Journal of Geriatric Cardiology (2017) 14: 473–480

Research Article · **Open Access** ·

Glycation of high-density lipoprotein triggers oxidative stress and promotes the proliferation and migration of vascular smooth muscle cells

Qian DU[1,*], Ming-Ming QIAN[1,*], Pin-Li LIU[2], Le ZHANG[1], Yan WANG[1], Dong-Hui LIU[1]

[1]*Department of Cardiology, the Affiliated Cardiovascular Hospital of Xiamen University, Medical College of Xiamen University, Xiamen, China*
[2]*Department of Endocrinology, the Second Hospital of Hebei Medical University, Shijiazhuang, China*

Abstract

Background In type 2 diabetes mellitus (T2DM), high-density lipoprotein (HDL) impairs its anti-atherogenic properties and even develops to a pro-inflammatory and pro-atherogenic phenotype because of abnormal compositions and modifications. In this study, we examined the effects and the related mechanisms of glycation of HDL on the proliferation and migration of vascular smooth muscle cells (VSMCs). **Methods & Results** Glycated HDL (G-HDL) was modified with D-glucose (25 mmol/L) *in vitro*. Diabetic HDL (D-HDL) was isolated from T2DM patients. Rat VSMCs were isolated from the thoracic aortas. Human VSMCs were obtained from ScienCell Research Laboratories. Alpha-actin was detected through immunofluorescence. VSMC proliferation was assayed by Cell Count. VSMC migration was determined by transwell chamber and scratch-wound assay. Intracellular reactive oxygen species (ROS) was detected based on ROS-mediated 2',7'-dichlorofluorescein (DCFH-DA) fluorescence. Compared to native HDL (N-HDL), G-HDL remarkably promoted VSMC proliferation and migration in the dose and time-dependent manners. In addition, G-HDL enhanced ROS generation in VSMCs. However, the ROS scavenger, N-acetylcysteine, efficiently decreased ROS production and subsequently inhibited the proliferation of VSMCs induced by G-HDL. Similarly, D-HDL from T2DM patients also promoted ROS release and VSMC proliferation and migration. **Conclusions** HDL either glycated *in vitro* or isolated from T2DM patients triggered VSMC proliferation, migration, and oxidative stress. These results might partly interpret the higher morbidity of cardiovascular disease in T2DM patients.

J Geriatr Cardiol 2017; 14: 473–480. doi:10.11909/j.issn.1671-5411.2017.07.003

Keywords: High-density lipoprotein; Glycation; Migration; Proliferation; Vascular smooth muscle cells

1 Introduction

The proliferation and migration of vascular smooth muscle cells (VSMCs) play pivotal roles during the progression of atherosclerotic plaque.[1] Under the condition of inflammation or oxidative stress, VSMC phenotype shifts from a quiescent contractile state to an active synthetic state.[2] The synthetic phenotype of VSMCs proliferates and migrates from the medial layer of vessels into the intima, which results in neointimal hyperplasia and even leads to restenosis of coronary arteries after angioplasty in patients with cardiovascular disease (CVD).[3]

High-density lipoprotein (HDL) exerts notably anti-atherogenic properties.[4] The protective effects of HDL are mainly attributed to its abilities of reverse cholesterol transport (RCT) as well as its anti-inflammatory, anti-oxidant, and anti-apoptotic activities.[5] In addition, HDL also inhibits the proliferation and migration of VSMCs and alleviates the pathogenic neointima formation.[6,7] Over-expression of endothelial lipase (EL) decreases plasma HDL levels, and increases VSMC proliferation and migration and neointima formation in impaired carotid arteries.[8] Therefore, HDL might be an efficient target for improving neointimal formation.

Type 2 diabetes mellitus (T2DM) is an independent risk factor for CVD, and T2DM patients are more preferred to suffer from atherogenesis and CVD compared to non-T2DM individuals.[9] In T2DM condition, HDL becomes deficient and even develops to a pro-atherogenic agent owing to triglycerides (TG) enrichment, cholesterol esters (CE) depletion, and apolipoprotein glycation.[10,11] Diabetic HDL from T2DM patients lost its ability to promote endothelial cell repair because of down-regulation of scavenger receptor class B type I (SR-BI).[12] Also, diabetic HDL causes abnormal actions on breast cancer cell adhesion to endothelial

*The first two authors contributed equally to this work.

Correspondence to: Dong-Hui LIU, MD, PhD, the Affiliated Cardiovascular Hospital of Xiamen University, Medical College of Xiamen University, Hubin South Rd., Siming District, Xiamen 361004, Fujian Province, China. E-mail: liudh@xmu.edu.cn

Telephone: +86-592-2292373 **Fax:** +86-592-2292704

Received: November 18, 2016 **Revised:** July 10, 2017

Accepted: July 18, 2017 **Published online:** July 28, 2017

cells, and subsequently promotes the metastasis of breast cancer cells.[13] Furthermore, glycation of HDL partly impairs its anti-inflammatory abilities to inhibit cytokine release in macrophages.[14] Thus, glycation of HDL could impair its protective effects and transform HDL to be a pro-atherosclerogenic agent.

