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Research Article

Differential regulation of collagen types I and III expression in cardiac fibroblasts by AGEs through TRB3/MAPK signaling pathway

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Abstract. Advanced glycation end products (AGEs) play an important role in collagen deposition in diabetic cardiomyopathy. TRB3, a mammalian homolog of Drosophila tribbles, functions to increase glucose intolerance and regulates cell proliferation. We demonstrated that AGEs induce collagen type I expression but inhibit collagen type III expression, accompanied by increased TRB3 expression. Furthermore, the collagen type I induced by AGEs was down-regulated after inhibition of ERK and p38-MAPK, the collagen type III reduced by AGEs was up-regulated after inhibition of ERK. The expression of collagen types I and III regulated by AGEs through MAPK was partly reversed after treatment with TRB3 siRNA. It suggests that the TRB3/MAPK signaling pathway participates in the regulation of collagen types I and III by AGEs and may provide new therapeutic strategies for diabetic cardiomyopathy.

Keywords. Advanced glycation end products, cardiac fibroblasts, collagen, tribble 3, mitogen-activated protein kinase.

Introduction

Diabetic cardiomyopathy is one of the leading causes of increased morbidity and mortality in patients with diabetes. It is characterized by myocyte loss and myocardial fibrosis, leading to decreased elasticity and impaired contractile function. There is accumulating evidence that advanced glycation end products (AGEs) have a pathogenic role in the development of diabetic cardiomyopathy. Excessive AGEs produced as a result of hyperglycemia are known to produce irreversible cross-links between extracellular matrix (ECM) proteins [1, 2], compromising tissue compliance and causing myocardial stiffness [3]. AGEs also exert their detrimental effect by interacting and up-regulating their receptors, including the receptor for advanced glycation end products (RAGE), Through these receptors, AGEs activate several critical molecular pathways which trigger

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production of profibrogenic growth factors, connective tissue growth factor (CTGF), and TGFβ1 [1, 4], stimulate the production of the ECM and inhibit its degradation.

The composition of ECM, a complex network of structural proteins, mainly collagen types I and III in the myocardium, provides architectural support for the myocardium and plays an important role in myocardial function [5]. Several studies have demonstrated an accumulation of collagens including collagen types I and III in diabetic cardiomyopathy, which has been related to left-ventricular diastolic and systolic dysfunction [6, 7]. However, the effect of AGEs on the expression of the subtype of collagens in cardiac fibroblasts and the intracellular mechanism(s) whereby AGEs induce cardiac fibrosis remain poorly understood.

AGEs lead to activation of multiple signaling pathways, including mitogen-activated protein kinases (MAPK), through interacting with the receptor for AGE (RAGE) [8, 9], and MAPK have been demonstrated to participate in collagen expression in fibroblasts and to be an important mediator of the fibrotic process [10, 11], which suggests that AGEs may participate in cardiac fibrosis through the MAPK signaling pathway.

The Drosophila protein Tribbles regulates string activity and, hence, mitosis during ventral furrow formation [12]. Its mammalian homolog, Tribble 3 (TRB3), was induced in mouse liver under fasting conditions and found to promote glucose output [13]. TRB3 also serves as a molecular switch and regulates the relative activation of the three classes of MAPK [14].We previously reported increased total collagen content in myocardium, along with significantly increased TRB3 mRNA expression in the hearts of streptozotocin-induced diabetic rats [15].

In light of the relationship between AGEs, MAPK and TRB3 in cardiac fibrosis, we hypothesized that TRB3 regulates collagen expression induced by AGEs in cardiac fibroblasts through an MAPK signaling pathway. We studied rat cardiac fibroblasts cultured in AGE-albumin to determine: 1) whether AGEs affect collagen production in these cells; 2) whether MAPK are involved in AGE-induced collagen production; 3) whether the expression of TRB3 is increased in cardiac fibroblasts induced by AGEs; 4) whether TRB3 is involved in AGE-induced MAPK activity and collagen production.

Materials and Methods

Chemicals and reagents. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum and trypsin were purchased from Gibco (Grand Island, NY, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA), PD98059, SB203580 and SP600125 were obtained from Cell Signaling Technology (Danves, MA, USA). Trizol reagent was from Invitrogen (Carlsbad, CA, USA). Reagents for real-time RT-PCR were obtained from Promega Biotech (Madison, WI, USA). Phospho-specific antibodies for ERK1/2, p38-MAPK, and JNK and antibodies against total ERK1/2, p38-MAPK, and JNK were purchased from Cell Signaling Technology (Danves, MA, USA), Anti-trb3 antibody was provided by Dr. Jingti Deng (Shandong University, Jinan, P.R.China). X-tremeGENE siRNA Transfection was from Roche Co. (Mannheim, Germany). The chemiluminescence (ECL) kit was obtained from Amersham Pharmacia (Piscataway, NY, USA). The enzyme-linked immunosorbent assay (ELISA) kits for procollagen types I and III C-terminal peptide (PICP) were purchased from Bionewtrans Pharmaceutical Biotechnology (Franklin, MA, USA). Other chemicals were of the highest purity grade.

