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Original Article

Advanced glycation end products upregulates the expression of connexin43 via ERK MAPK and PI3K/Akt pathways in human endothelial cells

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Abstract: Intercellular communication through gap junctions plays an essential role in maintaining the functional integrity of vascular endothelium. Despite some emerging evidences suggesting that advanced glycation end products (AGEs) may impair endothelial function, however, its effect on connexin43 (Cx43) gap junction in endothelial cells remains unexplored. Here we investigated the effect of AGE-bovine serum albumin (AGE-BSA) on Cx43 gap junction in endothelial cells. The levels of Cx43 protein in human umbilical vein endothelial cells (HUVECs) was significantly increased by AGE-BSA treatment, accompanied by receptor for advanced glycation endproducts (RAGE) overexpression. This augmentation can be reversed by RAGE knockdown. In addition, AGE-BSA treatment activated the expression of ERK, P38, and JNK mitogen-activated protein kinases (MAPKs), which are supposed to participate in the regulation of Cx43. A MEK inhibitor PD98059, but not SB203580 (a p38 kinase inhibitor) or SP600125 (a JNK kinase inhibitor), abolished the effects of AGE-BSA on Cx43 expression. Moreover, both Akt and PKC phosphorylation were time-dependently augmented by AGE-BSA and PI3K inhibitor LY294002 (but not GF109203X, a PKC inhibitor) blocked the effects of AGE-BSA on Cx43 gap junctions. Taken together, AGE-BSA upregulated Cx43 expression via AGE-RAGE signaling, ERK MAPK and PI3K/Akt Signal transduction pathways appear to be involved in these processes.

Keywords: Advanced glycation end products, RAGE, connexin43, gap junction, signal transduction pathways

Introduction

Gap junctions are intercellular-channels that allow the exchange of small molecules such as small metabolites, ions, and second messengers between adjacent cells [1, 2]. These channels consist of two hemi-channels, called connexons, which are located in the plasma membrane of two adjacent cells. Each connexon is composed of six subunits of the protein connexin. Gap junctions play important roles in a variety of cellular processes including homeostasis, morphogenesis, cell differentiation, and growth control [3]. It is widely believed that cellular gap junctions are associated with the development of vascular diseases, such as hypertension, atherosclerosis, or restenosis [2].

Three connexin subtypes are expressed in the endothelium of the blood vessels: Cx37, Cx40, and Cx43, which are predominant in cultured vascular endothelial cells [4, 5]. Although the development of atherosclerosis depends on paracrine cell-to-cell interactions critically, direct intercellular communication via gap junctions may be another factor controlling atherogenesis [6]. An increasing number of studies have demonstrated that Cx43 regulating in endothelial cells is subjected to a large number of risk factors for atherosclerosis, including ageing [7], nicotine [4, 8], homocysteine [9], diabetes [10, 11], and hypertension [12]. Kwak et al [6] reported that reduced Cx43 expression inhibits atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice, indicating a critical role of Cx43-mediated gap junctional communication in atherosclerotic plaque formation. More recently, it has been reported that down-expression of Cx43 limits neointima formation after balloon distention injury in hypercholesterolemic mice by decreas-

ing the inflammatory response and reducing SMC migration and proliferation [13]. These findings identify Cx43-mediated intercellular communication as a new potential therapeutic target in atherosclerosis.

Increased formation of advanced glycation end products (AGEs) is generally regarded as one of the main mechanisms responsible for vascular damage in patients with diabetes [14]. The expression of AGEs was upregulated in aged and diabetic patients, which is considered as a process associated with endothelial dysfunction. But the effects of AGEs on vascular endothelial gap junction have not been fully clarified until now. A recent study showed that AGEs down-regulate gap junctions in human hepatoma SKHep1 cells via the activation of Src-dependent ERK1/2 and JNK/SAPK/AP1 signaling pathways [15]. In the present study, we aimed to investigate the effect of AGE-bovine serum albumin (AGE-BSA) on Cx43 gap junction in endothelial cells and the implication of MAPKs signaling as well as PI3K/Akt signal transduction pathway.

Materials and methods

Cell culture and chemicals

As our previously reported, human umbilical vein endothelial cells (HUVECs) were obtained from ScienCell (Carlsbad, CA, USA) in RMPI 1640 supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). The process was performed in a humidified incubator maintained at 37°C with 5% CO_2 and 95% oxygen. 3-8 passages were used in all experiments [16].

Bovine serum albumin (BSA), D-glucose, and Lucifer Yellow CH were obtained from Sigma Chemical (St. Louis, MO, USA). LY294002, GF109203X, PD98059, SB203580 and SP-600125 were purchased from Calbiochem (La Jolla, CA, USA).

