carries more SREBP-2 to the Golgi for cleavage which...
increased levels of nSREBP-2, increasing expression of HMG-CoAR and LDLr even in the presence of a high concentration of LDL which would normally inhibit this process and prevent cholesterol overloading. These data suggest that CML disrupts cholesterol-mediated feedback regulation for HMG-CoAR and LDLr by increasing SCAP expression and translocation from the ER to the Golgi. SREBP-2 is a key regulatory factor for both LDLr and HMG-CoAR transcription. It seems that CML significantly increased SREBP2 expression, but less efficiently increased both LDLr and HMG-CoAR expression. This may be because CML also activates a factor inhibiting LDLr expression. For example, it has been reported that CML increases oxidation of LDL, producing more ox-LDL which may inhibit LDLr transcription (11a). This may explain why expression of SREBP, to a lesser degree than LDLr and HMG-CoAR, was increased by CML.

When SCAP translocates from the ER to the Golgi, its carbohydrates are successively modified by α-mannosidase I, GlcNAc transferase I, and α-mannosidase II before it cycles back to the ER (14, 25). Inhibitors of α-mannosidase I and α-mannosidase II inhibit this process (12). Recent studies have indicated that interaction of CML with RAGE causes oxidative stress and activation of NF-κB via multiple intracellular signal pathways, which can alter enzymic activity of mannosidases and modify the protein half-life. Our results demonstrated that CML increased mRNA expression and enzyme activity of Golgi α-mannosidases, which can result in glycosylation of SCAP as demonstrated previously (25). We have also shown that CML increased SCAP stability as shown in Fig. 6, probably by preventing SCAP degradation and prolonging SCAP half-life. It seems that SCAP Golgi glycosylation by CML enhances the stability and recycling of SCAP between the ER and Golgi, increasing intracellular cholesterol levels by activating HMG-CoAR and LDLr. Interestingly, kifunensine (an inhibitor of α-mannosidase I) and swainsonine (an inhibitor of α-mannosidase II) reduced CML-induced cholesterol levels by inhibiting SREBP-2 activation and expression of HMG-CoAR and LDLr.

In summary, this study demonstrates that CML increases expression and potential glycosylation of SCAP in the Golgi by increasing enzyme activity of Golgi α-mannosidases, thereby causing an abnormal translocation of SCAP from the ER to the Golgi and enhancing recycling of the SCAP-SREBP complex between the ER and the Golgi. These processes increase SREBP cleavage and produce more nSREBP-2, which consequently activates HMG-CoAR and LDLr expression and lipid accumulation in HMCs. These results may improve our understanding of the molecular mechanisms of diabetic glomerulosclerosis and also suggest that SCAP transcripption and intracellular translocation could be a useful biomarker for prediction of progression of diabetic nephropathy and that anti-AGEs or Golgi enzyme inhibitors may be useful adjunctive therapeutic agents in the management of diabetic glomerulosclerosis.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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