Cell cultures. Cardiac fibroblasts were prepared from the ventricles of adult male, 300–350 g Wistar rats and was identified according to the method described previously [16]. After digesting the minced left ventricular free wall with trypsin (6 mg/ml) and collagenase type 2 (100 U/ml), collected cells were preincubated in DMEM with 10% FBS for two hours to separate CFs from myocytes. The cells were then cultured in DMEM supplemented with 10% fetal bovine serum (FBS) in 5% CO_2 and 95% humidified air at 37 °C. The purity of these cultures was positive for $>95\%$ cardiac fibroblasts as measured by vimentin expression and negative by von Willebrand factor, α-smooth muscle actin, and α-sarcomeric actin as previously described [17]. All studies were performed with cells at passages 2–4. Animal experiments were conducted in accordance with guidelines established by the Animal Care and Use Committee of Shandong University. Cells were stimulated with AGEs or BSA at concentrations of 200 μg/ml. To examine the effect of the MAPK pathway on collagen expression by AGEs, a specific ERK (PD98059, 10 μmol/L), p38 inhibitor (SB203580, 10 μmol/L) and JNK inhibitor (SP600125, 10 μmol/L) were added 30 min. before stimulations. To examine the effect of TRB3 on collagen expression by AGEs, TRB3 siRNA (30 μmol/L) was transfected 24 h before stimulations.

Preparation of AGEs. AGE-BSA was prepared by incubating BSA in phosphate- buffered saline (PBS, 10 mmol/L, pH 7.4) with 50 mmol/L D-glucose for

Table 1. Primers used for RT-PCR.

Name	GenBank accession	Primers	Size (bp)
β-actin	NM_031144	Forward:5′AGACCTTCAACACCCCAG3′; Reverse: 5′CACGATTTCCCTCTC AGC3′	255
GAPDH	XR_001863.1	Forward:5′-CTGATGCCCCCATGTTTAAT-3′; Reverse: 5′- TTAATGGGCTCTCTGATGCC-3′	240
procollagen type I,α1	XM_213440	Forward:5′TTCACCTACAGCACGCTTGT3′; Reverse:5′TTGGGATGGAGGGAGTTTAC3′	196
procollagen type III,α1	NM_032085	Forward:5′GGTCACTTTCACTGGTTGACGA3′; Reverse:5′TTGAATATCAAACACGCAAGGC3′	201
TRB3	NM_144755	Forward: 5′TGATGCTGTCTGGATGACAA3′; Reverse: 5′GTGAATGGGGACTTTGGTCT3′	292

eight weeks at 37 °C as described previously [18, 19]. Unmodified BSA was incubated under the same conditions without glucose as control. Protein concentrations were measured by the Bradford method. All AGE-protein specific fluorescence intensities were measured at a protein concentration of 1 mg/ml. AGEs and control BSA contained 66.5 and 6.33 units of AGEs per milligram of protein, respectively. All reagents were prepared under endotoxin-free conditions. Each preparation was tested by Limulus amoebocyte lysate assay (EToxate, Sigma, St. Louis, MO, USA) for endotoxin content (<0.8 EU endotoxin per ml).

Small interfering RNA (siRNA) transfection. Twenty-one nucleotide long siRNAs for rat TRB3 were chemically synthesized by Invitrogen. The 5′-GGCACAGAGUA CACCUGCATT-3′ and 5′-UGCAGGUGUUACUCUGUGCCTT-3′ oligoribonucleo- tides were used to inhibit TRB3 synthesis, The TRB3 siRNA is located in the region of the TRB3 transcript (GenBank™ accession number NM_144755) of the sequence. The 5′-GUAUGACAACAGCCUCAAGTT-3′ and 5′-CUUGAGGCUGUUGUCAU ACTT-3′ oligoribonucleotides were used as a GAPDH postive control. As a negative control, we used a randomly mixed sequences of the TRB3 siRNA 5′-UUCUCCGAA CGUGUCACGUTT-3′ and 5′-ACGUGACACGUUCGGAGAATT-3′ oligoribonucleotides. siRNA was transiently transfected into cells grown in a six-well plate by using X-treme GENE siRNA Transfection according to the manufacturer's protocol. To determine if the transfection was toxic to cardiac fibroblasts, the cytotoxic effect of the treatment was assessed by cell viability determined with MTT assay. We tested this reaction after 4 h – 48 h using an ELISA reader (Bio-Rad) at 570 nm, with a 690 nm filter as reference. The result showed that siRNA for 24 h and 48 h had no significant effect on cell viability compared to the control (supplement 2B). The siRNA with SFM (a fluorochrome) was transiently transfected into cells growing in a six-well plate using X-treme GENE siRNA transfection according to the manufacturer's protocol, the efficiency of transfection reached 70 – 80 % at 24 h after transfection. In each experiment, a series of wells was dedicated to the evaluation of the silencing of the TRB3 by real-time quantitative RT-PCR and western blotting analysis. All RNAi experiments were performed on at least three independent occasions with comparable results.