Preparation of AGEs

The AGEs used in this study were prepared by incubating 50 mg/ml of Bovine serum albumin (BSA) with 0.5 M D-glucose in 0.2 mol/L phosphate buffered saline (PBS, pH 7.4) for 90 days according to previous studies [17, 18]. All incubations were required for sterile, anaerobic conditions and performed in the dark at 37°C. BSA incubated without glucose under the same conditions was used as the negative control. Before performing the experiments, we purified and concentrated the AGE-BSA solution with extensive dialysis. The identification of AGEs was performed by fluorescence spectrophotometry (Data not shown).

Determination of gap-junctional intercellular communic ation (GJIC) by scrape loading/dye transfer technique

The scrape loading/dye transfer (SL/DT) technique was performed according to our previous protocol. Cells (2×10^5) were incubated in 35 mm Petri dishes for 2 days before the experiment. Then we washed the confluent cell layer with PBS twice followed by covering with a preheated (37°C) solution of 0.05% Lucifer Yellow CH dissolved in PBS without Ca^{2+} and Mg^{2+} before scrape loading. 5~6 times cut with a surgical scaple was required to performed in each dish of cells. After 3.5 min scraping, the dye was discarded and the dishes were rinsed with PBS for three times before fixing the cells with 4% paraformaldehyde. Finally, we measure the distance traveled by the dye in a direction perpendicular to the scrape with inverted fluorescent microscope (Olympus, Tokyo, Japan).

Quantitative RT-PCR

For the RNA studies, cells were lysed with the TRIzol method (Invitrogen, Carlsbad, CA, USA) to extracted the total RNA before converting into cDNA using murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). Specific primers matching the published sequences were used to identify and amplify Cx43 (216 bp, sense primer: 5'-AAT TCA GAC AAG GCC CAC AG-3'; anti-sense primer: 5'-CAT GGC TTG ATT CCC TGA CT-3'), and RAGE (128 bp, sense primer: 5'-ACG GCT GGT GTT CCC AAT AA-3'; anti-sense primer: 5'-TGT TCC TTC ACA GAT ACT CCC TTC-3'). Glyceraldehyde-3-dehydrogenase (GAPDH, 136 bp, sense primer: 5'-GGG TGT GAA CCA TGA GAA GT-3'; anti-sense primer: 5'-GAC TGT GGT CAT GAG TCC T-3') was amplified as a reference. Relative quantitative measurement of mRNA was performed on the ABI 7500 cycler (Applied Biosystems, CA, USA) with SYBR green PCR mix (Takara, Shiga, Japan). Finally, fold change of relative mRNA

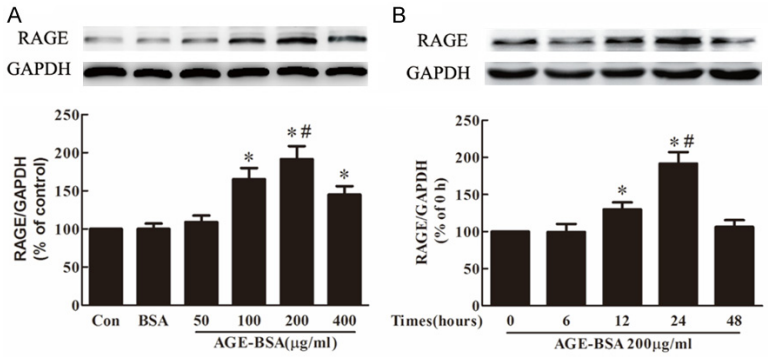

Figure 1. Effect of AGE-BSA on RAGE expression in HUVECs as assessed by western blotting. A. Cells were incubated with 0, 50, 100, 200, 400 μg/ml AGE-BSA for 24 h. **P* < 0.05 compared to control, #*P* < 0.05 compared to 50, 100, 400 μg/ml AGE-BSA. B. Time-dependent effects of AGE-BSA (200 μg/ml) on RAGE protein expressions. *P < 0.05 compared to control, #*P* < 0.05 compared to 6, 12, 48 h. Control, cells without any treatment; BSA, cells were treated with 200 μg/ml BSA for 24 h.

