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ARTICLE

Advanced Glycation End Products Down-regulate Gap Junctions in Human Hepatoma SKHep 1 Cells via the Activation of Src-Dependent ERK1/2 and JNK/SAPK/AP1 Signaling Pathways

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Hyperglycemia and advanced glycation end products (AGEs) are associated with an elevated risk of developing several cancers in diabetic patients. However, the detailed mechanisms remain to be elucidated. The mechanism of AGE−bovine serum albumin (BSA) on gap junction intercellular communication in human hepatoma cell line, SKHep 1, was investigated. Both Cx32 and Cx43 are major gap junction forming proteins in the liver, the loss of which has been shown to facilitate tumorigenesis. Although the MTT assay results showed that AGE−BSA significantly increased cell growth by 31%, AGE−BSA down-regulated Cx32 and Cx43 expression in a dose- and time-dependent manner. The present study also demonstrated that ERK1/2 and JNK/SAPK were significantly activated by AGE−BSA and that Src, ERK1/2, and JNK/SAPK inhibitors significantly reversed the reduction of Cx32 and Cx43 proteins by AGE−BSA. Taken together, these results strongly support the hypothesis that Src-dependent ERK1/2 and JNK/SAPK/AP1 signaling pathways play a key role in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells.

KEYWORDS: Advanced glycation end products (AGEs); connexin 32; connexin 43; ERK1/2; JNK/SAPK

INTRODUCTION

Hepatocellular carcinoma is the fifth most common tumor with high morbidity and mortality in Asia and South Africa (*1*). Many studies show that diabetes mellitus increases risks of recurrence of hepatocellular carcinoma after treatment (*2*), but the overall association between hepatocellular carcinoma and impaired glucose metabolism remains unknown. Glucose metabolism is a complex process that involves numerous regulatory pathways. In diabetes mellitus patients, aberrations in glucose metabolism might lead to abnormal cellular growth and regulation, leading to increased risk of tumorigenesis (*3*). Hyperglycemia and advanced glycation end products (AGEs) are key factors in the induction of microvascular complications in diabetes. The binding of AGEs to their receptors, RAGE, can impair the integrity of vessels through increasing permeability and accelerating abnormal absorptions (*4*). Although diabetes and cancer are major health concerns among the adult population, few studies have directly addressed the relationship between the two or the impact of diabetes on cancer outcomes.

Gap junctions are intercellular channels formed by the interaction of two hemichannel connexons, each from one cell, one of which is composed of six protein subunits (*5*−*7*). The major role of gap junction intercellular communication (GJIC) is considered to be the maintenance of homeostasis in multicellular organisms (*5*−*8*). Decreased expression of connectin (Cx) proteins contributes to abnormal GJIC (*9*). Uncontrolled tumor cell growth, because of the loss of GJIC, is due to the down-regulated expression of Cx genes (*6*, *10*−*12*). Previous papers have shown that overexpression of Cxs can inhibit proliferation of several cancers, making them very likely candidates for cancer therapy (*10*, *13*−*16*). Although many subtypes of connexins are known in mammalian cells, connectins Cx26, Cx32, and Cx43 are the most predominant in hepatocytes of humans and rats (*9*, *10*).

In the current study, the hepatocellular carcinoma cells SKHep 1 were treated with AGE−bovine serum albumin (BSA), and the results showed that AGE−BSA promoted proliferation of cells but down-regulated the expression levels of Cx32 and Cx43 proteins. This work further investigates whether mitogen-activated protein kinase (MAPK) signaling molecules play a role in the AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression. This investigation provided evidence for the first time that Src-dependent ERK1/2 and JNK/SAPK/AP1 signaling pathways play important roles in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells.

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MATERIALS AND METHODS

Cell Culture, Reagents, and Antibodies. SKHep 1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum (FBS), ampicillin (50 U/mL), and streptomycin (50 U/mL) at 37 °C. Cells at 70% confluence were transfected with plasmids using SuperFect reagents (Qiagen, Valencia, CA) according to the manufacturer's instructions. Following incubation, cells were harvested.

The Src and MAPK inhibitors (SU6656, PD98059, SP600125, and SB202190) for signaling molecules were purchased from Calbiochem (San Diego, CA). Monoclonal antibodies against Cx32, Cx43, and actin were obtained from Chemicon (Temecula, CA). Monoclonal antibodies against caspase 3, Src, p-Src, ERK1/2, p-ERK1/2, JNK/SAPK, p-JNK/SAPK, p38, and p-p38 were purchased from Cell Signaling Technology (Beverly, MA).

