Biochemical and Biophysical Research Communications 424 (2012) 663–668

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Angiotensin II induces apoptosis in intestinal epithelial cells through the AT2 receptor, GATA-6 and the Bax pathway

Lihua Sun, Wensheng Wang, Weidong Xiao, Hongyin Liang, Yang Yang, Hua Yang *

Department of General Surgery, Xingqiao Hospital, Third Military Medical University, Chongqing 400037, China

ARTICLE INFO

Article history:
Received 27 June 2012
Available online 7 July 2012

Keywords:
Angiotensin II
Apoptosis
Intestinal epithelial cells
AT2 receptor
GATA-6

ABSTRACT

Angiotensin II (Ang II) has been shown to play an important role in cell apoptosis. However, the mechanisms of Ang-II-induced apoptosis in intestinal epithelial cells are not fully understood. GATA-6 is a zinc finger transcription factor expressed in the colorectal epithelium, which directs cell proliferation, differentiation and apoptosis. In the present study we investigated the underlying mechanism of which GATA-6 affects Ang-II induced apoptosis in intestinal epithelial cells. The *in vitro* intestinal epithelial cell apoptosis model was established by co-culturing Caco-2 cells with Ang II. Pretreatment with Angiotensin type 2 (AT2) receptor antagonist, PD123319, significantly reduced the expression of Bax and prevented the Caco-2 cells apoptosis induced by Ang II. In addition, Ang II up-regulated the expression of GATA-6. Interestingly, GATA-6 short hairpin RNA prevented Ang II-induced intestinal epithelial cells apoptosis and reduced the expression of Bax, but not Bcl-2. Taken together, the present study suggests that Angiotensin II promotes apoptosis in intestinal epithelial cells through GATA-6 and the Bax pathway in an AT2 receptor-dependent manner.

1. Introduction

Massive resection of small intestine leads to the development of short bowel syndrome (SBS), which is characterized as a state of inadequate digestion and impaired absorption of nutrients. SBS is capable of causing a series of adaptive response to increase absorptive surface area and establish nutritional homeostasis in residual intestine [1,2]. This adaption is highlighted by an increase in villus length and crypt depth, and an increased number of microvillus. The adaptive processes include significant increases in epithelial cell (EC) proliferation and EC apoptosis rates [3–5]. To improve intestinal compensation after SBS, researchers usually focus on EC proliferation via an up-regulation in a number of growth factors including keratinocyte growth factor (KGF), glucagon like peptide 2 (GLP-2), epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) [2,6–9]. Also, a few studies showed that reducing intestinal epithelial cell apoptosis would significantly improve bowel adaptation [3]. For example, Wildhaber et al. reported that ACE inhibitors (ACE-I) significantly reduces EC apoptosis and enhances intestinal adaptation [10]. Furthermore, blockade of angiotensin II (Ang II) signaling by Ang II receptor antagonists reduced injury effects and apoptosis [2,11]. These studies suggested that Ang II plays a key role in apoptosis of intestinal epithelial cells. However, the mechanisms by which Ang II regulates intestinal epithelial cells apoptosis are still elusive.

Ang II exerts its biological effects via two major isoforms of receptor known as angiotensin type 1 (AT1) and type 2 (AT2) receptor, both of which are seven-transmembrane-spanning G protein-coupled receptors [11–13]. The signaling of AT1 and AT2 is associated with cell proliferation and apoptosis. The delicate balance between proliferation and apoptosis plays an essential role in the pathophysiology of various diseases [14,15]. Several studies demonstrated that Ang-II-induced apoptosis in coronary artery endothelial cells and transformed epithelial cells was mediated by the AT1 receptor [16–19]. By contrast, some other researchers showed that AT2-receptor activation induced apoptosis in smooth muscle cells, HUVECs and PC12W cells whereas AT1 activation resulted in proliferative and anti-apoptotic cellular responses in these cells [20–22]. Conclusively, Ang-II-induced signaling has been suggested to be cell-type dependent, and the signaling specificity has been considered to determine the biological response.