Real-time quantitative RT-PCR. Total RNA was extracted by using Trizol according to the manufacturer's procedures. Two micrograms of total RNA were reverse transcribed in a 20 μl volume containing 0.5 μg oligo-dT primer, 1 μl dNTP mixture, 1.25 μl RNase inhibitor, and 4 units reverse transcriptase. Real-time quantitative PCR involved the SYBR-based method in a 20 μl reaction in a Roche light-cycler. Reaction specificity was confirmed by analyzing melting curves and by electrophoresis on 2.0 % agarose gel analysis of products. The relative changes in gene expression were analyzed by the 2(-Delta Delta C(T)) method and normalized according to the expression of the housekeeping gene β-actin(26). Respective primer and product specifications are in Table 1.

Enzyme-linked immunosorbent assay (ELISA). Soluble collagen types I and III proteins were determined by secretion of procollagen types I and III C-terminal peptide (PICP), a marker for procollagen types I and III production by a PICP detection ELISA kit according to the manufacturer's protocol.

Western blot analysis. Cells were harvested in sample buffer, resolved by SDS-polyacrylamide gel electrophoresis, transferred to a polyvinylidene difluoride membrane, subjected to western blot using a 1 : 1000

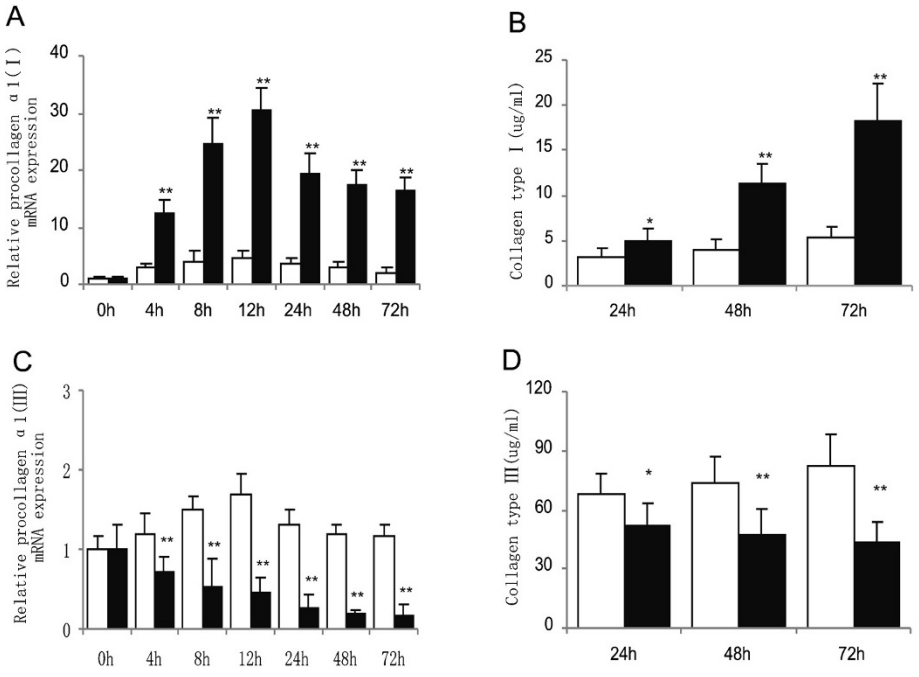

Figure 1. Real-time PCR and ELISA analysis demonstrate that AGEs induce collagen type I and induce loss of collagen type III in cardiac fibroblasts. (*A*) AGEs but not BSA induce the mRNA expression of procollagen type I in a time-dependent manner within 12 h. (*B*) AGEs but not BSA induce collagen type I protein expression 24 h – 72 h after treatment. (*C*) AGEs but not BSA significantly decrease mRNA expression of procollagen type III. (*D*) AGEs but not BSA decrease protein expression of collagen type III after 24 h – 72 h. Cardiac fibroblasts were treated with 200 μg/ml of AGEs (black squares) and 200 μg/ml of BSA (white squares). Data are means ± SEM from at least six independent experiments. $*P < 0.05$ and $**P < 0.01$ compared with BSA.