Preparation of AGE−BSA. The formation of AGE-modified BSA was validated with the following methods as described in ref *17*. BSA (50 mg/mL; fraction V, Sigma, St. Louis, MO) and 0.5 M glucose were incubated in 10 mM phosphate-buffered saline (PBS, pH 7.4) at 37 °C for 60 days. All incubations were performed in sterile and anaerobic conditions in the dark. Free glucose was removed by extensive dialysis. The molar ratio between BSA and glucose was 1 to 660. The formation of Maillard compounds was detected using the standard spectrum λ_{ex} 370 nm/λ_{em} 440 nm with a spectrofluorometer (Horiba Scientific Ltd., Kyoto, Japan). The ratio of relative fluorescence intensities of AGE−BSA to BSA was approximately 64:1.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blot Assay. Twenty microliters of sample was mixed with an equal volume of Laemmli buffer and analyzed by a 10% SDS-PAGE. Gels were stained with Coomassie brilliant blue (Sigma) or transferred to PVDF membranes. After blocking with 5% nonfat milk in phosphate-buffered saline, pH 7.4, with 0.05% Tween 20 (PBS-T), the target proteins were probed using respective antibodies and goat anti-mouse antibodies conjugated with horseradish peroxidase as secondary antibodies. Protein bands were developed by the ECL reagent (Amersham Pharmacia, U.K.).

Proliferative Activity. For proliferation of SKHep 1 cells, 3×10^4 cells/mL were cultured in 96-well plates. One set of cells was incubated with different concentrations of BSA, heated BSA, and AGE−BSA and another set with 250 and 500 μg/mL of AGE−BSA for different time periods at 37 °C. After discarding the medium, 0.5 mg/mL of MTT-based was added into individual wells. Plates were then incubated at 37 °C for 4 h. After incubation, 100 μL of DMSO was added to dissolve the formazan dye. The purple formazan solution was transferred to 96-well plates and read immediately at 540 nm with a spectrophotometer.

DNA Fragmentation Assay. Cells were treated with different concentrations of BSA, heated BSA, and AGE−BSA for 24 h and harvested in ice-cold PBS. After centrifugation (15000*g*) for 15 min at 4 °C, pellets were resuspended in 0.5 mL of isolation buffer (10 mM EDTA, 50 mM Tris-HCl, pH 8.0, 0.5% SDS, 0.5 mg/mL proteinase K) and incubated overnight at 50 °C. Lysates were centrifuged at 15000*g* for 15 min at 4 °C to separate soluble fragmented DNA from intact chromatins. Fragmented DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with ethanol. Purified DNA was treated with 1 μg/mL DNase-free RNase A for 1 h at 37 °C prior to electrophoresis.

Analysis of Cx32 and Cx43 Expression Levels in AGE−BSA-Treated SKHep 1 Cells. Cells were treated with different concentrations of AGE−BSA for 24 h or 250 μg/mL of AGE−BSA for 2, 4, 8, 12, and 24 h. Cells were then harvested and lysed in 0.5 mL of RIPA lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM PMSF). Proteins were separated on 5% polyacrylamide gels and transferred to PVDF membranes (Amersham Pharmacia, Hong Kong). Expression of specific proteins was detected using respective antibodies, followed by the secondary antibody, goat anti-mouse IgG conjugated with horseradish peroxidase. After incubation with enhanced chemiluminescence (ECL plus) (Amersham Biosciences, Little Chalfont, Buckinghamshire, U.K.), the result was exposed to X-ray films (Kodak, Rochester, NY).

Expression Levels of Cx32 and Cx43 Proteins on the Plasma Membrane of SKHep 1 Cells by Immunofluorescence/Confocal Microscopy. For immunofluorescence, SKHep 1 cells treated with AGE−BSA for 24 h were cultured on glass coverslips. At the indicated time points the cells were fixed with methanol at −20 °C for 5 min and incubated for 1 h with a 1:500 dilution of monoclonal antibodies against Cx32 or Cx43 protein at 37 °C, respectively. After that the cells were washed and incubated in buffer containing secondary antibodies in the dark for 50 min. After the nuclei were counterstained with bisbenzamide, the coverslips were sealed with 60% glycerol, and examined under Leica TCS SP and BD^{TM} CARV II confocal imaging.