Previously, we reported that oxidized HDL (ox-HDL) triggers reactive oxygen species (ROS) production and promotes the proliferation and migration of VSMCs.[15] However, in T2DM state, whether glycation of HDL also becomes an oxidative candidate and promotes VSMC proliferation and migration, which aggravates the progression of atherosclerotic plaque is unclear. In this study, we investigated this phenomenon and noted that glycated HDL (G-HDL) modified by glucose *in vitro* and diabetic HDL (D-HDL) from T2DM patients, induced ROS generation and promoted VSMC proliferation and migration, which may explain why T2DM patients have higher incidence of CVD.

2 Methods

2.1 Reagents

Dulbecco's modified Eagle's medium (DMEM), trypsin, and fetal bovine serum (FBS) were purchased from Gibco Co. (Carlsbad, CA, USA). N-acetyl-L-cysteine (NAC), 2', 7'-dichlorofluorescindiacetate (DCFH-DA) and crystal violet were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Cell Counting Kit-8 (CCK-8) was purchased from Enzo Biochem Inc. (Farmingdale, NY, USA). Transwell chamber with 8-μm pore polycarbonate membrane was obtained from Millipore Co. (Billerica, MA, USA). Antibody to α-actin was purchased from Beyotime (Haimen, China). Alexa Fluro-labeled anti-rabbit IgG was purchased from Cell Signaling Technology (Danvers, MA, USA). All other chemicals and reagents were obtained from commercial sources and were of analytical grade.

2.2 Patient characteristics

Ten healthy volunteers and eight T2DM patients were enrolled. Each recruited individual underwent the physical examination and laboratory tests. The inclusion criteria for T2DM patients were: (1) fasting glucose > 7.0 mmol/L; (2) no insulin therapy; and (3) haemoglobin A1c levels > 7%, respectively.[14] Healthy volunteers had normal glucose tolerance. The exclusion criteria were systemic infection, malignant tumor, and CVD. Each participant was given an informed consent after the procedure was explained. The study protocol was approved by the Institutional Review Board of the Second Hospital of Hebei Medical University.

2.3 HDL isolation and glycation

HDL (1.063–1.210 g/mL) was isolated by ultracentrifugation.[16] Briefly, the density of human plasma was adjusted to 1.3 g/mL with KBr, and saline (1.006 g/mL) was layered upper to form a discontinuous NaCl/KBr density gradient. Samples with the gradient were centrifuged at 350,000 g for 3.5 h at 4°C. HDL was collected, dialyzed, sterilized and stored in the dark at 4°C. To obtain G-HDL, HDL (1 mg/mL) was incubated with 25 mmol/L of D-glucose in PBS containing EDTA (1 mg/mL, PH 7.4) for seven days at 37°C *in vitro*.[14] To exclude the oxidation independent of glycation, butylated hydroxytoluene (BHT, 25 μmol/L) was added into the solution. Meanwhile, native HDL (N-HDL) was incubated with PBS in the absence of D-glucose under the same conditions. After seven days of incubation, both N-HDL and G-HDL were dialyzed again with PBS to remove the residual glucose in the buffer.

2.4 Cell culture

Rat VSMCs were isolated from male Sprague-Dawley rats (120–160 g) as described before.[15] Human VSMCs were obtained from ScienCell Research Laboratories (Santiago, CA, USA). VSMCs were grown in DMEM medium supplemented with 10% FBS and antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin) in an atmosphere of 5% CO_2 and 95% air at 37°C. Cells at passages 4–6 were used. Usually VSMCs were starved with serum-free DMEM medium for 12 h before treatment with HDL. All of the experiments followed the institution's instructions for animal care and usage. All of the procedures were approved by the Ethics Committee for the Use of Experimental Animals at Medical College of Xiamen University.

2.5 Cell proliferation assay

Cell proliferation was assayed by CCK-8 cell viability kit. Briefly, VSMCs were seeded at 3×10^3 cells/well in 96-well plates. After cells reached 70%–80% confluence, VSMCs were starved for 12 h with serum-free DMEM medium, and then incubated with N-HDL, G-HDL or D-HDL for 24 h to stimulate cell proliferation. Subsequently, CCK-8 reagent (10 μL) was added and cells were further incubated for 2 h at 37°C. The absorbance wavelength was read at 450 nm using spectrophotometric plate reader.

2.6 Transwell migration assay

Cell migration was tested using Transwell chamber with polycarbonate 8 μm pore membrane. VSMCs were starved with serum-free DMEM medium for 12 h. Subsequently, cells mixed with N-HDL, G-HDL or D-HDL were seeded in the upper wells (5×10^4 cells/200 μL of serum-free

DMEM with HDL), whereas the lower wells were filled with 800 μL of DMEM medium containing 10% FBS. After 6 h, cells migrated across the filters were fixed with 4% paraformaldehyde and stained with crystal violet. Cells migrating to the lower side of the filter were counted in three random fields per well (100 ×) using light microscopy (Nikon, Tokyo, Japan).

2.7 Scratch-wound assay

After 80% confluence, VSMCs were scratched gently with 200 μL pipette tips. Cells were rinsed twice with PBS to remove the debris, and the linear wound was recorded. VSMCs were treated with 50 μg/mL of N-HDL or G-HDL for 48 h. Then cell images were taken. The number of cells that migrated into the wound space was manually counted in three random fields (100 ×) using light microscopy (Nikon, Tokyo, Japan).

