Lipolytic inhibitor G0/G1 switch gene 2 inhibits reactive oxygen species production and apoptosis in endothelial cells

Yinfang Wang,1 Yahui Zhang,2 Yichun Zhu,1* and Peng Zhang3*

1Department of Physiology and Pathophysiology, Fudan University Shanghai Medical College, Shanghai, China; 2Department of Pathophysiology, Hubei University of Medicine, Hubei, China; and 3Central Laboratory, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

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Wang Y, Zhang Y, Zhu Y, Zhang P. Lipolytic inhibitor G0/G1 switch gene 2 inhibits reactive oxygen species production and apoptosis in endothelial cells. Am J Physiol Cell Physiol 308: C496–C504, 2015. First published January 14, 2015; doi:10.1152/ajpcell.00317.2014.—G0/G1 switch gene 2 (G0S2), a novel target gene of peroxisome proliferator-activated receptor, is highly expressed in fat tissues. G0S2 acts as proapoptotic factor toward human cancer cells. Endothelial cell (EC) apoptosis may be an initiating event in the development of atherosclerosis. However, the expression and function of G0S2 in vascular ECs remain unknown. Here, we reported for the first time that G0S2 is expressed in arterial ECs. Ectopic expression of G0S2 increased neutral lipid accumulation in cultured ECs. However, G0S2 prevented ECs from serum-free starvation stress- and hydrogen peroxide (H2O2)-induced apoptosis. G0S2 blocked the H2O2-induced dissipation of mitochondrial membrane potential. G0S2 decreased the release of cytochrome c from mitochondria into the cytosol, followed by activation of caspase-9 and caspase-3. The anti-apoptotic effect of G0S2 was Bcl-2 and adipose triglyceride lipase independent. In contrast, gene silence of G0S2 increased serum-free starvation stress-induced EC apoptosis and decreased the formation of capillary-like structures. We further found that G0S2 couples with the F0F1-ATP synthase in ECs. Levels of ATP were elevated, whereas reactive oxygen species levels were reduced in G0S2-expressing ECs. G0S2 can inhibit endothelial denudation secondary to H2O2-induced injury to ECs in vivo. These results indicate that G0S2 acts as a pro-survival molecule in ECs. Taken together, our results indicate that G0S2 has a protective function in ECs and may be a potential target for the treatment of cardiovascular diseases associated with reactive oxygen species-induced EC injury, such as atherosclerosis and restenosis.

G0S2; endothelial cell; mitochondria; apoptosis; atherosclerosis

ATHEROSCLEROSIS IS RESPONSIBLE for coronary artery diseases and stroke. It is characterized by endothelial dysfunction, thickening of the intima, vascular inflammation, and deposits of fatty substances, cholesterol, calcium, and cellular waste products within the intima of the vessel wall. The mechanisms of atherosclerosis remain uncertain.

The endothelium is a key modulator of hemostatic and homeostatic function within the blood vessel. There is increased apoptosis of endothelial cells (EC) in atherosclerotic lesions (22, 28). Accumulating evidence suggests that EC apoptosis may be the initiating event in the development of atherosclerosis (29). Oxidative stress and many of proatherogenic factors, including oxidized low-density lipoproteins, an- giotensin II, and tumor necrosis factor-α, are known to induce EC apoptosis (4, 16, 23, 27). The apoptotic ECs are prothrombotic and pro-proliferative and presumably contribute to the ensuing atherogenic processes, such as the proliferation and migration of smooth muscle cells and the infiltration of leukocyte and blood coagulation. On the contrary, rapid reendothelialization may be essential for preventing atherosclerosis. Therefore, understanding the mechanisms of EC apoptosis is important for treatments for coronary heart disease.