Involvement of Src-Dependent ERK1/2 and JNK/SAPK Signaling Pathways in AGE−BSA-Mediated Down-regulation of Cx32 and Cx43 Protein Expression in SKHep 1 Cells. In this study, we investigated the role of Src and MAPKs in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells and the involvement of their downstream targets. First, we blocked Src and MAPK signalings using Src and MAPK inhibitors (*18*−*21*). The working concentrations of the various inhibitors were as follows: 5 μM SU6656, 20 μM PD98059, 5 μM SB202190, and 10 μM SP600125. Cells were pretreated with various inhibitors for 30 min, then AGE−BSA (250 μg/mL) was added to cells for 24 h. Second, we determined the response of AP-1, which is the downstream target of JNK/SAPK. In the present study, we determined whether AGE−BSA regulates the AP-1 signaling by using a reporter gene assay. AP-1 luciferase reporter plasmids were purchased from Stratagene (La Jolla, CA). Plasmid AP-1-Luc is cis-reporting systems containing the firefly luciferase reporter gene, which is controlled by a synthetic promoter that contains direct repeats of the transcription recognition sequences for AP-1. The luciferase reporter plasmid and pRKβGAL were cotransfected into the cells. Twenty-four hours after transfection, the cells were harvested. Luciferase and β-galactosidase activities were quantified by the Luciferase Assay System and the β-galactosidase Enzyme Assay System (Promega Corp.), respectively. The luciferase activity was normalized to β-galactosidase activity to account for the transfection efficiency.

Statistical Analysis. All data were analyzed using independent sample *t* test and are expressed as averages of three independent experiments. A *p* value of < 0.05 was considered to be significant.

RESULTS

Preparation of AGE−BSA. The molecular weight of AGE−BSA was approximately 78 (monomer), whereas control BSA (monomer) was only 69 kDa (monomer) (**Figure 1**). Trace amounts of molecules with molecular weights higher than 156 kDa were observed on the SDS-PAGE. This was the characteristics of BSA after glycation for 6 weeks. The potential of AGE compounds to produce intra- and intermolecular cross-linking yields those glycated BSA monomers and dimers. A significant part of AGE−BSA in the form of monomer was speculated to be able to enter the cell. Our results were consistent with the previous findings of Andreea et al. (*17*).

Effects of AGE−BSA on SKHep 1 Cell Proliferation. DNA fragmentation assay and detection of activated caspase 3 were carried out to assess apoptosis in SKHep 1 cells. Apoptosis was not observed at all tested concentrations (**Figure 2A**). The MTT assay was used to evaluate the effects of AGE−BSA on the proliferation rate of SKHep1 cells, and the results showed that there was no difference in cytoviability among the different concentrations of BSA and heated BSA (**Figure 2B**). However, there was marked increase in the cytoviability of $> 18.3 \pm 1.0\%$ when the cells were treated with at least 50 and 100 μg/mL AGE−BSA compared to the negative controls (BSA and heated BSA) 24 and 48 h post-treatment (**Figure 2B**), suggesting a dose-dependent manner on SKHep 1 cell proliferation. In addition, when SKHep 1 cells were treated with 250 and 500 μg/mL AGE−BSA at indicated time periods, the MTT assay showed a time-dependent increase in the number of cells (**Figure 2C**). Differences could be observed as early as 4 h but were more pronounced at 12 h and 24 h after AGE−BSA treatment as compared to the zero time point. In addition, our results showed that BSA and heated BSA did not affect cytoviability (**Figure 2B**,**C**).

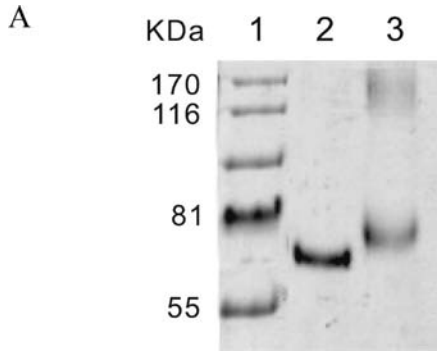

Figure 1. SDS-PAGE profiles of 6 weeks of glycation of BSA (100 mg/mL) at 37 °C in PBS (10 mM, pH 7.4). Lanes 1, 2, and 3 indicate molecular weight marker, unglycated BSA (control), and AGE−BSA, respectively. Only 2 μg of protein was loaded in each lane. The molecular weight of AGE−BSA was approximately 78 (monomer), whereas that of control BSA (monomer) was only 69 kDa (monomer). Trace amounts of molecules with molecular mass higher than 156 kDa were observed on the SDS-PAGE. This was carried out using a 4% stacking and 7.5% resolving gel and Coomassie blue staining.

Effects of AGE−BSA on Cx32 and Cx43 Protein Levels in SKHep 1 Cells. Figure 3 shows the effects of different concentrations of AGE−BSA on the levels of Cx32 and Cx43 proteins as detected by Western blot assay 24 h after treatment. Western blot assay indicated a dose-dependent decrease in the levels of Cx32 and Cx43 proteins (**Figures 3A**,**B**) as compared to the zero time point. Significant differences appeared at 100, 250, and 500 μg/mL AGE−BSA, and the relative levels of Cx32 protein were 77.3 ± 11.0, 53.4 ± 7.8, and 47.2 ± 11.3%, respectively (**Figure 3A**). Significant differences appeared at 100, 250, and 500 μg/mL AGE−BSA for Cx43 protein, and the relative protein levels were 55.1 ± 15.8% for 250 μg/mL and 32.6 ± 2.8% for 500 μg/mL (**Figure 3B**). The effects were similar for both 100 and 250 ug/mL AGE−BSA on Cx43 protein levels in SKHep 1 cells.