2.8 Measurement of intracellular ROS

Intracellular ROS was detected using the oxidant-sensitive probe DCFH-DA.[15] VSMCs were seeded on glass coverslips. After starving with serum-free DMEM medium for 12 h, cells were washed twice with PBS and incubated with 5 μmol/L of DCFH-DA for 30 min. Subsequently, VSMCs were treated with 50 μg/mL of N-HDL, G-HDL or D-HDL for 15 min, and the relative DCF fluorescence intensity was detected by fluorescent microscopy (Nikon, Tokyo, Japan). The examination wavelength was 488 nm and the emission wavelength was 530 nm, respectively. The fluorescence intensity was determined using Image-Pro Plus/IOD.

2.9 Immunofluorescence assay

For immunofluorescent staining, VSMCs were seeded on glass coverslips. Rabbit polyclonal anti-α-actin antibody and Alexa Fluro-labeled anti-rabbit IgG were used. Nuclear localization was counterstained with DAPI. Images were obtained under fluorescent microscopy (200 ×) (Nikon, Tokyo, Japan).

2.10 Statistical analysis

All experiments were repeated 3–4 times. Data were presented as mean ± SD. Differences were compared with *t* test or one-way ANOVA using GraphPad Prism (5.0). Values of $P < 0.05$ were considered statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

3 Results

3.1 G-HDL promoted VSMC proliferation

The expression of α-actin was detected through immunofluorescence assay (Figure 1A and E). VSMC proliferation was assayed using CCK-8 kits. G-HDL promoted the proliferation of human and rat VSMCs, however, N-HDL almost had no effects on VSMC proliferation (Figure 1B and F). Moreover, the pro-proliferative ability of G-HDL was in the dose and time-dependent manners (Figure 1C, D, G and H). Thus, compared to N-HDL, G-HDL remarkably promoted VSMC proliferation.

3.2 G-HDL promoted rat VSMC migration

The pro-migrative effects of G-HDL on rat VSMCs were tested by Transwell chamber and wound-healing assay. Compared to control group, N-HDL did not cause changes on VSMC migration, however, G-HDL notably facilitated VSMC migration (Figure 2A and C). And the pro-migrative ability of G-HDL was also dose-dependent (Figure 2B and D). Therefore, G-HDL significantly promoted VSMC migration.

3.3 Diabetic HDL promoted rat VSMC proliferation and migration

To further investigate whether diabetic HDL from T2DM patients also displayed the pro-proliferative and pro-migrative effects on rat VSMCs, we isolated healthy HDL from ten normal volunteers and diabetic HDL from eight T2DM patients. VSMC proliferation was assayed by CCK-8 kits, and VSMC migration was tested through Transwell chamber as mentioned above. Similarly to G-HDL, diabetic HDL also showed more pro-proliferative and pro-migrative abilities than that of HDL from healthy volunteers (Figure 3A and B).

3.4 G-HDL and D-HDL promoted ROS generation in rat VSMCs

ROS levels in rat VSMCs were measured through monitoring the intracellular fluorescence intensity of DCFH-DA oxidation. Compared to control group, N-HDL nearly had no influence on ROS production, however, G-HDL significantly increased intracellular ROS generation in VSMCs (Figure 4A). Similarly to G-HDL, diabetic HDL (D-HDL) also induced ROS release in VSMCs compared to healthy HDL (Figure 4B). These results demonstrated that both G-HDL and D-HDL triggered oxidative stress in VSMCs.

3.5 NAC inhibited ROS release and cell proliferation in rat VSMCs

To investigate whether ROS was involved in VSMC proliferation triggered by G-HDL, an ROS scavenger, NAC, was employed. ROS generation and cell proliferation were assayed as mentioned above. NAC efficiently decreased intracellular ROS production, and inhibited VSMC proliferation induced by G-HDL (Figure 5A and B).