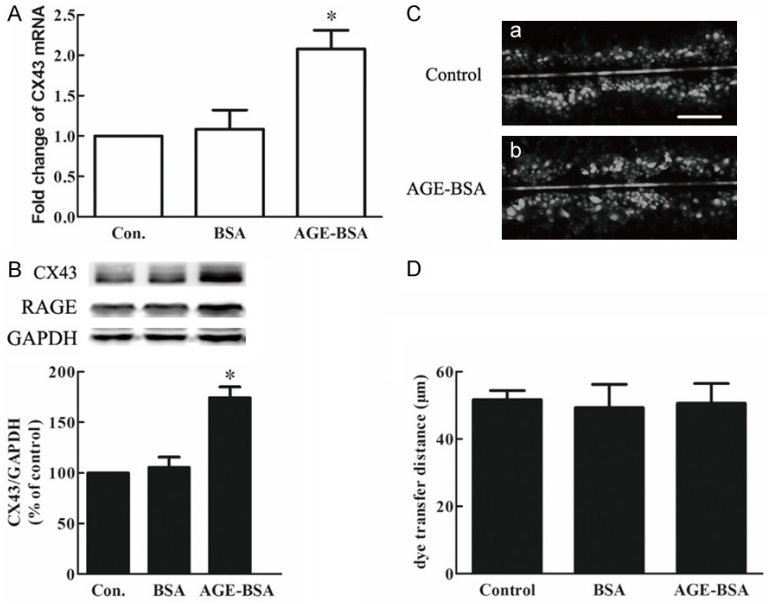

Figure 2. Effects of AGE-BSA on Cx43 gene and protein expressions. A. Effects of AGE-BSA (200 μg/ml, 24 h) on Cx43 gene expressions. B. Effects of AGE-BSA (200 μg/ml, 24 h) on Cx43 protein expressions. *P < 0.05 compared to control. C. Effect of EGCG on intercellular communication between HUVECs as assessed by SL/DT technique. Cells were loaded for 3.5 minutes with a 0.05% (w/v) Lucifer Yellow CH solution. Confluent cells were treated control (a), 200 μg/ml AGE-BSA 24 h (b). D. Quantification of dye transfer distance. Bar, 50 μm.

expression of Cx43 was statistically analyzed in the $2^{-\Delta\Delta Ct}$ method as described before [19].

Small interference RNA transfection

The transfection process was described in our previous studies [16]. The siRNA duplexes were supported by GenePharma (Shanghai, China). RAGE sense siRNA sequence is 5'-GAATCCTCCCCAATGGTTCA-3' and anti-sense siRNA sequence is 5'-GCCCGACACCGGAAAGT-3'. Negative control is a non-silencing siRNA (sense: 5'-UUC UCC GAA CGU GUC ACG UdTT-3'; anti-sense: 5'-ACG UGA CAC GUU CGG AGA AdTT-3'). Then we mixed the siRNAs with Hiperfect transfection reagent (Qiagen, Hilden, Germany) and transfected them into the cells incubating in the six-well. Briefly, 100 pmol siRNA against Cx43 or control siRNA was diluted in 500 μl non-serum culture medium, and mixed with 12 μl Hiperfect transfection reagent by vortexing for 10 s. one milliliter culture medium with serum was added into the cells after 3 h of incubating with the transfection complexes under normal conditions. Verification of siRNA efficacy was confirmed by real-time polymerase chain reaction and Western blot test.

Western blot and antibody

Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA) supplemented with 1 mM PMSF, 2 μg/ml aprotinin, 2 μg/ml pepstatin, 2 μg/ml leupeptin, 1 mM Na_3VO_4, and 10 mM NaF was prepared for the protein extraction from the cells. And then we determined the concentration by using Bio-Rad DC protein assay reagents (Hercules, CA). Proteins (20~40 μg) were separated by 10% SDS-polyacrylamide gels and electrotransferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA). After blocking the membranes in 5% milk-TBST, we incubated them with primary antibody at the appropriate dilution at 4°C overnight, followed by with incubation for 1 h with a secondary

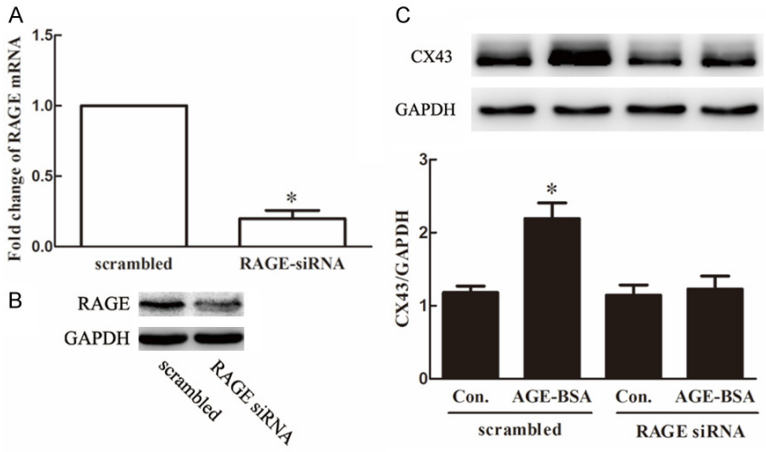

Figure 3. RAGE-specific siRNA abrogated the effect of AGE-BSA on Cx43 expression. Uptake of RAGE siRNA reduces RAGE expression, as confirmed using quantitative RT-PCR (A), and western blotting (B). (C) Representative example and quantification of Cx43 protein expression in HUVECs exposed to AGE-BSA (0, 200 μg/ml) for 24 h after transfection with scrambled and RAGE-specific siRNA. Data are expressed as a ratio of Cx43 to GAPDH. *P < 0.05 compared with control.

antibody conjugated to horseradish peroxidase (1:10000). After reaction with enhanced chemiluminescence reagent (Amersham, Haemek, Isreal), the images were captured on the image reader LAS-4000 system (Fujifilm, Tokyo, Japan).