dilution of antibody for MAPKs, 1:500 dilution of antibody for TRB3, the membrane was then incubated with 1:3000 horseradish peroxidase-conjugated anti-mouse antibodies, and detected using an enhanced chemilumine- scence detection system. The density of the resulting protein bands was analyzed using the ImageMaster totallab software (version 2005, Amersham Pharmacia Biotech, USA).

Statistical analysis. All values are shown as means ± SEM. Differences between two groups were determined by unpaired Student's *t*-test. ANOVA was used for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

AGEs regulate the expression of collagen types I and III in cardiac fibroblasts. Several studies have suggested that AGEs participate in cardiac fibrosis in diabetic cardiomyopathy [1, 20]; however, the effect of AGEs on the collagen subtype expression is still undetermined. In the present study we first examined the expression of collagen types I and III after treatment with AGEs. As shown in Figure1, compared with the BSA group, AGEs increased both the mRNA expression of procollagen type I (Fig. 1A) and the protein expression of collagen type I (Fig. 1B) in cardiac fibroblasts in a time-dependent manner within 12 h for mRNA and 72 h for protein. However, AGEs decreased both the mRNA expression of procollagen type III (Fig. 1C) and protein expression of collagen type III (Fig. 1D) by cardiac fibroblasts. Thus, AGEs induce the expression of collagen types I but reduce slightly the expression of collagen types III by cardiac fibroblasts. These findings corroborate with previous studies showing that AGEs breakers inhibit cardiac remodeling in diabetic rats [1].

AGEs activate MAPK signaling pathways in cardiac fibroblasts. MAPK is known to be an important mediator which participates in collagen expression by fibroblasts [10, 11, 21], while the p38 MAPK signaling cascade is an important pathway in the progression of left ventricular dysfunction and pathologic remodeling [21]. In the previous study we found that high glucose induced collagen expression via the ERK1/2 signaling pathway [22], Therefore, we further examined the activation of the MAPK signaling pathway after treatment with AGEs. As shown in Figure 2, AGEs inducing the activity of ERK1/2 and p38-MAPK were increased significantly by AGEs in a time-dependent manner within 30 min., decreasing to baseline levels at 60 min. (Figs. 2A and 2B), and JNK was increased significantly by AGEs in a time-dependent manner at 30 min., decreasing to baseline levels at 60 min. (Fig. 2C). These data indicate that AGEs promote the phosphorylation of MAPK in rat cardiac fibroblasts.

Blocking of MAPK modifies the expression of collagen types I and III in cardiac fibroblasts by AGEs. To further determine whether MAPK are