G0/G1 switch gene 2 (G0S2) was initially found to be differentially expressed in lymphocytes during the lectin-induced switch from the G0 to the G1 phase of the cell cycle (24). G0S2 gene encodes a protein of 103 amino acids with a 78% of sequence homology between mouse and human. Later, it was reported that G0S2 mRNA is highly expressed in brown and white adipose tissue and associated with growth arrest in 3T3-L1 fibroblasts (35). Activation of peroxisome proliferator-activated receptor (PPAR)-γ, PPAR-β/δ, and retinoic acid, as well as insulin stimulation, can upregulate G0S2 expression in 3T3-L1 cells and human acute promyelocytic leukemia cells (10, 30, 34, 35). Yang et al. (34) reported that G0S2 was localized in lipid droplets. It inhibited the triglyceride hydrolysis activity of adipose triglyceride lipase (ATGL) and played an important role in regulating lipolysis in adipocytes. Recently, our laboratory and other research groups (32, 38) have found that G0S2 contributes to liver steatosis. A previous study showed that G0S2 interacts with Bcl-2 and promotes apoptosis in tumor cells (33). Moreover, Kioka et al. (9) showed that G0S2 prevents cells from ATP depletion and induces a cellular tolerance for hypoxic stress. Improved mitochondrial fitness, as evidenced by increased ATP production, is associated with prolonged life span of cultured ECs (17). However, the expression and function of G0S2 in ECs are still unknown at the present time. In this study, we examined the role of G0S2 in apoptosis in ECs.

MATERIALS AND METHODS

Cell culture and reagents. Human umbilical vein ECs were isolated and cultured according to the procedure our laboratory described previously (37). Human umbilical vein ECs from passage 3–5 were used for all experiments. Fetal bovine serum, medium 199, OPTI-MEM, lipofectamine LTX reagent, TRIZOL reagent, Moloney murine leukemia virus reverse transcriptase (RT), and ribonuclease inhibitor were purchased from Invitrogen (Carlsbad, CA). Collagen I, acidic fibroblast growth factor, thiazolyl blue tetrazolium bromide (MTT), carbonyl cyanide p-trifluoromethoxyphenylhydrazone, and Hoechst 33258 were from Sigma (St. Louis, MO). Antibodies against CD31 and Myc-Tag (9B11) mouse MAb and Myc-Tag (71D10) rabbit MAb were purchased from Cell Signal-
ing Technology, (Beverly, MA). Antibody against cytochrome c was from Millipore (Billerica, MA). Polyclonal antibody against ATPSA1 and tetramethylrhodamine isothiocyanate (TRITC)-conjugated donkey anti-goat and -rabbit antibodies were from ProteinTech Group (Chicago, IL).

Animal experiments. All investigations were conducted conforming to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and approved by the Animal Care and Use Committee of Fudan University Shanghai Medical College. Male Sprague-Dawley rats (weighing 200–250 g) were anesthetized with pentobarbital (50 mg/kg, intraperitoneal injection). The carotid arteries of rats were removed for immunofluorescent assay. Our laboratory previously reported the adenovirus expressing a Myc-Tagged G0S2 protein (32). The adenoviruses were expanded and purified by cesium chloride method. Adenoviral infection of rat carotid arteries was performed as our laboratory previously reported (37). Briefly, the left common carotid artery of rat was surgically exposed, and an arteriotomy was made on the external carotid artery. Fifty microliters of viral solution containing adenovirus expressing β-galactosidase (Ad-LacZ) or G0S2 (Ad-G0S2) at a titer of 1 × 10^9 plaque-forming units were infused into the isolated carotid segment and remained for 15 min. Then the viral solution was withdrawn, the external carotid artery was ligated, and the blood flow to the common and internal carotid artery restored. The rats were anesthetized again 2 days later. Some animals were killed to histologically assess transgene expression in carotid arteries. The others were treated with H_2O_2 to induce endothelial injury in the carotid artery. The adenovirus-infected common carotid artery was exposed as described. After the common carotid lumen was flushed with saline, we replaced the saline with 20 μM H_2O_2 for 5 min. H_2O_2 solution was subsequently removed. The external carotid artery was ligated, and the wound was closed. Eight hours after injury, common carotid arteries were washed free of blood by perfusing via the left ventricle with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde and stained with Oil Red O solution for 30 min at room temperature. The cells were fractionated with 30% isopropanol. The accumulation of neutral lipid droplets, ECs were fixed with 4% paraformaldehyde and stained with Oil Red O solution for 30 min at room temperature. The cells were fractionated with 30% isopropanol. After staining, cells were washed with PBS to remove unbound dye. To quantify the Oil Red O content levels, the samples were treated with isopropanol and read spectrophotometrically at 510 nm. Triglyceride isopropanol solution (Sigma, MO). The formazan crystals were solubilized in dimethyl sulfoxide. The product was quantified spectrophotometrically by measuring absorbance at 490 nm.