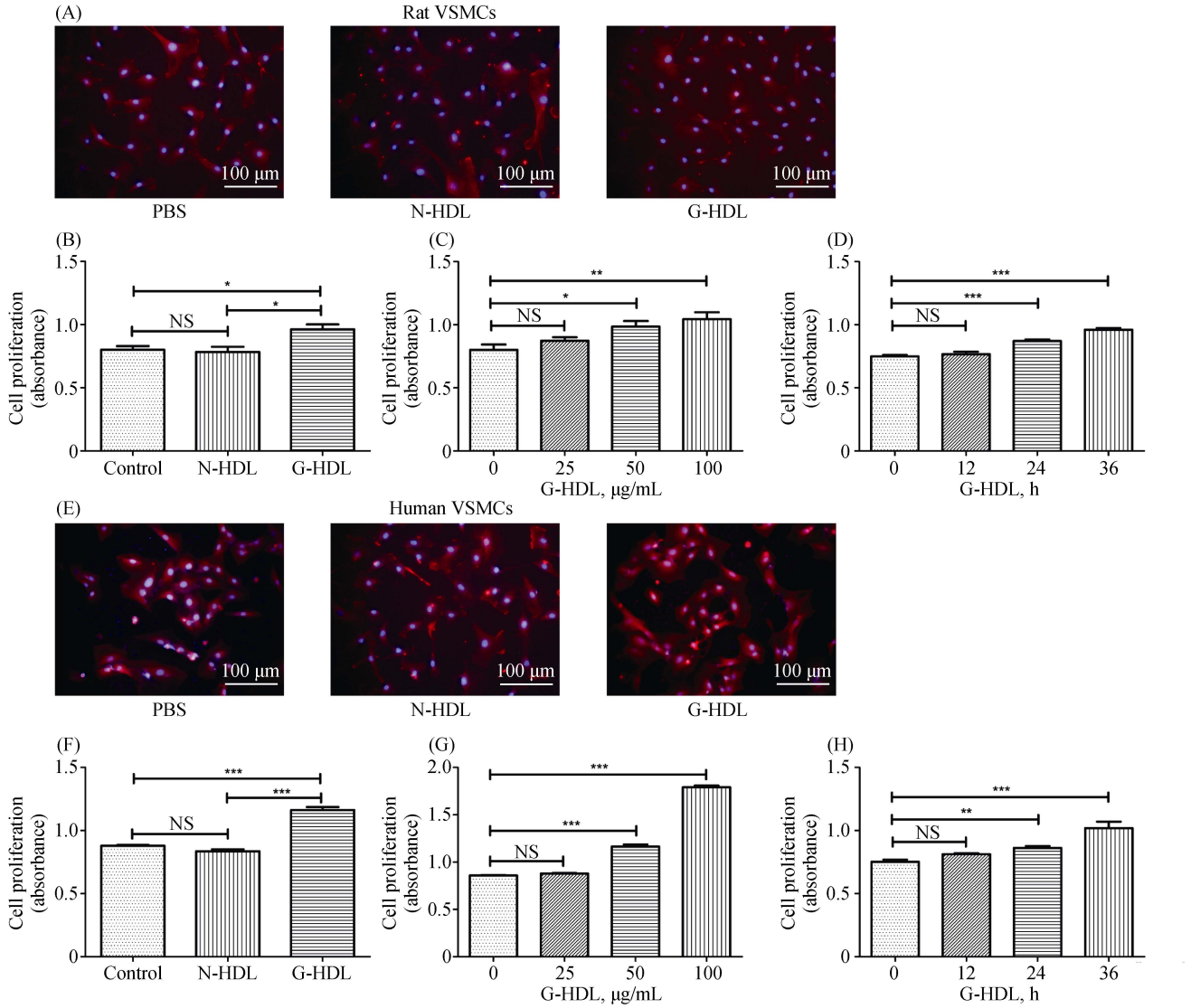

Figure 1. G-HDL promoted VSMC proliferation. (A & E): Human and rat VSMCs were incubated with PBS, N-HDL (50 μg/mL), or G-HDL (50 μg/mL) for 12 h, and the expression of α-actin was detected through immunofluorescence assay (× 200); (B & F): VSMCs were incubated with PBS, N-HDL (50 μg/mL), or G-HDL (50 μg/mL) for 24 h, and cell proliferation was assayed by CCK-8 kits; (C & G): VSMCs were incubated with 0, 25, 50 or 100 μg/mL of G-HDL for 24 h, and cell proliferation was assayed; (D & H): VSMCs were incubated with 50 μg/mL of G-HDL for 0, 12, 24, or 36 h, and cell proliferation was assayed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. CCK-8: cell counting kit-8; G-HDL: glycated high-density lipoprotein; N-HDL: native high-density lipoprotein; NS: not significant; VSMCs: vascular smooth muscle cells.

4 Discussion

In this study, we found that glycation of HDL, either modified with glucose *in vitro* or from T2DM patients, remarkably promoted VSMC proliferation and migration, and oxidative stress might play key roles in these processes.

CVD remains the principal cause of morbidity and mortality in T2DM patients, despite significant advances in managing strategies to lessen CVD risk factors.[9] In T2DM state, the vasculature persistently exposes in the disturbed metabolic circumstance, which potentially underlies the macrovascular complications in T2DM patients.[17] VSMCs shift from the contractile phenotype to the synthetic phenotype, which drives VSMCs to proliferate and migrate from the medial layer to the intima of vessels.[2] Consequently, the synthetic phenotype of VSMCs results in neointima formation and extracellular matrix deposition in vessel wall, which contributes to the narrowing of vessel lumen after coronary angioplasty.[3]

Although HDL is considered to be an anti-atherosclerotic candidate, HDL shows a pro-atherogenic phenotype in T2DM conditions.[11,18] Diabetic HDL carries higher levels of sphin-