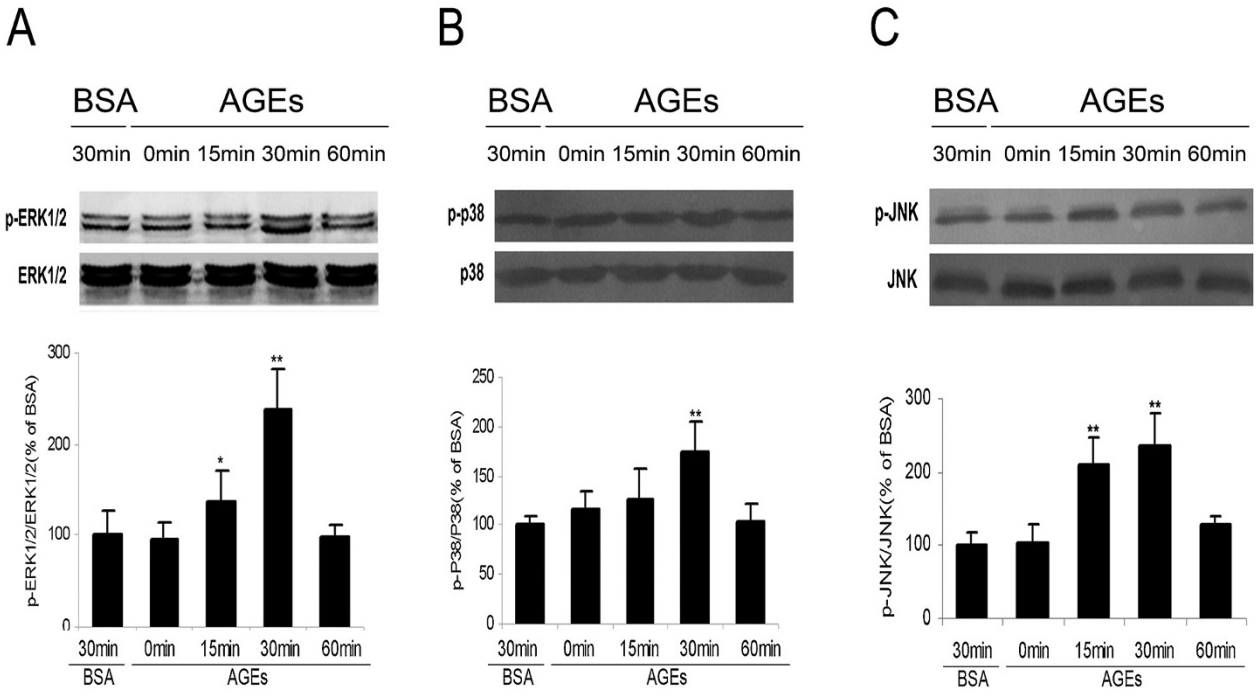

Figure 2. Western blot analysis demonstrates that AGEs activate the MAPK signaling pathway. (*A*) Representative western blots performed with anti-phospho-ERK1/2 (p-ERK1/2) and anti-ERK1/2 (ERK1/2) antibodies in cardiac fibroblasts incubated for 0 min. – 60 min. in the presence of AGEs and BSA. (*B*) Representative western blots performed with anti-phospho-p38 (p-p38) and anti-p38 (p38) antibodies in cardiac fibroblasts incubated for 0 min. – 60 min. in the presence of AGEs and BSA. (*C*) Representative western blots performed with anti–phospho-JNK (JNK) and anti-JNK (JNK) antibodies in cardiac fibroblasts incubated for 0 min. – 60 min. in the presence of AGEs and BSA. Cardiac fibroblasts were treated with 200 μg/ml of AGEs (black squares) and 200 μg/ml of BSA (white squares). Data represent results from six independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with BSA.

involved in the expression of collagen types I and III in cardiac fibroblasts by AGEs, we tested the mRNA expression of procollagen types I and III at 12 h and the protein expression of collagen types I and III at 24 h by AGEs after blockade of MAPK. As shown in Figure 3, both the mRNA and protein expression of collagen type I up-regulated by AGEs were reduced by a specific p38-MAPK inhibitor, SB203580 ($P < 0.01$) and a specific ERK inhibitor, PD98059 ($P < 0.01$), the inhibitory effect of PD98059 was greater than that of SB203580 (Figs. 3A and 3B). Both the mRNA and protein expression of collagen type III down-regulated by AGEs were slightly decreased by PD98059 ($P < 0.05$), but SB203580 partly reversed the effects of AGEs ($P < 0.01$) (Figs. 3C and 3D). However, a specific JNK inhibitor SP600125 had no significant effect on the expression of collagen types I and III (Figs. 3A–3D). These results demonstrate that AGEs regulate the production of collagen types I and III mainly via the ERK1/2 and p38-MAPK pathway.

TRB3 is involved in the expression of collagen types I and III regulated by AGEs in cardiac fibroblasts through activation of ERK1/2 and p38-MAPK. To test the efficacy of the selected siRNA sequence, we measured the mRNA level of TRB3 after 24 h transfection with TRB3 siRNA. The TRB3 mRNA expression in the cells transfected with TRB3 siRNA (30 μM) was lower than that in the cells transfected with the control siRNA. The addition of AGEs to cardiac fibroblasts increased TRB3 mRNA expression 4.53-fold that of BSA (Fig. 4A) and increased TRB3 protein expression 2.3-fold that of BSA at 30 min (Fig. 4B). After inhibiting the expression of TRB3 by TRB3 siRNA, both the mRNA expression of procollagen type I (Fig. 4C) and protein expression of collagen type I (Fig. 4D) were decreased markedly ($P < 0.01$), and the mRNA expression of procollagen type III (Fig. 4E) and protein expression of collagen type III (Fig. 4F) were slightly increased ($P < 0.05$). After transfection with TRB3 siRNA, the activity of ERK1/2 and p38-MAPK induced by AGEs was depressed significantly at 30 min. (Fig. 5A and 5B). These data suggest that the TRB3- activated ERK1/2 and p38-MAPK signaling pathway play a pivotal role in AGE-induced expression of collagen types I and III by cardiac fibroblasts.