When SKHep1 cells treated with 250 μg/mL AGE−BSA at indicated time periods were analyzed by Western blot assay, there was a time-dependent decrease in both Cx32 and Cx43 protein levels (**Figures 3C**,**D**). However, as shown in **Figure 3C**, the decrease seemed to diminish with time as indicted by the fact that at 12 and 24 h, Cx32 protein levels were 64.5 ± 19.6 and 38.8 ± 1.1%, respectively. **Figure 3D** shows that the decrease in Cx43 could be observed as early as 8 h post-treatment but the decrease was more significant after 12 and 24 h post-treatment as compared to the zero time point.

There was no difference in Cx32, Cx43, p-ERK 1/2, p-JNK/SAPK, and p-p38 protein levels among BSA and heated BSA treatments (**Figure 3E**).

Detection of Cx32 and Cx43 Proteins on the Plasma Membrane of SKHep 1 Cells by Immunofluorescence/Confocal Microscopy. Confirmation of Cx32 and Cx43 protein expression on the plasma membrane of SKHep 1 cells was done by immunofluorescence/confocal microscopy. **Figure 4** shows the cell nucleus in blue color stained with bisbenzamide and plaques of Cx32 and Cx43 in the plasma membrane. The plaques decreased as AGE−BSA concentrations increased, suggesting a dose-dependent down-regulation of Cx32 and Cx43 protein levels (**Figure 4**).

Involvement of Src-Dependent ERK1/2 and JNK/SAPK Signaling Pathways in AGE−BSA-Mediated Down-regulation of Cx32 and Cx43 Protein Expression in SKHep 1 Cells. Having demonstrated AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells, we next want to investigate