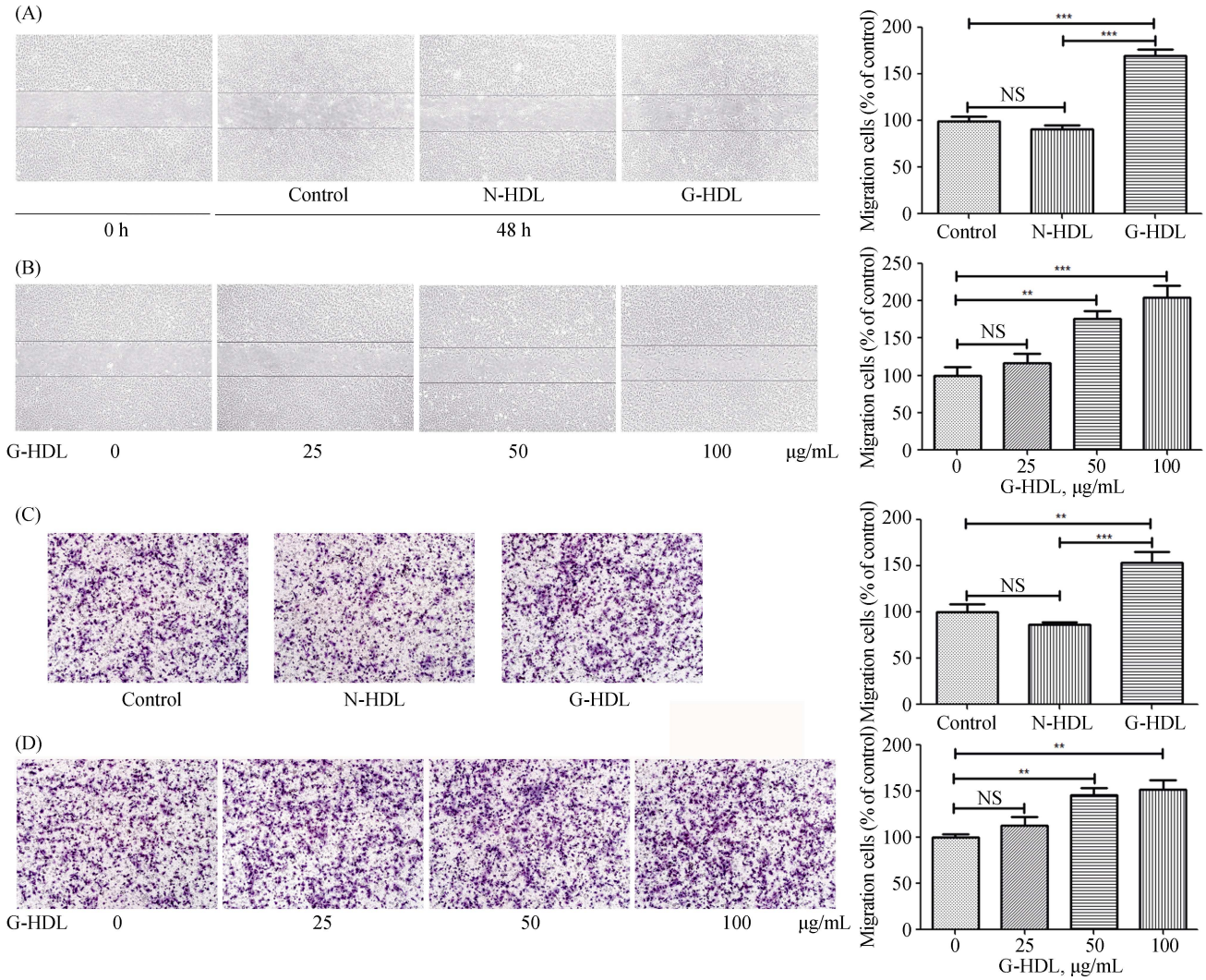

Figure 2. G-HDL promoted rat VSMC migration. (A&B): The confluent monolayer of VSMCs was scratched and a linear wound was recorded. VSMCs were incubated with 50 μg/mL of N-HDL or G-HDL for 48 h (A), or incubated with 25, 50, and 100 μg/mL of G-HDL for 48 h (B). After incubation, cell images were taken (× 100), and the number of cells migrated into the wound space were manually counted in three random fields per well with light microscope. (C & D): The migration of VSMCs was assayed using Transwell chamber. VSMCs were incubated with N-HDL (50 μg/mL), or G-HDL (50 μg/mL) for 6 h (C), or incubated with 25, 50, and 100 μg/mL of G-HDL for 6 h (D). Cell numbers translocated to the lower layer were counted after staining with crystal violet. $^{**}P < 0.01$, $^{***}P < 0.001$. G-HDL: glycated high-density lipoprotein; N-HDL: native high-density lipoprotein; NS: not significant; VSMCs: vascular smooth muscle cells.

gosine 1-phosphate (S1P) than that of native HDL, which could partly be responsible for the abnormal functions of diabetic HDL.[19] In addition, we also found that both G-HDL and D-HDL show higher glycation levels, which notably impair their anti-inflammatory effects and endothelial cell protections.[12,14] In this study, we demonstrated that both G-HDL *in vitro* and D-HDL from T2DM patients, promoted VSMC proliferation and migration, which implied that glycation of HDL might accelerate the progression of atherosclerotic plaque (Figures 1–3).

Oxidative stress plays a pivotal role in the initiation and progression of diabetes-associated CVD.[20] Intracellular ROS is involved in the regulation of VSMC proliferation, migration, contraction and differentiation.[21,22] Angiotensin II (Ang-II) and oxidized low-density lipoprotein (ox-LDL) induce oxidative stress and promote VSMC proliferation and migration.[23,24] Oxidized HDL (ox-HDL) induces ROS production and cell apoptosis, and impairs cell migration and neovascularization in endothelial progenitor cells by activation of CD36-MAPK-TSP-1 pathways.[25] Previously, we found that ox-HDL facilitates NADPH oxidase activation and ROS generation in VSMCs, and consequently