Immunofluorescent assay. Snap-frozen tissue samples were cut into 5-μm-thick sections. The sections were subjected to immunostaining by using antibody specific for G0S2 or CD31. For immunofluorescence or immunohistochemistry, sections were treated with 3% bovine serum albumin for 60 min and then incubated with PBS for 10 min at room temperature. The cells were fractionated with 30% isopropanol. After staining, cells were washed with PBS to remove unbound dye. To quantify the Oil Red O content levels, the samples were treated with isopropanol and read spectrophotometrically at 510 nm. Triglyceride content in cells was measured using colloidal gold method. Kits from Abcam (Cambridge, MA), according to the manufacturer’s instruction.

Mitochondrial transmembrane potential. Mitochondrial membrane potential was measured using MitoLight (Millipore) was used to detect mitochondrial membrane potential in ECs. Following treatment, the cells were stained with MitoLight, according to the manufacturer’s instruction.

Statistical analysis. Data are expressed as means ± SE. Differences were analyzed by ANOVA or Student’s t-test. P values <0.05 were considered statistically significant.

RESULTS

G0S2 inhibits serum-free starvation stress- and H_2O_2-induced EC apoptosis. We first examined the expression of G0S2 within the vessel walls of Sprague-Dawley rats. ECs were identified by positive immunofluorescent staining for CD31.
Immunofluorescent evidences revealed that G0S2 was present in carotid arteries. G0S2 was found to colocalize with CD31-positive cells. The resulting data confirmed G0S2 was expressed in arterial ECs (Fig. 1A). Weak staining of G0S2 was also detected in the vascular smooth muscle cells. No cell-specific staining was observed with normal IgG. Immunohistochemical study also showed that G0S2 is expressed in the intact rat blood vessels (Fig. 1B).
The subcellular localization of G0S2 in cultured ECs was further investigated. In cultured ECs, the immunostaining for G0S2 exhibited a granular cytoplasmic distribution (Fig. 1C). The plasmid expressing GFP-G0S2 fusion protein was transfected into ECs by electroporation. As shown in Fig. 1D, the GFP-G0S2 fusion protein was also localized to a specific granular cytoplasmic compartment, enriched around the nuclear periphery. To examine whether G0S2 is associated with mitochondria, MitoLight, a lipophilic cationic dye that stains healthy living cell mitochondria, was used to identify mitochondria in cultured ECs. Figure 1D clearly showed that part of MitoLight stains the same cellular compartment as the GFP fusion protein. Confluent ECs were infected with adenovirus expressing β-galactosidase (Ad-LacZ) or G0S2 (Ad-G0S2) (bar = 10 μm). E: G0S2 protein levels in the cells were determined with Western blotting. F: the 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) cell viability assay was performed to study the protective effect of G0S2 on serum-deprivated ECs. G: ECs were treated with varying concentrations of H2O2 for 24 h. Apoptosis morphological changes in ECs were observed by fluorescence microscopy after staining with Hoechst 33258 (original magnification × 200) (bar = 20 μm). H: the percentages of apoptotic cells were shown. I: ECs were treated with 300 μM H2O2 for indicated time and then subjected to annexin V/propidium iodide (PI) staining. J: representative fluorescence-activated cell sorting (FACS) analyses showing the inhibition effect of G0S2 on apoptosis rate in ECs. K: the effect of serum deprivation on capillary-like structure formation was evaluated (bar = 1 mm). Values are means ± SE from 3 independent experiments. **P < 0.01; *P < 0.05.