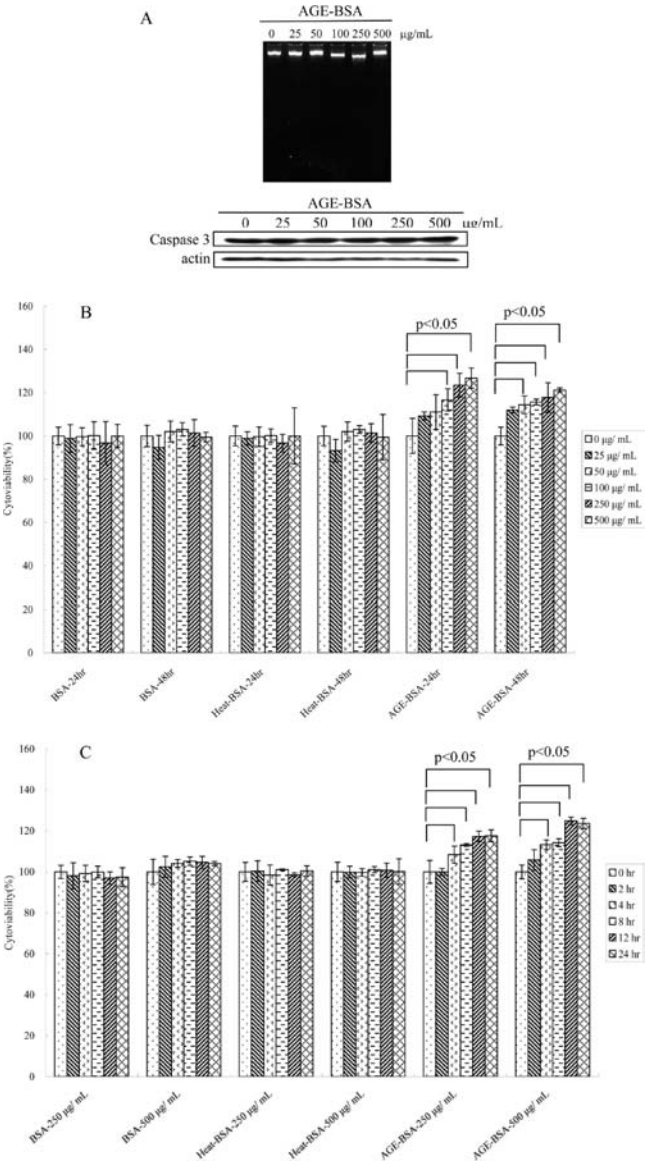

Figure 2. Proliferative effects of AGE−BSA on SKHep 1 cells. (**A**) To identify whether AGE−BSA induces apoptosis, DNA fragmentation (upper panel) and detection of caspase 3 activation (lower panel) were carried out. The chromosomal DNA was separated on a 1.5% agarose gel. DNA molecular weight marker is shown at the left. The AGE−BSA-treated cells did not produce an oligonucleosomal DNA ladder. The activated caspase 3 was not observed by detection of the disappearance of the full-length proenzyme (procaspase 3). The increase in SKHep 1 cell viability after treatment with different concentrations of AGE−BSA for 24 h and 48 h is shown in **B**, and treatment with 250 and 500 μg/mL AGE−BSA for indicated time periods is shown in **C**. There were marked increases in the cytoviability of up to 25.6 ± 1.0 and 26.7 ± 6.9%, respectively, after treatment with 250 and 500 μg/mL AGE−BSA compared to the nontreated control. The results are the mean ± standard deviation (SD) of three separate experiments (*, $p < 0.05$). MTT assay indicated that AGE−BSA can promote tumorigenesis.

which signaling molecules are involved. The results revealed that ERK 1/2 and JNK/SAPK, but not p38, were significantly activated by AGE−BSA (**Figure 5**). Treatment of the AGE−BSA-treated SKHep 1 cells with both ERK1/2 inhibitor (PD98059) and JNK/SAPK inhibitor (SP600125) significantly reversed the reduction of Cx32 and Cx43 proteins by AGE−BSA (**Figure 5B**,**C**). In addition, cells treated with p38 inhibitor (SB202190) were