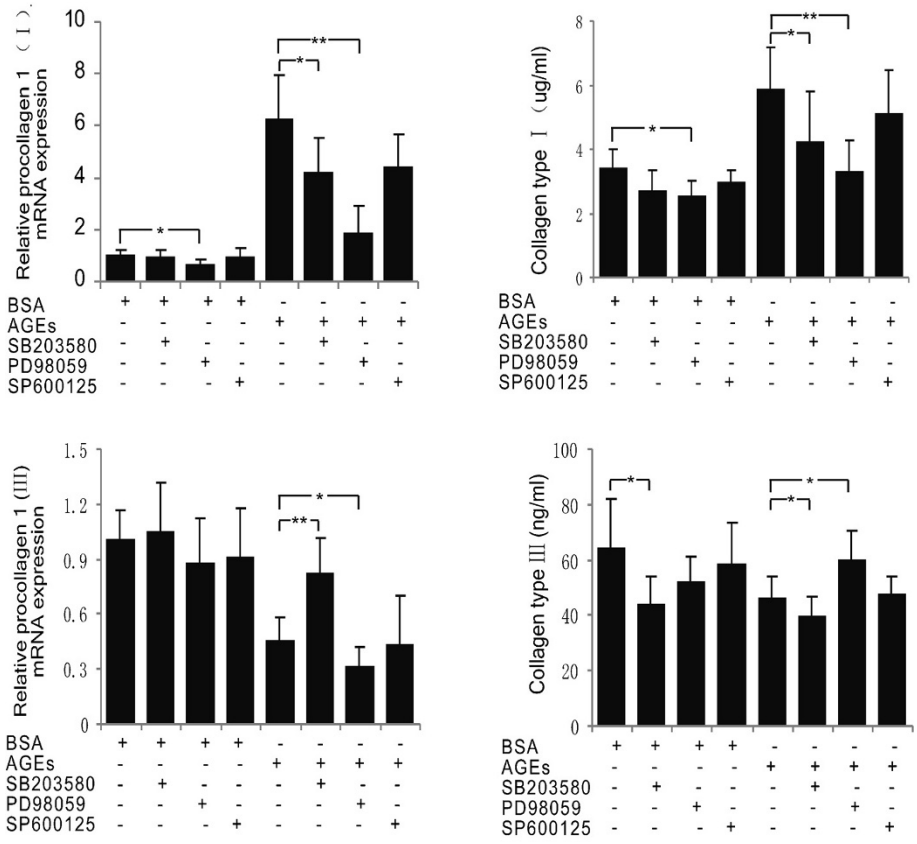

Figure 3. Effect of MAPK inhibitors on the expression of collagen type I and collagen type III in rat cardiac fibroblasts by AGEs. AGE-induced procollagen type I mRNA expression at 12 h (*A*) and protein expression of collagen type I at 24 h (*B*) are blocked by a specific ERK1/2 inhibitor (PD98059) and p38-MAPK inhibitor (SB203580). AGE-reduced procollagen type III mRNA expression at 12 h (*C*) and protein expression of collagen type III at 24 h (*D*) are blocked by SB203580, but PD98059 decreases the expression of collagen type III. Cardiac fibroblasts were treated with AGEs (200 μg/ml) and BSA (200 μg/ml) after being given a specific MAPK inhibitors. Data are means ± SEM from six independent experiments. $*P < 0.05$ and $**P < 0.01$ compared with BSA control and treatments with the MAPK inhibitors.

Discussion

In the present study we demonstrated that AGEs up-regulated the expression of collagen type I in rat cardiac fibroblasts through activation of ERK1/2 and p38-MAPK and down-regulated the expression of collagen type III through activation of p38-MAPK; however, ERK1/2 activated by AGEs induce the expression of collegen type III slightly ,which is different from the role of p38-MAPK. Moreover, AGEs significantly up-regulated the expression of TRB3, the mammalian homolog of the mitosis regulator Tribbles, in cardiac fibroblasts. After inhibition of TRB3 with TRB3 siRNA, both phosphorylation of ERK1/2 and p38-MAPK by AGEs were attenuated and the AGE-induced expression of collagen type I was markedly decreased, whereas the expression of collagen type III down-regulated by AGEs was significantly increased. Thus, the differential regulation of collagen types I and III in cardiac fibroblasts by AGEs through TRB3 mediated activation of a MAPK pathway ERK1/2 and p38-MAPK.