The subcellular localization of G0S2 in cultured ECs was further investigated. In cultured ECs, the immunostaining for G0S2 exhibited a granular cytoplasmic distribution (Fig. 1C). The plasmid expressing GFP-G0S2 fusion protein was transfected into ECs by electroporation. As shown in Fig. 1D, the GFP-G0S2 fusion protein was also localized to a specific granular cytoplasmic compartment, enriched around the nuclear periphery. To examine whether G0S2 is associated with mitochondria, MitoLight, a lipophilic cationic dye that stains healthy living cell mitochondria, was used to identify mitochondria in cultured ECs. Figure 1D clearly showed that part of MitoLight stains the same cellular compartment as the GFP fusion protein. Confluent ECs were infected with adenovirus expressing β-galactosidase (Ad-LacZ) or G0S2 (Ad-G0S2) (bar = 10 μm). E: G0S2 protein levels in the cells were determined with Western blotting. F: the 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) cell viability assay was performed to study the protective effect of G0S2 on serum-deprivated ECs. G: ECs were treated with varying concentrations of H2O2 for 24 h. Apoptosis morphological changes in ECs were observed by fluorescence microscopy after staining with Hoechst 33258 (original magnification × 200) (bar = 20 μm). H: the percentages of apoptotic cells were shown. I: ECs were treated with 300 μM H2O2 for indicated time and then subjected to annexin V/propidium iodide (PI) staining. J: representative fluorescence-activated cell sorting (FACS) analyses showing the inhibition effect of G0S2 on apoptosis rate in ECs. K: the effect of serum deprivation on capillary-like structure formation was evaluated (bar = 1 mm). Values are means ± SE from 3 independent experiments. **P < 0.01; *P < 0.05.
fusion protein indicated, confirming that G0S2 was partly localized within the mitochondria in ECs.

Studies have identified G0S2 as a selective inhibitor of adipose lipolysis. To better understand the physiological role of G0S2, cultured ECs were infected with a recombinant adenovirus expressing G0S2, as we previously report. The expression of G0S2 in ECs was verified by Western blotting analysis (Fig. 1E). Compared with control, G0S2 overexpression resulted in neutral lipid accumulation in ECs in vitro. It caused an ~1.7-fold increase in triglyceride content in ECs (data not shown). Mitochondria plays an important role in energy metabolism and regulation of cell death. We next investigated whether G0S2 participates in apoptotic regulation in ECs. ECs were infected with the adenoviruses expressing LacZ or G0S2 before serum starvation and H2O2 treatment. The cell survival rates were reduced by serum deprivation, as assessed by MTT assay. G0S2 overexpression inhibited serum-deprivation induced EC apoptosis (Fig. 1F). Hoechst 33258 staining demonstrated chromatin condensation and nuclear fragmentation, morphological features of apoptosis, in H2O2− (100, 300 μM) treated cells. Compared with cells infected with the control adenovirus, ECs infected with Ad-G0S2 showed significant decreases in apoptosis after H2O2 exposure (Fig. 1, G and H). These cells were also stained with annexin V and PI without a process of fixation and further analyzed using flow cytometer. Figure 1, I and J, showed that Ad-G0S2-infected cells demonstrated significant decrease in H2O2-induced apoptotic cells compared with Ad-LacZ-infected cells (26.57 ± 5.73 vs. 41.01 ± 4.21% and 47.19 ± 3.73 vs. 70.58 ± 9.61% at 18 and 36 h, respectively). Furthermore, we assessed the effect of G0S2 on EC tube-like structure formation under serum-free culture condition. The resulting data showed that this process was significantly enhanced in G0S2 overexpressed ECs (Figs. 1K).