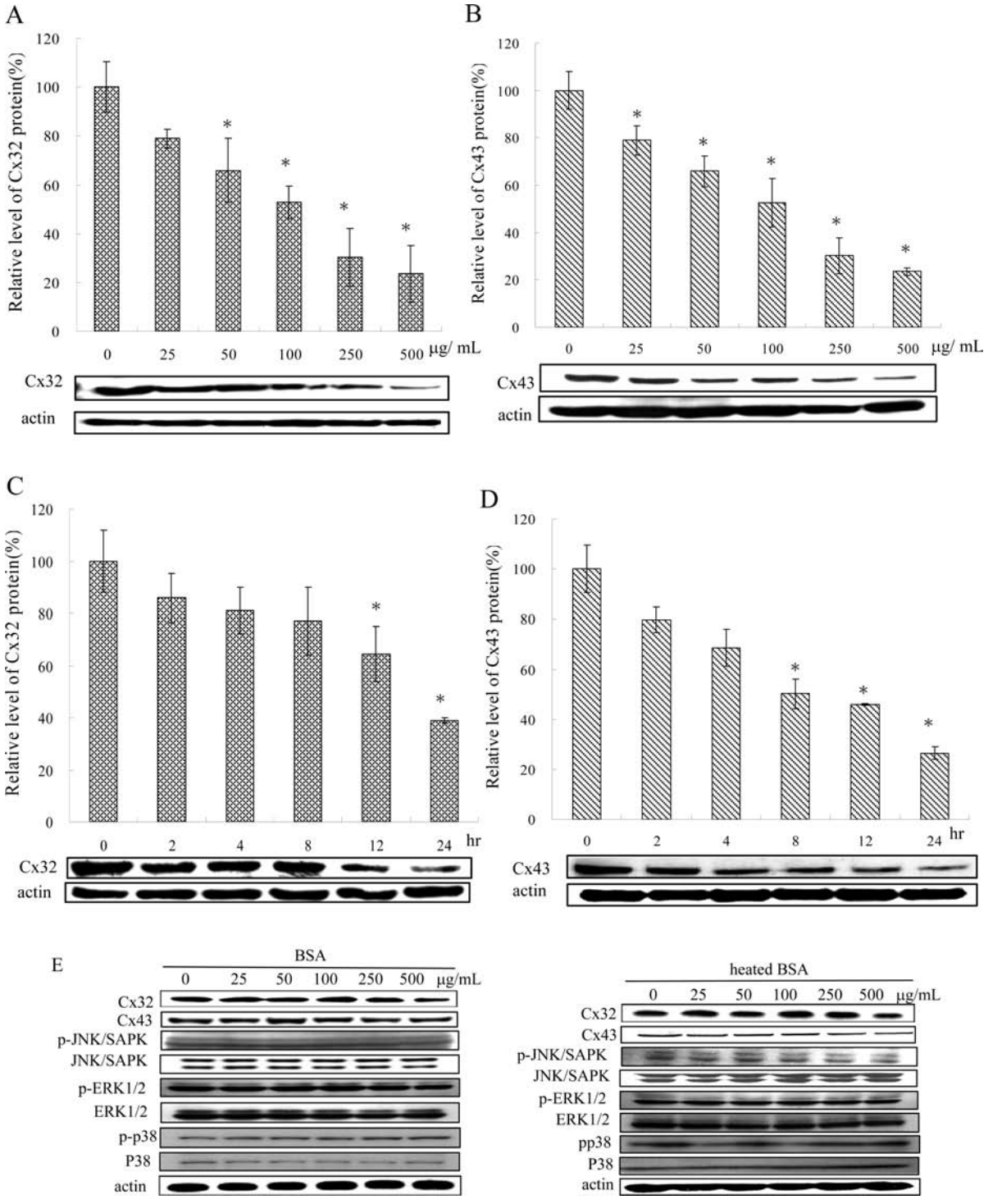

Figure 3. AGE−BSA affects Cx32 and Cx43 protein levels in SKHep 1 cells in a dose- and time-dependent manner. Down-regulation of Cx32 (**A**) and Cx43 (**B**) protein levels by sequential treatment with AGE−BSA for 24 h was detected by Western blot. Down-regulation of levels of Cx32 (**C**) and Cx43 (**D**) proteins with 250 μg/mL AGE−BSA for indicated time periods was detected by Western blotting. The protein levels of Cx32, Cx43, and MAPKs were examined after BSA and heated BSA treatments (**E**). In this study, the relative levels of Cx32 and Cx43 proteins were the average of three independent experiments, as shown by the histogram at the top of the blot. Error bars represent mean ± SD (∗, $p < 0.05$).

unaffected (**Figure 5A**). These results suggest that both ERK1/2 and JNK/SAPK, but not p38, are involved in AGE−BSA mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells.

In this study, inhibition of Src alters both ERK 1/2 and JNK/SAPK activation (**Figure 6A**). Src inhibitor (SU6656) significantly reversed Cx32 and Cx43 protein expression in AGE−BSA-treated SKHep 1 cells (**Figure 6B**). A reporter gene assay validates that AGE−BSA increases functional AP-1 activity, and AP-1 activity was also inhibited by JNK/SAPK inhibitor. Our results demonstrate that AGE−BSA treatment corresponded to JNK/SAPK phosphorylation, affecting AP-1 (**Figure 6C**). Taken together, all of the data suggested that Src-dependent ERK1/2 and JNK/SAPK signaling pathways were involved in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep1 cells.