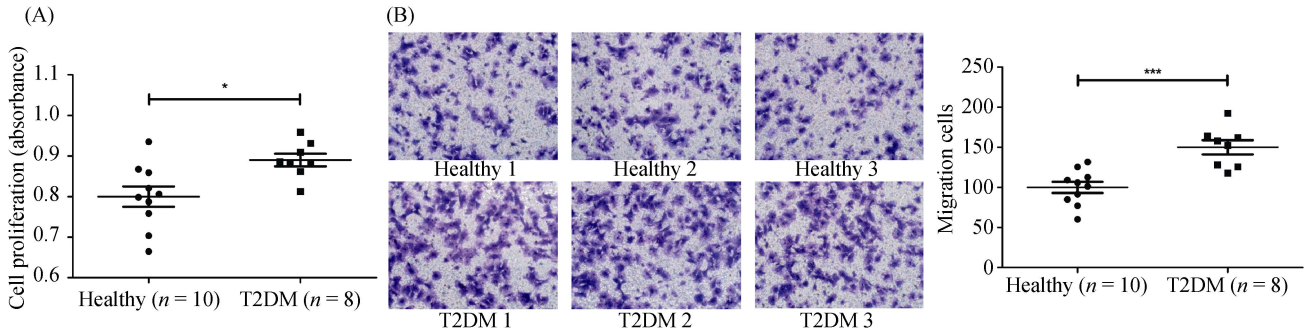

Figure 3. D-HDL promoted rat VSMCs proliferation and migration. (A): VSMCs were incubated with 50 μg/mL of healthy HDL (n = 10) or diabetic HDL (n = 8) for 24 h, and cell proliferation was assayed by CCK-8 kits; (B): VSMCs were incubated with 50 μg/mL of healthy HDL (n = 10) or D-HDL (n = 8) for 6 h, and cell migration was tested using Transwell chamber. Cells translocated to the lower layer were stained with crystal violet, and counted in three random fields (× 100). *P < 0.05, ***P < 0.001. CCK-8: cell counting kit-8; D-HDL: diabetic high-density lipoprotein; T2DM: type 2 diabetes mellitus; VSMCs: vascular smooth muscle cells.

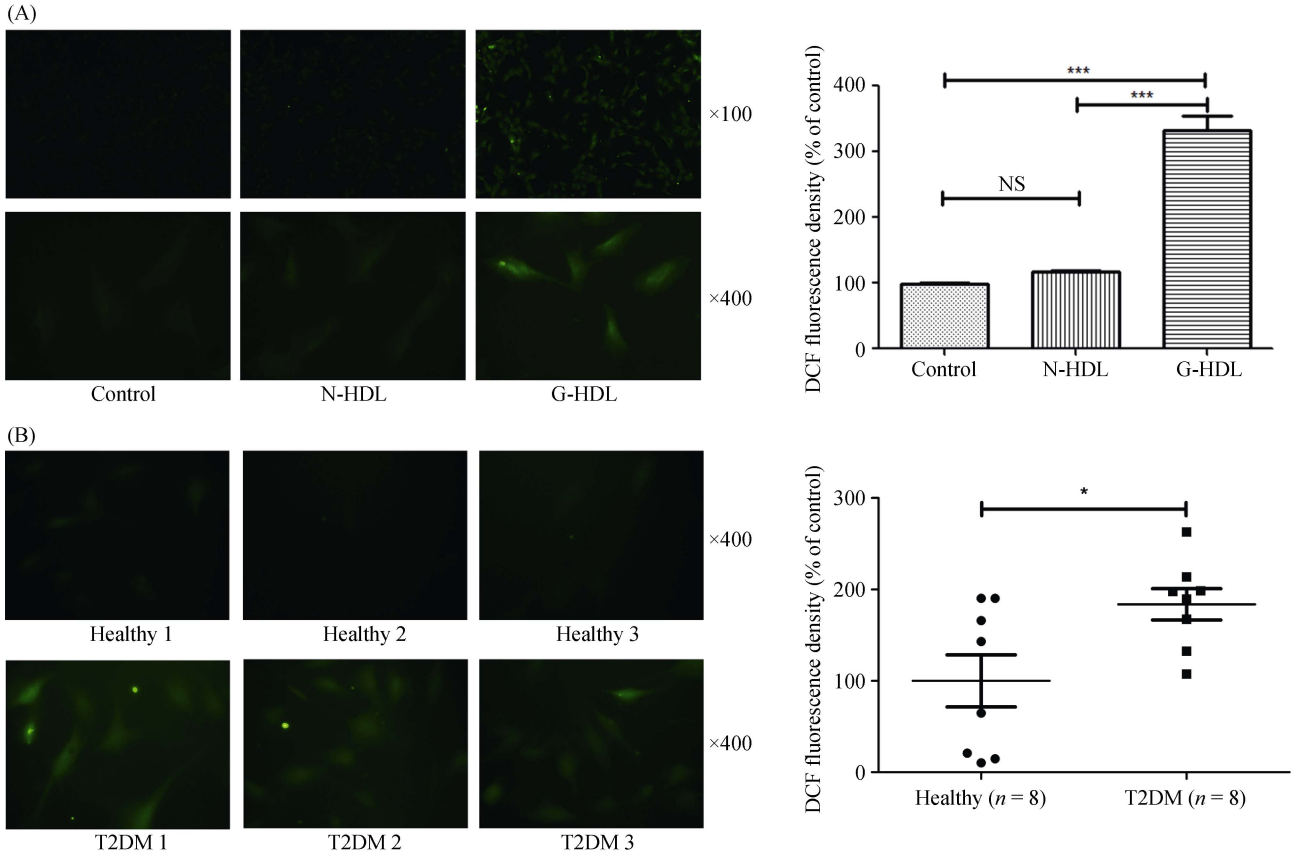

Figure 4. G-HDL and D-HDL promoted ROS generation in rat VSMCs. (A): VSMCs were incubated with PBS, N-HDL (50 μg/mL), or G-HDL (50 μg/mL) for 15 min, and intracellular ROS was assayed by monitoring the fluorescence intensity of DCF oxidation; (B): VSMCs were incubated with healthy HDL (50 μg/mL), or T2DM HDL (50 μg/mL) for 15 min, and intracellular ROS was detected. Fluorescent images of cells were taken with fluorescence microscope (× 100 and × 400). DCF fluorescence intensity in VSMCs was quantified using Image-Pro Plus/IOD. *P < 0.05, ***P < 0.001. DCF: 2′,7′-dichlorofluorescin; D-HDL: diabetic high-density lipoprotein; G-HDL: glycated high-density lipoprotein; N-HDL: native high-density lipoprotein; NS: not significant; ROS: reactive oxygen species; T2DM: type 2 diabetes mellitus; VSMCs: vascular smooth muscle cells.