Cardiac fibroblasts are the predominant secretary cells of collagen in the heart and the key mediators of normal and pathological cardiac remodeling [23]. However, their role, mainly to produce collagen types I and III [24], has not been recognized in diabetic conditions. Our previous study showed that high glucose induced the expression of collagen types I and III in rat cardiac fibroblasts [22]. Here, we showed that AGEs significantly up-regulated both the mRNA expression of procollagen type I and the protein expression of collagen type I in adult rat cardiac fibroblasts, but the mRNA expression of procollagen type III and the protein expression of collagen type III were down-regulated by AGEs. These results suggest that AGEs might be one of the causes which improve the ratio of collagen type I / collagen type III, a signal index of cardiac remodeling in the later stage of heart failure [5]. This is different from the effect of high glucose, which increases both the production of collagen type I and collagen type III in rat cardiac fibroblasts [22].

AGEs regulate collagen types I and III by an ERK1/2- and p38-MAPK-dependent pathway. MAPK plays an important role in collagen production in cardiac fibroblasts [22, 25, 26] and activation of MAPK has previously been demonstrated in various cell types in response to AGEs [9, 27, 28]. In the present study, AGEs activated ERK1/2 and p38-MAPK within 30 min. after treatment. Furthermore, both the mRNA expression of procollagen type I and the protein expression of collagen type I induced by AGEs were significantly inhibited by the addition of

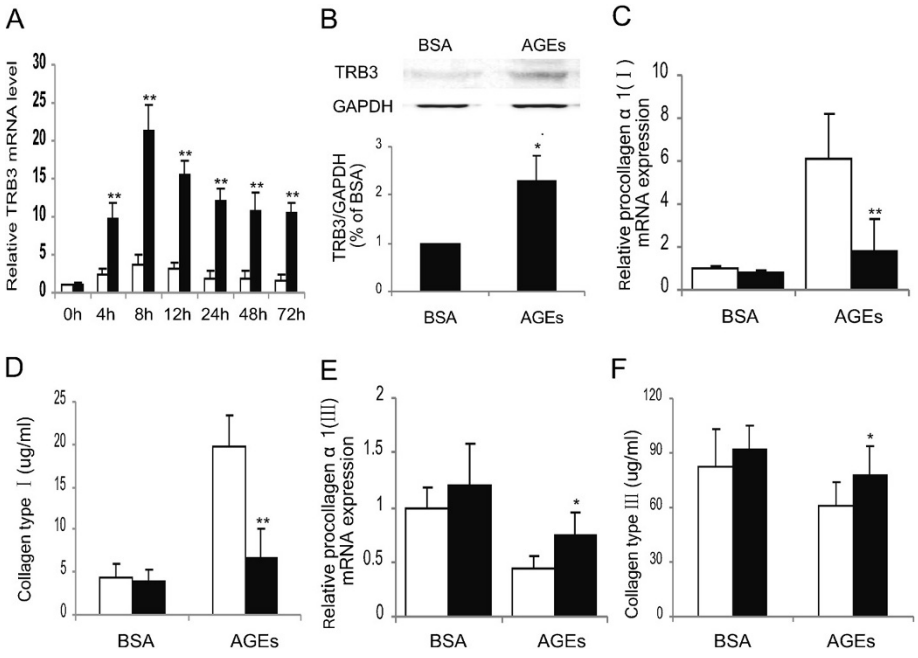

Figure 4. Effect of TRB3 on the expression of collagen type I and collagen type III by AGEs (200 μg/ml) in rat cardiac fibroblasts. (*A*) Real time RT-PCR revealed that AGEs induce TRB3 mRNA expression in cardiac fibroblasts. Cardiac fibroblasts were treated with 200 μg/ml of AGEs (black squares) and 200 μg/ml of BSA(white squares). (*B*) Representative western blots performed with anti-TRB3 and anti-GAPDH antibodies in cardiac fibroblasts incubated for 15 min. in the presence of AGEs and BSA. (*C*) and (*D*) AGE-induced mRNA expression of procollagen type I at 12 h and the protein expression of collagen type I at 72 h are inhibited after silencing TRB3 with TRB3 siRNA. (*E*) and (*F*) AGE-induced mRNA expression of procollagen type III at 12 h and the protein expression of collagen type III at 72 h are inhibited after silencing TRB3 with TRB3 siRNA. Cardiac fibroblasts were treated with AGEs (200 μg/ml) and BSA(200 μg/ml) after blockade of TRB3 with TRB3 siRNA. (*C*)–(*F*) Cardiac fibroblasts were treated with 30 μmol/L TRB3 siRNA (black squares) and 30 μmol/L control siRNA (white squares). Data represent means ± SEM from at least six independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with BSA control or treatment with AGEs.