G0S2 prevents ECs from apoptosis via inhibition of caspase pathway. H2O2 could induce apoptosis through the mitochondrial dysfunction/caspase activation pathway. We next tested

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Fig. 3. G0S2 silencing induces EC apoptosis. Confluent ECs were transfected with G0S2 siRNA or scramble siRNA for 24 h. A: G0S2 gene knockdown was confirmed by RT-PCR method. B: cell viability was measured at 24 h by MTT. Apoptotic cells were determined by annexin V/PI staining. C and D: annexin V/PI positive populations were quantified by FACS. E: ECs were seeded in growth factor-reduced, matrigel-coated 24-well plate, and their ability to form a network of capillary-like structures was evaluated (bar = 1 mm). Results represent the means ± SE of three experiments. *P < 0.05.
whether G0S2 inhibits H$_2$O$_2$-induced mitochondrial dysfunction in ECs. We examined whether H$_2$O$_2$ increased caspase-3 and -9 activities by using DEVD-pNA as a substrate. H$_2$O$_2$ exposure increased caspase-3 and -9 activities in ECs by 3.7 ± 0.4- and 3.7 ± 1.5-fold, respectively. G0S2 overexpression significantly inhibited H$_2$O$_2$-induced increase in caspase-3 and -9 activities (Fig. 2, A and B). EC mitochondrial-membrane integrity was further investigated by evaluating the uptake of a lipophilic cationic dye. Red fluorescence represented dimeric dye that had accumulated in the intact mitochondrial membrane. In apoptotic cells with altered mitochondrial membrane potential, the dye in its monomeric form stays in the cytoplasm, fluorescing green. Compared with Ad-LacZ-infected cells, Ad-G0S2-infected ECs displayed substantially less H$_2$O$_2$-induced apoptotic mitochondria, as indicated by relatively few green-labeled cells (Fig. 2C). EC mitochondria and cytosolic fractions were also isolated. We found that G0S2 overexpression significantly prevented the release of cytochrome c (Fig. 2D). These results suggested that G0S2 protects ECs from serum-free starvation stress- and H$_2$O$_2$-induced mitochondrial damage.

We next examined whether G0S2 exerts its anti-apoptotic effect through Bcl-2 or ATGL. ECs were transfected with siRNA duplexes targeting Bcl-2 or ATGL. Bcl-2 and ATGL knockdown were confirmed by using Western blotting assay. Our results showed that ectopic expression of G0S2 protected cells against H$_2$O$_2$-induced apoptosis in Bcl-2 or ATGL-silenced ECs. The data indicated that G0S2 prevents ECs from apoptosis through a Bcl-2 or ATGL-independent mechanism (Fig. 2, E and F).

G0S2 silencing induces EC apoptosis. Moreover, we attempted to investigate whether endogenous G0S2 contributes to EC survival. G0S2 silencing was confirmed by RT-PCR (Fig. 3A). The MTT assay indicated about a 30% loss of viability in G0S2-silenced ECs (Fig. 3B). EC apoptosis was also analyzed using annexin V/PI staining and FACS analysis. Apoptosis was observed in a modest fraction of cells transfected with the control siRNA (8.0 ± 2.5% of the overall cell population). A significant increase in the percentage of apoptotic cells was observed in the G0S2-silenced ECs (20.1 ± 4.1%) (Fig. 3, C and D). Finally, the result data showed that EC tube-like structure formation was significantly delayed in G0S2-deficient ECs (Fig. 3E). These data indicated that G0S2 is necessary for the maintenance of EC survival.