GATA is a family of six transcriptional regulation proteins that plays important role in both developmental processed and tissue- or cell-specific gene transcription [23,24]. Previous studies have indicated that GATA-1, GATA-2 and GATA-3 function mainly in hematopoietic cell lineages whereas GATA-4, GATA-5 and GATA-6 are found in tissues of the endodermal origin [25–27]. Among the GATA family members, GATA-6 is highly expressed in gastric, colonic, ovary, pulmonary and cardiovascular cell lines [25,28–31]. In the intestinal tract, GATA-6 expression is gradually

* Corresponding author.
E-mail address: hwbyang@126.com (H. Yang).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.003

increased along the crypt–villus axis [26,27,32]. Studies have suggested that GATA-6 is associated with proliferation, differentiation and apoptosis by regulating genes expression in gastrointestinal tissue [28,32–34]. It has been shown that GATA-6 regulated the apoptosis-related proteins FasL in smooth muscle cells [21]. Moreover, GATA-6 has been shown to regulate Bax and Bcl-2 in human fetal testis [35]. But there is no report on the relationship between GATA-6 and apoptosis related genes in intestine so far. In this study, we explore the mechanism of Ang II induced apoptosis in intestinal epithelium cells involving GATA-6 transcriptional regulation of Bax and Bcl-2 expression.

2. Materials and methods

2.1. Materials

Caco-2 (human colon carcinoma) cells were purchased from China Center for Type Culture Collection (Beijing, China). Angiotensin II was purchased from Sigma (St. Louis, MO, USA). AT1 receptor blocker losartan was purchased from Merck, Co. (USA). AT2 receptor blocker PD123319 was purchased from Wako Pure Chemical Industries, Ltd. (Japan). Anti-bax and anti-bcl-2 antibodies were purchased from Millipore (USA). Anti-GAPDH antibody was purchased from Goodhere Biotechnology (Hangzhou, China). Anti-GATA-6 antibody was purchased from Abcam (USA).

2.2. Cell culture and treatments

Caco-2 cell were grown in Eagle's Minimum Essential Medium, supplemented with 20% fetal bovine serum (Gemini Bioproducts, Woodland, CA), 100 U/100ug/ml penicillin and streptomycin (Invitrogen, Carlsbad, CA). Cells were incubated in a 5% CO_2 humidified incubator at 37 °C. The following groups were analyzed: (1) untreated cells (control); (2) Ang II (10^{-6} mol/L) alone; (3) AT1 receptor blocker losartan (10^{-6} mol/L) and then Ang II; (4) AT2 receptor blocker PD123319 (10^{-6} mol/L) and then Ang II. Prior to stimulation with Ang II, Caco-2 cells were pretreated for 1 h with losartan or PD123319. Ang II was then added for 48 h. All treatments were performed in triplicates.

2.3. Flow cytometry using Annexin V staining to identify apoptotic cells

The apoptotic ratios of Caco-2 in different groups were measured by flow cytometry according to the protocol provided by the manufacturer. Double staining for FITC–Annexin V binding and for cellular DNA using propidium iodide were performed as described in the protocol. Cells were washed twice with phosphate-buffered saline (PBS, pH 7.4) and lifted by adding trypsin–EDTA. After this treatment, cells were centrifuged and washed twice with ice-cold PBS. Then the cells were pelleted at 1000 rpm for 5 min and resuspended in 200 μl blinding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl and 2.5 mM CaCl2), followed by adding 5 μl of FITC-labeled Annexin V and 10 μl of propidium iodide. The cells were incubated on ice for 15 min in the dark. Prior to flow cytometry of stained cells, 200 μl binding buffer was transferred into tubes and analyzed using a FACScalibur flow cytometer (Becton Dickinson, USA) with an emission wavelength of 488 nm. All experiments were repeated three times. Results were expressed as the percentage of Annexin V + cells in the total cell population.