promotes VSMC proliferation and migration.[15] However, inhibition of ROS production depresses the proliferation and migration of VSMCs.[15] In hyperglycemia condition, glucose reacts with amino acids of proteins to form Schiff bases, and then rearrange into the irreversible formation of advanced glycosylation end products (AGEs), which con-

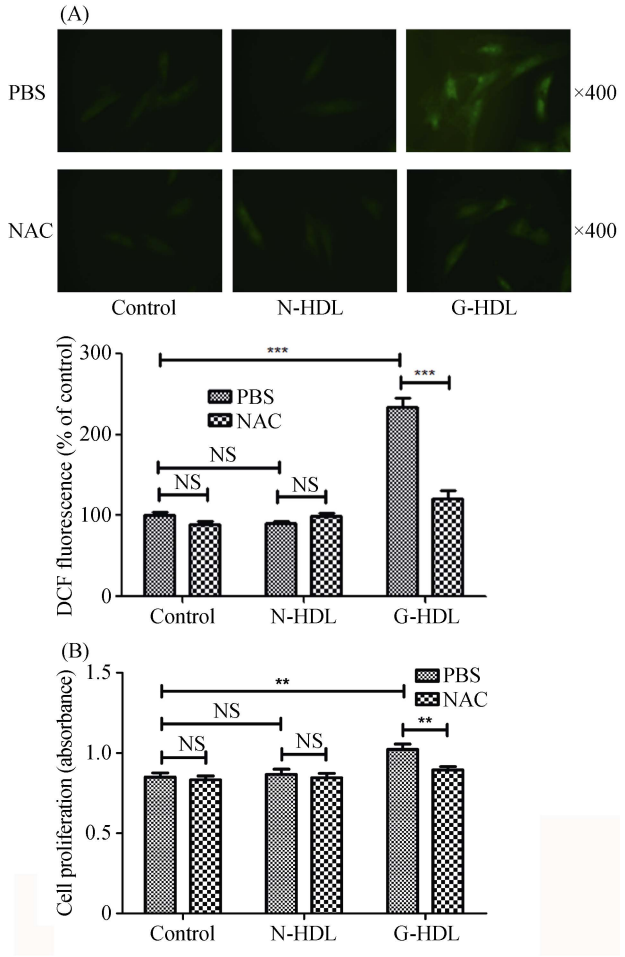

Figure 5. NAC inhibited ROS release and cell proliferation in rat VSMCs. VSMCs were pre-incubated with NAC (20 mmol/L) for 30 min, and then treated with PBS, N-HDL (50 μg/mL) or G-HDL (50 μg/mL) for 15 min. Intracellular ROS production was assayed by monitoring the fluorescence intensity of DCF oxidation (A). Fluorescent images of cells were taken with fluorescence microscope (× 400). After incubation with NAC, VSMCs were treated with PBS, N-HDL (50 μg/mL) or G-HDL (50 μg/mL) for 24 h for cell proliferation assay (B). $^{**}P < 0.01$, $^{***}P < 0.001$. DCF: 2′,7′-dichlorofluorescin; G-HDL: glycated high-density lipoprotein; NAC: N-acetyl-L-cysteine; N-HDL: native high-density lipoprotein; NS: not significant; ROS: reactive oxygen species; VSMCs: vascular smooth muscle cells.

tributes to the development of diabetic microvascular and macrovascular complications.[26,27] AGEs activate the receptor for advanced end-products (RAGE) and increase nuclear factor kappa B (NF-κB) activity, which transform VSMCs to the synthetic phenotype.[28] AGEs also trigger ROS release via stimulating NADPH oxidase, and induce the expression of cell adhesion molecules and monocyte chemoattractant protein-1(MCP-1) in endothelial cells.[29] We also found that both G-HDL and D-HDL have higher levels of AGEs than that of native HDL.[14] In this study, G-HDL significantly increased the production of ROS in VSMCs, and depression of ROS release could inhibit VSMC proliferation (Figures 4 & 5), which might partly explain the pro-proliferative effects of HDL glycation.

In summary, HDL either glycated with glucose *in vitro* or isolated from T2DM patients, induced VSMC proliferation and migration. The change of HDL from the anti- atherosclerotic phenotype to the pro-atherogenic phenotype might partly interpret the higher morbidity of CVD and the re-narrowing of vessels following coronary angioplasty and stents in T2DM patients.

Acknowledgements

This project was supported by Grant 31200884 from the National Natural Science Foundation of China; Grant 2016D016, 2016-ZQN-92, and 2016–2-75 from the Natural Science Foundation of Fujian; and Grant 3502Z20154048, 3502Z20144061, and 3502Z20154047 from the Natural Science Foundation of Xiamen.