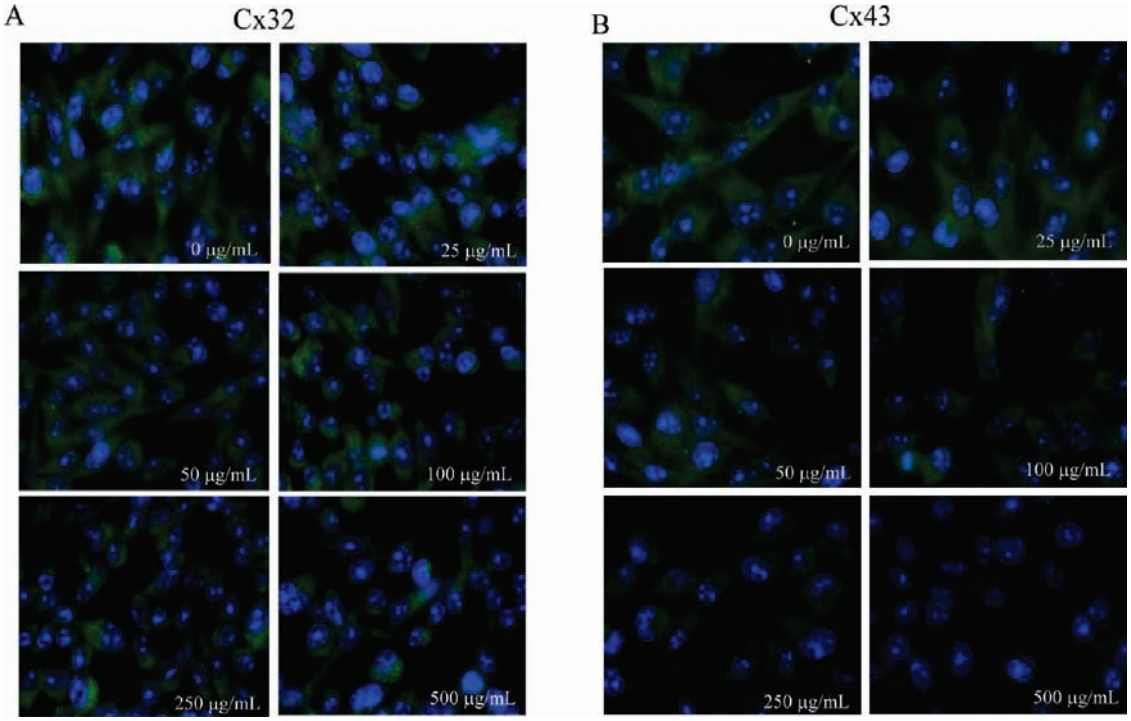

Figure 4. Expression of Cx32 and Cx43 proteins on the plasma membrane of SKHep 1 cells by immunofluorescence/confocal microscopy. Down-regulation of Cx32 (**A**) and Cx43 (**B**) proteins by sequential treatment with AGE−BSA for 24 h is shown by immunolabeling. Compared with the control group (0 μg/mL), Cx32 and Cx43 gap junctions (green spots) are reduced as the doses of AGE−BSA increase (25−500 μg/mL); blue label, nucleus. The figure is representative of triplicate experiments.

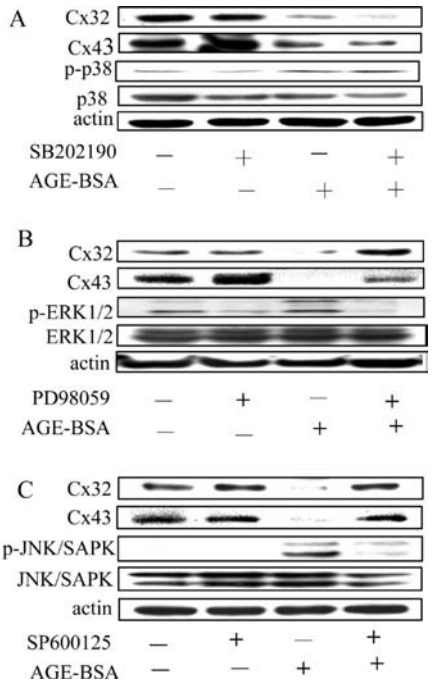

Figure 5. Activation of ERK1/2 and JNK/SAPK signaling pathways in AGE−BSA-treated SKHep 1 cells. The AGE−BSA down-regulated Cx32 and Cx43 protein expression in SKHep 1 cells. Both ERK1/2 inhibitor PD98059 and JNK/SAPK inhibitor SP600125, but not p38 inhibitor SB202190, significantly reversed the reduction of Cx32 and Cx43 proteins by AGE−BSA. The figure is representative of triplicate experiments. Error bars represent standard deviation.

DISCUSSION

This paper is the first to demonstrate that AGE−BSA promoted SKHep 1 cell proliferation but down-regulated the levels of both Cx32 and Cx43 gap junctional proteins. Gap junctional intercellular communication has been hypothesized to regulate growth control, differentiation, and apoptosis. Most normal, contact-inhibited cells have functional GJIC, whereas most, if not all, tumor cells have dysfunctional GJIC (*6*, *7*, *11*). Cancer cells are characterized by the lack of growth control, by the inability to terminally differentiate, and by resistance to apoptosis. Various oncogenes (e.g., ras, raf, neu, src, and mos) down-regulate GJIC (*11*, *22*−*24*), whereas several tumor suppressor genes can up-regulate GJIC (*15*, *25*). Transfection of gap junction genes ("connexins") into GJIC-deficient tumor cells has been seen to restore GJIC, growth control, and reduce tumorigenicity (*16*, *26*). Besides, up-regulation of gap junction has been linked to some tumor suppressor genes. Connexin proteins have been known to function as tumor suppressors, and transfection of Cx43 decreases neoplastic potential as evidenced by attenuated anchorage-independent growth and increased tumorigenesis in mice (*16*).

The present results demonstrate a direct stimulation of proliferation on SKHep 1 cells by AGE−BSA. Few AGE molecular structures are known in detail, and of these fewer still have been demonstrated to exist in vivo. AGE−BSA was prepared by means of a prolonged incubation of BSA and glucose because this leads to the formation of a mixture of intermediate and late AGEs that is believed to be representative of the spectrum of adducts existing in vivo (*4*). AGE formation proceeds slowly under normal ambient sugar concentration, but is enhanced in the presence of hyperglycemia. Early glucosylated proteins (Schiff bases) are rearranged to form irreversible metabolites, AGEs, which exert deleterious effects on various tissues. The previous study suggested that high glucose levels triggered apoptosis in endothelial cells (*27*). We speculated that AGEs formed in a hyperglycemic environment would have an aggregative effect on SKHep 1 cells that may result in apoptosis, but that was not the