We purchased the Anti-Akt, anti-phospho-Akt, Anti-PKC, anti-phospho-PKC, anti-ERK 1/2, anti-phospho-ERK 1/2, anti-JNK 1/2, anti-phospho-JNK 1/2, anti-p38 MAPK and anti-phospho-p38 MAPK from Cell Signaling Technology (Beverly, MA, USA). Anti-Cx43 was from Sigma. Anti-GAPDH polyclonal antibody was obtained from Santa Cruz (CA, USA). Rabbit polyclonal antibody against RAGE was from Millipore (Minneapolis, USA).

Statistical analysis

All experiments were performed at least three times. Data were expressed as means ± SEM and analyzed by unpaired Student's t-test for comparisons between two groups or Newman-Keuls test was performed for further comparisons between each two individuals. P values < 0.05 were considered statistically significant.

Results

AGE-BSA upregulates the expression of RAGE

We observed the effect of AGE-BSA on the expression of RAGE. In accordance with previous studies [17, 20], Western blot analysis revealed that AGE-BSA treatment to HUVECs resulted in significant augmentation of RAGE in time and dose-dependent manner, reached a maximum at 24 h and 200 μg/ml respectively (**Figure 1A**, **1B**).

AGE-BSA increases the expression of Cx43

As shown in **Figure 2A**, **2B**, mRNA and protein expression of Cx43 in HUVECs, which were determined by Real-time RT-PCR and western blotting respectively, were significantly up-regulated by AGE-BSA (200 μg/ml, 24 h) as compared to control. To investigate the effect of AGE-BSA on GJIC in endothelial cells, the GJIC activity of HUVECs was performed with SL/DT assay. No significant change of GJIC was observed after AGE-BSA treatment as compared to control (**Figure 2C**).

RAGE mediates the augmentation of Cx43 induced by AGE-BSA

To evaluate the effect of RAGE knock-down mediated by siRNA on AGE-BSA induced up-regulation of Cx43 in HUVECs, siRNAs were transfected into the cells. As shown in **Figure 3A**, **3B**, we observed that RAGE expression was decreased approximately 85% compared with the controls without silencing, which were confirmed by real-time PCR and western blot tests as well. The augmentation of Cx43 by AGE-BSA treatment was completely abrogated by RAGE silencing (**Figure 3C**).

AGE-BSA mediated increase of Cx43 depends on the activation of ERK/MAPK

We examined the activation of several MAPKs (ERK1/2, p38 and JNK) to identify the effect of AGE-BSA on the Cx43 in HUVECs. As shown in **Figure 4A**, AGE-BSA could activate both ERK1 and ERK2. The phospho-ERK1/2 level reached a maximum 60 min after 200 μg/ml AGE-BSA treatment. AGE-BSA treatment also induced P38 (**Figure 4B**) and JNK (**Figure 4C**) activation with peaks at 30 min and 60 min respectively, which were similar to ERK1/2 phosphorylation.