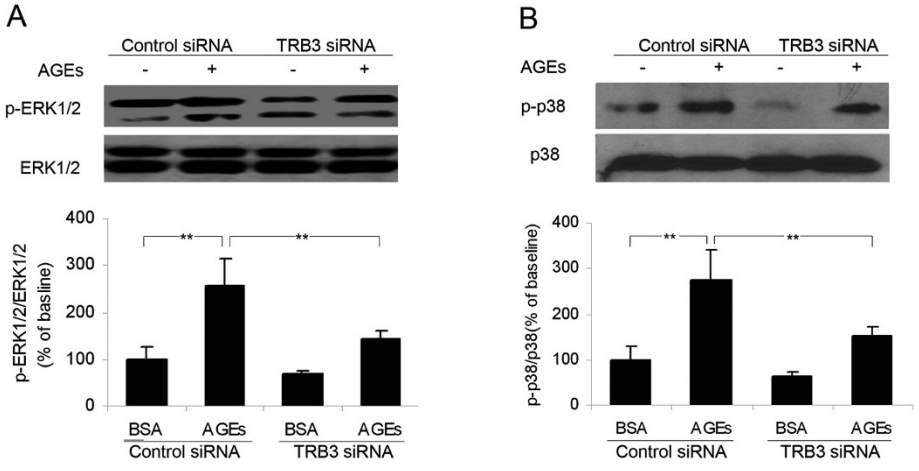

Figure 5. TRB3 siRNA inhibit the phosphorylation of ERK1/2 and p38-MAPK activated by AGEs. Representative western blot showing that the phosphorylation of ERK1/2 (*A*) and p38-MAPK (*B*) induced by AGEs (200 μg/ml) at 30 min. is blocked by a TRB3 siRNA (30 μmol/L). Data represent the results from three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the control siRNA.

PD98059 or SB203580, the inhibitory role of PD98059 was more significant than that of SB203580. The mRNA expression of procollagen type III and the protein expression of collagen type III reduced by AGEs were increased after treatment with SB203580 but PD98059 enhanced the inhibitory effect of AGEs on the expression of collagen type III. Though JNK was slightly activated by AGEs, the inhibitor of JNK has no significant effect on the production of collagen types I and III. Thus, AGEs likely mediate the expression of collagen types I and III directly through the following MAPK pathway: AGE-induced collagen type I expression requires activation of ERK 1/2 and p38- MAPK; however, decreased collagen type III expression by AGEs requires ERK 1/2 activation and the p38-MAPK pathway up-regulates collagen type III expression slightly in rat cardiac fibroblasts.

A novel and significant finding is that TRB3 is involved in the expression of collagen types I and III regulated by AGEs through activation of ERK1/2 and p38-MAPK pathways. Firstly, AGEs induced TRB3 mRNA expression as early as four hours after treatment, after the phosphorylation of ERK1/2 and p38-MAPK. Secondly, after blockade of TRB3 with TRB3 siRNA, the AGE-induced phosphorylation of ERK1/2 and p38-MAPK was decreased significantly. Finally, knockdown of TRB3 expression by TRB3 siRNA suppressed the AGE-induced production of collagen type I and slightly increased the production of collagen type III. Activation of MAPK signaling pathways occurs in response to a wide range of stimuli, ultimately leads to changes in gene expression, and the *de novo* synthesized proteins contribute to the cellular response. TRB3 proteins could act either as activators or inhibitors of MAPK activity, depending on the ratio of TRB3 to MAPKK in the cell [29]. In mammalian cells, TRB3 at a relatively low level can bind to and regulate MAPKK and hence activate MAPK phosphorylation, especially ERK1/2 phosphorylation [14].

Our results appear to contradict previous results that Tribbles inhibits cell proliferation in Drosophila and human cells [12, 30, 31]. However, a similar result in Hela cells showed that overexpression of TRB3 interferes with starvation-induced apoptosis and restores the growth ability, and it was postulated that overexpression of TRB3 interferes with apoptosis by titrating out potential growth inhibitory binding partners [32]. TRB3 may have a different effect in various cell types and dosages, and more detailed studies in different cell types is required to clarify the precise function of TRB3.

In summary, the present study presents evidence that AGEs stimulate the expression of collagen type I and decrease the expression of collagen type III through an ERK1/2- and p38-MAPK-dependent pathway. Moreover, our results suggest that TRB3 is involved in these processes through regulating the activity of the ERK1/2 and p38-MAPK pathways. Although our results suggest that blockage of TRB3 activity could be a therapeutic approach to inhibit AGE-induced collagen expression, future study is required to elucidate the role of the TRB3-MAPK pathway on cardiac fibrosis in diabetic animal studies.

Electronic supplementary material. Supplementary material is available in the online version of this article at springerlink.com (DOI 10.1007/s00018-008-8255-3) and is accessible for authorized users.

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