G0S2 interacts with F0F1-ATP synthase in ECs. ATP and ROS play a role in apoptotic death. The effect of G0S2 overexpression on intracellular ATP and ROS contents was assessed. Figure 4A showed that the intracellular ATP level was increased by G0S2. The addition of carbonyl cyanide p-trifluoromethoxyphenylhydrazone, an oxidative phosphorylation uncoupler, reversed G0S2-induced ATP production. In contrast, G0S2 drastically reduced cellular ROS content (Fig. 4B). F0F1-ATP synthase, a complex V of the electron transport chain, is an important constituent of mitochondria-dependent signaling pathways involved in apoptosis. We investigated whether G0S2 can physically interact with
F0F1-ATP synthase in ECs. Immunoprecipitation was performed with anti-Myc-Tag or anti-ATP5A1 antibodies. The data showed that the Myc-tagged G0S2 communoprecipitated with ATP5A1, and ATP5A1 communoprecipitated with Myc-tagged G0S2 (Fig. 4, C and D). Furthermore, we examined intracellular localization of G0S2 and ATP5A1 by using indirect immunofluorescence. The resulting data showed that G0S2 partially colocalizes with ATP5A1 (Fig. 4E). On the contrary, G0S2 silencing reduced intracellular ATP level in ECs (Fig. 4F).

**G0S2 protects ECs against H2O2-induced apoptosis in vivo.** Using carotid artery injury model, we examined the effect of G0S2 overexpression on EC apoptosis in rats. Rat carotid arteries were adenovirally transduced to express LacZ or G0S2, then stimulated with H2O2 to induce apoptosis in ECs. The expression of G0S2 in the endothelium was confirmed by en face immunofluorescence staining with the anti-Myc-Tag antibody (Fig. 5A). Endothelium was identified by using anti-CD31 antibody. Denudation of ECs after H2O2 exposure was determined by en face staining (Fig. 5B). The number of ECs was decreased in Ad-LacZ groups. In contrast, G0S2 overexpression inhibited EC denudation (Fig. 5C).

**DISCUSSION**

In this study, we reported for the first time that G0S2 is expressed in ECs. G0S2 was partly localized in EC mitochondria. We also provided both in vitro and in vivo evidences that G0S2 can protect ECs against serum starvation- and H2O2-induced apoptosis. G0S2 played a role in regulating ATP and ROS contents in ECs.

Previous data revealed that many normal tissues, as well as cancer cells, express G0S2 (12, 33–35). In vitro and in vivo evidences in the present study showed that G0S2 is expressed in ECs at both mRNA and protein levels. This is the first report on the expression of G0S2 in the vascular wall. Reportedly, G0S2 exhibited variable subcellular localization in different cells. Zandbergen et al. (35) have demonstrated that G0S2 protein was present in the endoplasmic reticulum of HEK-293 and 3T3-L1 cells. Endogenous G0S2 was also found to be present in the mitochondria in H1299 non-small cell lung cancer cells (33). Several studies have shown that G0S2 localizes to lipid droplets in adipocytes and hepatocytes and prevents their degradation mediated by ATGL (8, 32, 34). Recently, Kioka et al. (9) reported that G0S2 acts in the mitochondria of cardiomyocytes to promote activity of F0F1-ATP synthase. We...
revealed here that G0S2 partly displays a staining pattern that is characteristic of the mitochondria. Our unpublished data also showed that G0S2 localizes to both mitochondria and the surface of lipid droplets in adipogenic differentiating mesenchymal stem cells. These data indicated that G0S2 protein has diverse physiological functions in different cells. Subcellular distribution of G0S2 protein is altered in various pathophysiological conditions.

Experimental animal models and clinical studies had demonstrated that ROS are increased in atherosclerosis (3, 18). Proatherosclerosis factors, angiotensin II, interleukin-1β, intercellular adhesion molecule-1, and oxidized low-density lipoproteins can stimulate ROS formation (5, 21, 31, 36). ROS play an important role in the development of atherosclerosis. Excessive production of ROS under pathophysiological conditions induces stress responses and causes EC apoptosis (3, 11).