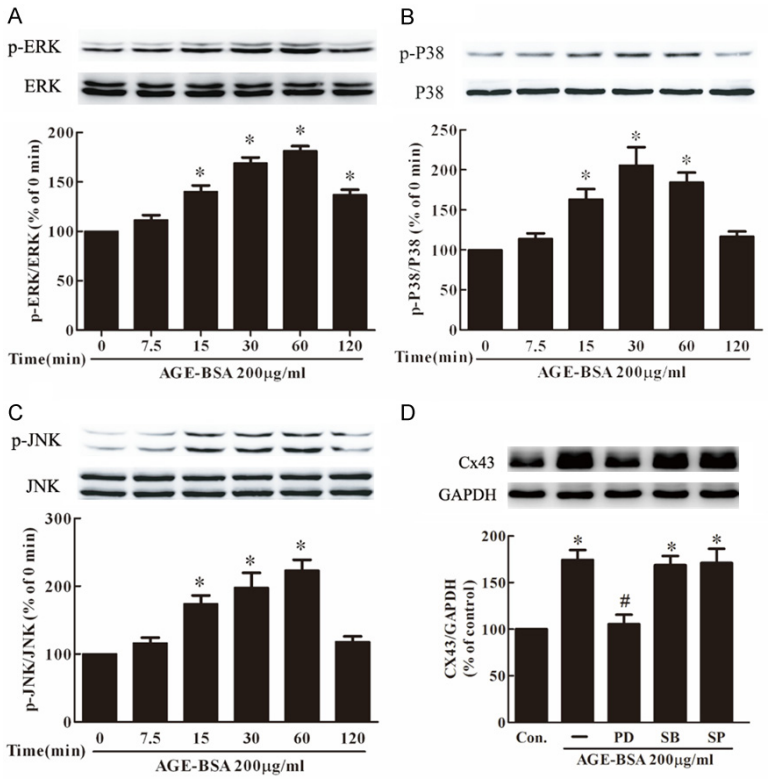

Figure 4. ERK MAPK plays a role in the effects of AGE-BSA on Cx43 expression within HUVECs. A-C. Effects of EGCG on phosphorylated-ERK (p-ERK), Phosphorylated-p38 (p-p38), and phosphorylated-JNK (p-JNK) MAPK pathways in HUVECs. *P < 0.05 compared to 0 min, Data are represented as mean ± SEM. D. Representative example and quantification of the Western blot analysis of Cx43 protein expression in HUVECs exposed to AGE-BSA treatment with or without pre-treatment with inhibitors (20 μM PD98059, 10 μM SB203580, 20 μM SP600125) for 30 min. Each bar represents mean ± SEM. of three independent experiments. Each bar represents mean ± SEM. (n=3). *$P < 0.05$ compared to control, #$P < 0.05$ compared to AGE-BSA (200 μg/ml, 24 h) treatment.

To further investigate whether MAPKs was involved in the process of AGE-BSA mediated increase of Cx43, 20 μM PD98059, a selective inhibitor of MEK1/2, or 10 μM SB203580, a specific inhibitor of p38 MAPK, or 10 μM SP600125, a specific inhibitor of JNK were pretreated to the confluent cells. As shown in **Figure 4D**, the pharmacological inhibitor of MEK1/2 (PD98059) abolished the effect of AGE-BSA on Cx43 in HUVECs completely. However, other two MAPK inhibitors (SB203580, SP600125) failed to attenuate the effect of AGE-BSA on Cx43.

PI3K/Akt pathway may also participate in the up-regulation of Cx43 by AGE-BSA

We also investigated the effects of AGE-BSA on Akt and PKC phosphorylation. As illustrated in **Figure 5A**, **5B**. 200 μg/ml AGE-BSA treatment of HUVECs stimulated Akt phosphorylation in a time-dependent manner. The phospho-Akt-Ser473 level reached a maximum 30 min. However, the total Akt expression was not observed any significant changes over the course of the experiments. The result of the AGE-BSA induced PKC phosphorylation was similar with the Akt phosphorylation while peaked at 15 min. Pretreatment of HUVECs with PI3K inhibitor LY294002 (10 μM), other than GF109203X (a PKC inhibitor, 5 μM) for 30 min, drastically abolished the augmentation of Cx43 by AGE-BSA treatment (**Figure 5C**).

Discussion

Cx43 gene upregulation has been previously found in several proatherosclerotic pathophysiological conditions [9, 21-24]. It has been reported that high glucose induces downregulation of Cx43 in endothelial cells [10, 11]. However, coronary endothelial cells isolated from streptozotocin-induced diabetic mice exhibit low protein expression of Cx37 and Cx40, but not Cx43 [25]. In this report, we demonstrated that AGE-BSA induced Cx43 over-expression, which was not associated with an increase in gap junctional intercellular communication. The fact that intercellular communication was not affected despite the changes in expression of Cx43 is not unique. Several precedents exist when overexpression of Cx43 did not lead to the increased intercellular communication [9, 26]. Our findings may indicate that Cx43 is not exclusive for intercellular communication in HUVEC in vitro. Alternatively, conformational changes of Cx43, such as changes in phosphorylation and/or ability to form hemichannels, may result in changes of cell-to-cell communication. The functional consequences of such an increase of Cx43 induced by AGE-BSA