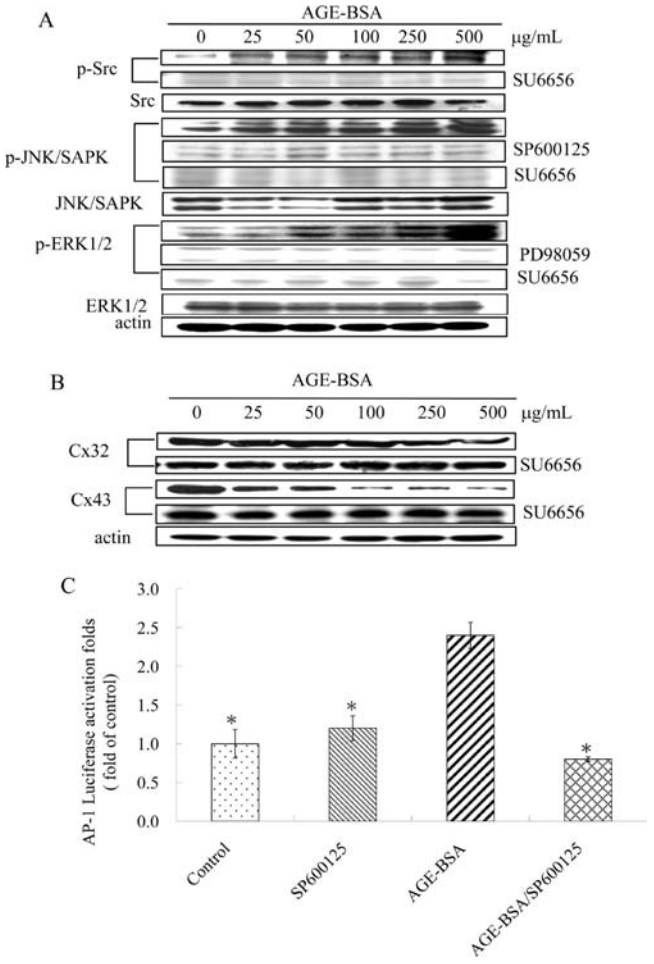

Figure 6. Src-dependent ERK1/2 and JNK/SAPK signaling pathways play a key role in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells. AGE−BSA-induced Src-dependent ERK1/2 and JNK/SAPK signaling can be inhibited by Src, ERK1/2, or JNK/SAPK inhibitors (**A**). The AGE−BSA-mediated down-regulation of Cx32 and Cx 43 protein expression was also suppressed by Src inhibitor (SU6656) (**B**). An equal amount of total cell lysate from each sample was subjected to Western blot analysis with respective antibodies. The same blot was reprobed for actin as an internal control. Fifty nanograms of each AP-1-Luc and pRKβGAL was cotransfected into SKHep 1 cells. Luciferase and β-galactosidase activities were quantified 24 h after transfection, and β-gal was used to normalize for transfection efficiency. The AP-1 activation in AGE−BSA-treated cells and suppressed in the cells pretreated with JNK/SAPK inhibitor (**C**). The figure is representative of triplicate experiments. The values shown are the means of three independent experiments, and error bars indicate standard deviations ($*$, $p < 0.05$).

case here. Because treatment of SKHep 1 cells with AGE−BSA did not cause apoptosis as speculated, this strongly suggests that different cell types have different sensitivities to AGE−BSA as far as the pathogenesis of diabetes mellitus is concerned.

Several studies have suggested that Cx32 expression has inhibitory effects on hepatocarcinogenesis and that transfection with Cx32 cDNA inhibits hepatoma cellular proliferation (*9*). This study also demonstrated that high concentrations of AGEs down-regulate Cx32 and Cx43 protein levels and promote SKHep 1 cell proliferation in vitro. AGEs have been strongly implicated in the initiation and acceleration of chronic complications in diabetes (*28*). AGE-induced damage is initiated, in most cases, by interaction with cell-membrane specific multiligand receptors for AGEs called RAGE (*29*). Our study indicated that Cxs could be further down-regulated by exposure of SKHep 1 cells to an excess of AGE−BSA, leading to possible induction of hepatocellular carcinogenesis and tumorigenesis. This study suggests a role for protein−AGEs in the pathogenesis of HCC carcinoma in diabetic patients.

To elucidate the mechanism of AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells, we investigated the role of Src and MAPKs in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells and their downstream targets. Our current findings provide some information regarding the molecular mechanism of AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells. All data suggested that a Src-dependent pathway is involved in the activation of ERK1/2 and JNK/SAPK/AP1 signaling in AGE−BSA-treated cells and that multiple and independent pathways may be involved in AGE−BSA-mediated down-regulation of Cx32 and Cx43 protein expression in SKHep 1 cells. Further studies will be performed using a systemic approach to identify other possible cellular factors involved and analyze their biological roles.

ABBREVIATIONS USED

AGEs, advanced glycation end products; Cx32, connexin 32; Cx43, connexin 43; GJIC, gap junction intercellular communication; BSA, bovine serum albumin; MTT, tetrazolium; MAPK, mitogen-activated protein kinase.

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