Previous data had shown that ectopic expression of G0S2 induces apoptosis in H1299 lung cancer cells and HCT116 colorectal cells (33). However, studies also showed that overexpression of G0S2 in squamous lung cancer cell lines, LC-1 sq, does not induce apoptosis (12, 13). G0S2 interacted with F0F1-ATP synthase and prevented cells from hypoxic stress-induced apoptosis in cardiomyocytes (9). Our in vivo and in vitro data showed that G0S2 protects ECs against serum starvation- and H2O2-induced apoptosis. This is the first report to address the role of G0S2 in EC apoptosis. Probably, G0S2 acts as a pro- or anti-apoptotic factor in different tissues and cells. Therefore, these data indicate its potential protection role in preventing vascular diseases. G0S2 played an important role in regulating lipolysis in adipocytes and hepatocytes. It can couple with ATGL and prevent ATGL-mediated triglyceride degradation. We found here that overexpression of G0S2 induces triglyceride accumulation in cultured ECs. Therefore, G0S2 is also likely to play a role in the maintenance of the endothelial triglyceride pool. We also found that depletion of triglyceride by incubation with the Triacsin C induces apoptosis in ECs (data not shown). These data indicate that G0S2 may also maintain intracellular triglyceride level, which is necessary for EC survival.

It is becoming increasingly clear that mitochondria plays an important role in the regulation of cell death. They contain many pro- and anti-apoptotic proteins. Free-radical damage or growth factor deprivation can activate proapoptotic members of the Bcl-2 gene. Apoptosis inducing factor, Smac/DIABLO, and cytochrome c are subsequently released from the mitochondria through permeability transition pore. This in turn leads to the formation of the apoptosome and the activation of the caspase cascade. They can directly or indirectly inhibit permeability transition pore opening and the release of cytochrome c. Maintenance of proper mitochondrial transmembrane potential is essential for the survival of the cell, because it drives the synthesis of ATP and maintains oxidative phosphorylation. Reportedly, inhibition of F0F1-ATP synthase activity causes depletion of mitochondrial ATP levels and significant stimulation of mitochondrial ROS production, followed by depolarization of mitochondrial membrane potential (7). G0S2 was found to interact with F0F1-ATP synthase in cardiomyocytes (9). We demonstrated here that G0S2 overexpression prevented ECs from ATP depletion and ROS accumulation induced by serum-free starvation stress. The promotion of F0F1-ATP synthase by G0S2 likely represents an essential event responsible for the ATP production, ROS depletion, and can act as a central regulator of the apoptotic machineries.

Many signaling molecules, which include Bcl-2, Bcl-xL, TAMP12, UCP-2, and Mcl-1, can inhibit mitochondrial apoptosis (15, 19, 20). It was reported that G0S2 can specifically interact with Bcl-2 and prevent the formation of Bcl-2/Bax heterodimers in cancer cells (33). We still observed that G0S2 can protect Bcl-2 silenced ECs from apoptosis. These data indicated that this protection does not seem to involve a Bcl-2-mediated resistance. ATGL was also associated with G0S2 protein in cells. Previously, ATGL was identified as a stress resistance mediator in adipocytes (14). Apoptosis was increased in ATGL-deficient macrophages (1). On the other hands, ATGL-deficient mice with tumors resisted the increased myocyte apoptosis (6). We demonstrated that G0S2 protects ATGL-silenced ECs from apoptosis. It is suggested that G0S2 prevents ECs from apoptosis, independent of ATGL. Reportedly, serum withdrawal and H2O2 may modulate either mitochondrial or nonmitochondrial apoptotic pathways (25, 26). So, the role of G0S2 on the nonmitochondria-mediated apoptosis signal pathway should be further evaluated.

In summary, we have demonstrated, for the first time, that up-regulation of G0S2 can protect ECs from mitochondrial oxidative damage. G0S2 can regulate the activity of F0F1-ATP synthase and induce EC tolerance for apoptosis. Our findings indicated that G0S2 may have therapeutic potential in the treatment of atherosclerosis.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.W. and Y. Zhang performed experiments; Y. Zhu interpreted results of experiments; P.Z. conception and design of research; P.Z. analyzed data; P.Z. drafted manuscript; P.Z. edited and revised manuscript; P.Z. approved final version of manuscript.

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