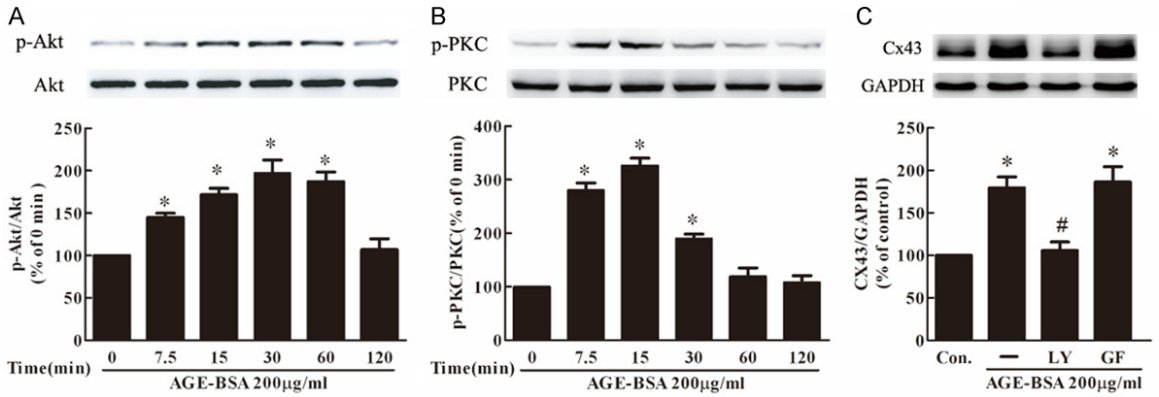

Figure 5. PI3K/Akt signal transduction pathway is also involved in the effects of AGE-BSA on Cx43 within HUVECs. A, B. Effects of AGE-BSA on phosphorylated-AKT (p-AKT) and phosphorylated-PKC (p-PKC) in HUVECs. Cells were treated with 200 μg/ml AGE-BSA for 0, 7.5, 15, 30, 60, 120 min. **P* < 0.05 compared to 0 min, Data are represented as mean ± SEM. C. After cells were treated with inhibitors (10 μM LY294002 or 5 μM GF109203X) alone or in the present of AGE-BSA (200 μg/ml, 24 h), western blot analysis were performed to examine Cx43 protein expression. Representative example and quantification of the Western blot analysis of Cx43 protein expression. Each bar represents mean ± SEM. of three independent experiments. Each bar represents mean ± SEM. (n=3). **P* < 0.05 compared to control, #*P* < 0.05 compared to AGE-BSA (200 μg/ml, 24 h) treatment.

are unknown, have not been pursued in the present work, and require further studies.

The mechanisms of AGEs linked to cellular stress and tissue dysfunction include receptor-independent and receptor-dependent processes. The receptor-dependent action is mediated by binding to specific cell surface molecules such as RAGE and others [27]. AGEs bind to RAGE, thereby leading to activation of a range of inflammatory and fibrotic pathways causing tissue injury. There is increasing evidence that RAGE overexpression was associated with increased vascular injury, nephropathy, and retinopathy [28]. Whether RAGE overexpression participates in the regulation of Cx43 gap junction is unknown. Our data presented here demonstrate that AGE-BSA treatment induced RAGE overexpression in HUVECs. The augmentation of Cx43 expression induced by AGE-BSA was completely abolished by RAGE knockdown, indicating that RAGE overexpression plays a critical role in the regulation of Cx43 gap junction within endothelial cells.

That MAPKs play essential roles in regulating Cx43 expression is becoming consensus. Cho et al [29] reported that mushroom Phellinus linteus extract prevent inhibition of GJIC by hydrogen peroxide via the ERK1/2 and p38 MAP kinases. The author believed that the loss of Cx43 in WB-F344 cells with anisomycin treatment was possibly owing to accelerated degradation. Moreover, the reduction in numbers of gap junctions and intercellular communication were contributed to the cellular losses as well [30]. In addition, JNK, another stress-activated kinase, has been implicated in the regulation of Cx43 in cardiomyocytes, which indicates occuring through the down-regulation of Cx43 protein. This decrease in GJIC may be a contributing factor for cardiac dysfunction in the failing heart [31]. In human hepatoma SKHep 1 cells, AGE-BSA significantly down-regulates the Cx32 and Cx43 expression in a dose- and time-dependent manner through the activation of Src-dependent ERK and JNK/SPAK/AP1 signaling pathways play a key role in this process [15]. In the present study, we showed that AGE-BSA activates the time-dependent phosphorylation of MAPK family proteins (ERK, p38, JNK) in HUVECs. Inhibition of ERK by co-treatment of PD98059 is able to completely abolish the augmentation of Cx43 by AGE-BSA treatment in HUVECs. However, SB203580 (an inhibitor of p38 MAPK) and sp600125 (an inhibitor of JNK MAPK) failed to block the effects of AGE-BSA on Cx43 gap junction. Taken together, the data presented here suggest that ERK MAPK is, at least partially, responsible for the effects of AGE-BSA on Cx43 expression within HUVECs.

Besides MAPKs signaling, other signal transduction pathways including PI3K/Akt and PKC

signaling may also participate in the regulation of Cx43 gap junction [32-35]. Tacheau et al [35] demonstrated that TGF-beta1 induces the expression of Cx43 in normal murine mammary gland epithelial cells via activation of P38 and PI3K/Akt signaling pathways. A recent study showed that both PI3K/Akt and PKC signaling pathways participate in the regulation of Cx43 induced by 5'-N-ethylcarboxamide in mouse embryonic stem cells [34]. It has been reported that treatment with AGE-BSA induces the endothelial-to-mesenchymal transition in cultured human and monkey endothelial cells via Akt signaling cascades [36]. In RAW 264.7 macrophages, AGE-BSA might activate PKC and subsequently induce iNOS expression and NO release [37]. In accordance with previous studies [36, 38], the results of our study discovered that treatment of AGE-BSA time-dependently activates both PI3K/Akt and PKC signaling. Inhibition of PI3K/Akt by pretreatment with LY294002, drastically abrogates the effects of AGE-BSA on Cx43, suggesting that the activation of Akt by AGE-BSA treatment may participate in the regulation of Cx43 gap junction as well.

In conclusion, the present study demonstrated for the first time that AGEs in vitro upregulate Cx43 expression of endothelial cells via AGE-RAGE signaling, ERK MAPK and PI3K/Akt signal transduction pathways also participated in this process.