References

1 Lowe HC, Oesterle SN, Khachigian LM. Coronary in-stent restenosis: current status and future strategies. *J Am Coll Cardiol* 2002; 39: 183–193.
2 Chistiakov DA, Orekhov AN, Bobryshev YV. Vascular smooth muscle cell in atherosclerosis. *Acta Physiol* (*Oxf*) 2015; 214: 33–50.
3 Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. *Acta Med Indones* 2007; 39: 86–93.
4 Feig JE, Hewing B, Smith JD, *et al*. High-density lipoprotein and atherosclerosis regression: evidence from preclinical and clinical studies. *Circ Res* 2014; 114: 205–213.
5 Rosenson RS, Brewer HB, Jr., Ansell B, *et al*. Translation of high-density lipoprotein function into clinical practice: current prospects and future challenges. *Circulation* 2013; 128: 1256–1267.
6 Tamama K, Tomura H, Sato K, *et al*. High-density lipoprotein inhibits migration of vascular smooth muscle cells through its sphingosine 1-phosphate component. *Atherosclerosis* 2005; 178: 19–23.
7 van der Vorst EP, Vanags LZ, Dunn LL, *et al*. High-density lipoproteins suppress chemokine expression and proliferation in human vascular smooth muscle cells. *FASEB J* 2013; 27: 1413–1425.
8 Sun L, Ishida T, Okada T, *et al*. Expression of Endothelial Lipase Correlates with the Size of Neointima in a Murine Model of Vascular Remodeling. *J Atheroscler Thromb* 2012; 19: 1110–1127.

9 Tousoulis D, Papageorgiou N, Androulakis E, *et al*. Diabetes mellitus-associated vascular impairment: novel circulating biomarkers and therapeutic approaches. *J Am Coll Cardiol* 2013; 62: 667–676.
10 Kontush A, Chapman MJ. Why is HDL functionally deficient in type 2 diabetes? *Curr Diab Rep* 2008; 8: 51–59.
11 Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract Endocrinol Metab* 2009; 5: 150–159.
12 Pan B, Ma Y, Ren H, *et al*. Diabetic HDL is dysfunctional in stimulating endothelial cell migration and proliferation due to down regulation of SR-BI expression. *PLoS One* 2012; 7: e48530.
13 Pan B, Ren H, He Y, *et al*. HDL of patients with type 2 diabetes mellitus elevates the capability of promoting breast cancer metastasis. *Clin Cancer Res* 2012; 18: 1246–1256.
14 Liu D, Ji L, Zhang D, *et al*. Nonenzymatic glycation of HDL impairs its anti-inflammatory effects in innate immunity. *Diabetes Metab Res Rev* 2011; 28: 186–195.
15 Wang Y, Ji L, Jiang R, *et al*. Oxidized high-density lipoprotein induces the proliferation and migration of vascular smooth muscle cells by promoting the production of ROS. *J Atheroscler Thromb* 2014; 21: 204–216.
16 Liu D, Ji L, Tong X, *et al*. Human apolipoprotein A-I induces cyclooxygenase-2 expression and prostaglandin I-2 release in endothelial cells through ATP-binding cassette transporter A1. *Am J Physiol Cell Physiol* 2011; 301: C739–C748.
17 Hwang MH, Kim S. Type 2 Diabetes: Endothelial dysfunction and Exercise. *J Exerc Nutrition Biochem* 2014; 18: 239–247.
18 Taskinen MR, Boren J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. *Atherosclerosis* 2015; 239: 483–495.
19 Tong X, Peng H, Liu D, *et al*. High-density lipoprotein of patients with type 2 diabetes mellitus upregulates cyclooxgenase-2 expression and prostacyclin I-2 release in endothelial cells: relationship with HDL-associated sphingosine-1-phosphate. *Cardiovasc Diabetol* 2013; 12: 27.
20 Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; 107: 1058–1070.
21 Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. *Cardiovasc Res* 2006; 71: 216–225.
22 Wang J, Shi N, Chen SY. Manganese superoxide dismutase inhibits neointima formation through attenuation of migration and proliferation of vascular smooth muscle cells. *Free Radic Biol Med* 2012; 52: 173–181.
23 Yaghini FA, Song CY, Lavrentyev EN, *et al*. Angiotensin II-induced vascular smooth muscle cell migration and growth are mediated by cytochrome P450 1B1-dependent superoxide generation. *Hypertension* 2010; 55: 1461–1467.
24 Robbesyn F, Garcia V, Auge N, *et al*. HDL counterbalance the proinflammatory effect of oxidized LDL by inhibiting intracellular reactive oxygen species rise, proteasome activation, and subsequent NF-kappaB activation in smooth muscle cells. *FASEB J* 2003; 17: 743–745.
25 Wu J, He Z, Gao X, *et al*. Oxidized high-density lipoprotein impairs endothelial progenitor cells' function by activation of CD36-MAPK-TSP-1 pathways. *Antioxid Redox Signal* 2015; 22: 308–324.
26 Vlassara H. Recent progress on the biologic and clinical significance of advanced glycosylation end products. *J Lab Clin Med* 1994; 124: 19–30.
27 Ott C, Jacobs K, Haucke E, *et al*. Role of advanced glycation end products in cellular signaling. *Redox Biology* 2014; 2: 411–429.
28 Simard E, Sollradl T, Maltais JS, *et al*. Receptor for advanced glycation end-products signaling interferes with the vascular smooth muscle cell contractile phenotype and function. *PLoS One* 2015; 10: e0128881.
29 Wautier JL, Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 2004; 95: 233–238.