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Disclosure of conflict of interest

None.

Abbreviations

AGEs, advanced glycation end products; HUVECs, human umbilical vein endothelial cells; Cx43, connexin43; siRNA, small interfering RNA; RAGE, receptor for advanced glycation endproducts.

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References

[1] Saez JC, Berthoud VM, Branes MC, Martinez AD and Beyer EC. Plasma membrane channels formed by connexins: their regulation and functions. Physiol Rev 2003; 83: 1359-1400.

[2] Brisset AC, Isakson BE and Kwak BR. Connexins in vascular physiology and pathology. Antioxid Redox Signal 2009; 11: 267-282.

[3] Simon AM and Goodenough DA. Diverse functions of vertebrate gap junctions. Trends Cell Biol 1998; 8: 477-483.

[4] Tsai CH, Yeh HI, Tian TY, Lee YN, Lu CS and Ko YS. Down-regulating effect of nicotine on connexin43 gap junctions in human umbilical vein endothelial cells is attenuated by statins. Eur J Cell Biol 2004; 82: 589-595.

[5] Wang HH, Kung CI, Tseng YY, Lin YC, Chen CH, Tsai CH and Yeh HI. Activation of endothelial cells to pathological status by down-regulation of connexin43. Cardiovasc Res 2008; 79: 509-518.

[6] Kwak BR, Veillard N, Pelli G, Mulhaupt F, James RW, Chanson M and Mach F. Reduced connexin43 expression inhibits atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice. Circulation 2003; 107: 1033-1039.

[7] Yeh HI, Chang HM, Lu WW, Lee YN, Ko YS, Severs NJ and Tsai CH. Age-related alteration of gap junction distribution and connexin expression in rat aortic endothelium. J Histochem Cytochem 2000; 48: 1377-1389.

[8] Haussig S, Schubert A, Mohr FW and Dhein S. Sub-chronic nicotine exposure induces intercellular communication failure and differential down-regulation of connexins in cultured human endothelial cells. Atherosclerosis 2008; 196: 210-218.

[9] Li H, Brodsky S, Kumari S, Valiunas V, Brink P, Kaide J, Nasjletti A and Goligorsky MS. Paradoxical overexpression and translocation of connexin43 in homocysteine-treated endothelial cells. Am J Physiol Heart Circ Physiol 2002; 282: H2124-2133.

[10] Sato T, Haimovici R, Kao R, Li AF and Roy S. Downregulation of connexin 43 expression by high glucose reduces gap junction activity in microvascular endothelial cells. Diabetes 2002; 51: 1565-1571.

[11] Li AF and Roy S. High glucose-induced downregulation of connexin 43 expression promotes apoptosis in microvascular endothelial cells. Invest Ophthalmol Vis Sci 2009; 50: 1400-1407.
[12] Yeh HI, Lee PY, Su CH, Tian TY, Ko YS and Tsai CH. Reduced expression of endothelial connexins 43 and 37 in hypertensive rats is rectified after 7-day carvedilol treatment. Am J Hypertens 2006; 19: 129-135.
[13] Chadjichristos CE, Matter CM, Roth I, Sutter E, Pelli G, Luscher TF, Chanson M and Kwak BR. Reduced connexin43 expression limits neointima formation after balloon distension injury in hypercholesterolemic mice. Circulation 2006; 113: 2835-2843.
[14] Brownlee M. Negative consequences of glycation. Metabolism 2000; 49: 9-13.
[15] Lin FL, Chang CI, Chuang KP, Wang CY and Liu HJ. Advanced glycation end products downregulate gap junctions in human hepatoma SKHep 1 cells via the activation of Src-dependent ERK1/2 and JNK/SAPK/AP1 signaling pathways. J Agric Food Chem 2010; 58: 8636-8642.
[16] Zhao Y, Yu L, Xu S, Qiu F, Fan Y and Fu G. Downregulation of connexin43 gap junction by serum deprivation in human endothelial cells was improved by (-)-Epigallocatechin gallate via ERK MAP kinase pathway. Biochem Biophys Res Commun 2011; 404: 217-222.
[17] Zhang FL, Gao HQ and Shen L. Inhibitory effect of GSPE on RAGE expression induced by advanced glycation end products in endothelial cells. J Cardiovasc Pharmacol 2007; 50: 434-440.
[18] Xu B, Chibber R, Ruggiero D, Kohner E, Ritter J and Ferro A. Impairment of vascular endothelial nitric oxide synthase activity by advanced glycation end products. FASEB J 2003; 17: 1289-1291.
[19] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
[20] Sun C, Liang C, Ren Y, Zhen Y, He Z, Wang H, Tan H, Pan X and Wu Z. Advanced glycation end products depress function of endothelial progenitor cells via p38 and ERK 1/2 mitogen-activated protein kinase pathways. Basic Res Cardiol 2009; 104: 42-49.
[21] Ashton AW, Yokota R, John G, Zhao S, Suadicani SO, Spray DC and Ware JA. Inhibition of endothelial cell migration, intercellular communication, and vascular tube formation by thromboxane A(2). J Biol Chem 1999; 274: 35562-35570.
[22] DePaola N, Davies PF, Pritchard WF Jr, Florez L, Harbeck N and Polacek DC. Spatial and temporal regulation of gap junction connexin43 in vascular endothelial cells exposed to controlled disturbed flows in vitro. Proc Natl Acad Sci U S A 1999; 96: 3154-3159.
[23] Polacek D, Bech F, McKinsey JF and Davies PF. Connexin43 gene expression in the rabbit arterial wall: effects of hypercholesterolemia, balloon injury and their combination. J Vasc Res 1997; 34: 19-30.
[24] Yeh HI, Lai YJ, Chang HM, Ko YS, Severs NJ and Tsai CH. Multiple connexin expression in regenerating arterial endothelial gap junctions. Arterioscler Thromb Vasc Biol 2000; 20: 1753-1762.
[25] Makino A, Platoshyn O, Suarez J, Yuan JX and Dillmann WH. Downregulation of connexin40 is associated with coronary endothelial cell dysfunction in streptozotocin-induced diabetic mice. Am J Physiol Cell Physiol 2008; 295: C221-230.
[26] Huang RP, Hossain MZ, Huang R, Gano J, Fan Y and Boynton AL. Connexin 43 (cx43) enhances chemotherapy-induced apoptosis in human glioblastoma cells. Int J Cancer 2001; 92: 130-138.
[27] Wendt T, Tanji N, Guo J, Hudson BI, Bierhaus A, Ramasamy R, Arnold B, Nawroth PP, Yan SF, D'Agati V and Schmidt AM. Glucose, glycation, and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. J Am Soc Nephrol 2003; 14: 1383-1395.
[28] Jandeleit-Dahm K, Watson A and Soro-Paavonen A. The AGE/RAGE axis in diabetes-accelerated atherosclerosis. Clin Exp Pharmacol Physiol 2008; 35: 329-334.
[29] Cho JH, Cho SD, Hu H, Kim SH, Lee SK, Lee YS and Kang KS. The roles of ERK1/2 and p38 MAP kinases in the preventive mechanisms of mushroom phellinus linteus against the inhibition of gap junctional intercellular communication by hydrogen peroxide. Carcinogenesis 2002; 23: 1163-1169.
[30] Ogawa T, Hayashi T, Kyoizumi S, Kusunoki Y, Nakachi K, MacPhee DG, Trosko JE, Kataoka K and Yorioka N. Anisomycin downregulates gap-junctional intercellular communication via the p38 MAP-kinase pathway. J Cell Sci 2004; 117: 2087-2096.
[31] Petrich BG, Gong X, Lerner DL, Wang X, Brown JH, Saffitz JE and Wang Y. c-Jun N-terminal kinase activation mediates downregulation of connexin43 in cardiomyocytes. Circ Res 2002; 91: 640-647.
[32] Bhattacharjee R, Kaneda M, Nakahama K and Morita I. The steady-state expression of connexin43 is maintained by the PI3K/Akt in osteoblasts. Biochem Biophys Res Commun 2009; 382: 440-444.
[33] Xia X, Batra N, Shi Q, Bonewald LF, Sprague E and Jiang JX. Prostaglandin promotion of osteocyte gap junction function through tran-

scriptional regulation of connexin 43 by glycogen synthase kinase 3/beta-catenin signaling. Mol Cell Biol 2010; 30: 206-219.
[34] Kim MO, Lee YJ and Han HJ. Involvement of Cx43 phosphorylation in 5'-N-ethylcarboxamide-induced migration and proliferation of mouse embryonic stem cells. J Cell Physiol 2010; 224: 187-194.
[35] Tacheau C, Fontaine J, Loy J, Mauviel A and Verrecchia F. TGF-beta induces connexin43 gene expression in normal murine mammary gland epithelial cells via activation of p38 and PI3K/AKT signaling pathways. J Cell Physiol 2008; 217: 759-768.
[36] Ma J, Liu T and Dong X. Advanced glycation end products of bovine serum albumin-induced endothelial-to-mesenchymal transition in cultured human and monkey endothelial cells via protein kinase B signaling cascades. Mol Vis 2010; 16: 2669-2679.
[37] Wu CH, Chang CH, Lin HC, Chen CM, Lin CH and Lee HM. Role of protein kinase C in BSA-AGE-mediated inducible nitric oxide synthase expression in RAW 264.7 macrophages. Biochem Pharmacol 2003; 66: 203-212.
[38] Xu Y, Wang S, Feng L, Zhu Q, Xiang P and He B. Blockade of PKC-beta protects HUVEC from advanced glycation end products induced inflammation. Int Immunopharmacol 2010; 10: 1552-1559.