2.4. Quantitative real-time PCR

Total RNA was extracted from cultured Caco-2 cells using Trizol (Invitrogen) following the manufacturer's instructions. Briefly, RNA was reverse-transcribed into complementary DNA (cDNA) with a SuperScript First-Strand Synthesis System RT-PCR kit (Invitrogen) and served as a template for amplification of Bax, Bcl-2, GATA-6 and β-actin. The forward and reverse primer (Table 1) were synthesized by TaKaRa. Gene expression was carried out by real-time PCR using the ABI 7500 and the SYBR® Premix Ex TaqTM II (TaKaRa) starting with 2 ng of reverse-transcribed total RNA. The thermal cycling conditions for reverse transcription and amplification activation were set at 45 °C for 30 min and 95 °C for 10 min, respectively. PCR denaturing was set at 94 °C for 5 min and annealing/extending at 59 °C for 1 min, for a total of 40 cycles. The expression of each gene was normalized to β-actin expression in the individual samples.

Table 1
Primer sequences of oligonucleotides used for PCR.

Gene	Primers		Size of product (bp)
Bax	Forward	5′-TTTTGCTTCAGGGTTTCATCCA-3′	117
	Reverse	5′-TGAGACACTCGCTCAGCTTCTTG-3′	
Bcl-2	Forward	5′-GTGGATGACTGAGTACCTGAACC-3′	133
	Reverse	5′-AGTCTTCAGAGACAGCCAGGAG-3′	
GATA-6	Forward	5′-TCCCATGACTCCAACTTCCACC-3′	170
	Reverse	5′-AGAGCCCATCTTGACCCGAAT-3′	
β-Actin	Forward	5′-CCAC G A AACTACCTTC A ACTCC-3′	132
	Reverse	5′-GTGATCTCCTTCTGCATCCTGT-3′	

2.5. Western blot analysis

The cells were washed twice with PBS prior to lysis in cold RIPA buffer (PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1ug/ml APMSF, 1.0 mM sodium orthovandate, 1× mammalian protease inhibitor cocktail; Sigma–Aldrich). The protein concentration was determined according to the Bradford method using BCA assay reagent (Beyotime). Samples (25 μg protein) were loaded onto SDS–PAGE gels and the gels were transferred to polyvinylidene difluoride membrane (Millipore) after electrophoresis. Membranes were blocked by 5% bovine serum albumin in TBS-T (50 mM Tris–HCl pH 7.5, 140 mM NaCl, 0.1% Tween) and then incubate with the following primary antibodies at 4 °C: mouse monoclonal anti-bax (1:200), rabbit monoclonal anti-bcl-2 (1:500), rabbit polyclonal anti-GATA-6 (1:1000), rabbit polyclonal anti-GAPDH (1:1000). The membranes were then washed three times and incubated with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies. Membrane imaging was performed using the enhanced chemiluminescence detection system (ECL, Boster) according to the manufacturer's instructions.

2.6. Short Hairpin RNA Assay

For inhibition of GATA-6 function, a transient transfection assay was performed. The custom shRNA directed against the 19 nucleotides of the human GATA-6 sequence, 5′-GCAATGCTTGTGGACTCTA-3′, was purchased from Shanghai Sunbio Medical Biotechnology Co. Ltd. Cells cultured to 90–95% confluence in a 6-well plates were transfected with the shRNA expression plasmid (4 μg for single transfections) using Lipofectamine 2000 reagent (Invitrogen) in antibiotics-free and serum-free Opti-MEM medium according to the manufacturer's instructions. After 6 h, the medium was replaced with normal culture medium with Ang II, and the cells were incubated for 48 h before harvested for western blot analysis of GATA-6, Bax and Bcl-2, or for apoptosis detection by flow cytometry.

2.7. Statistical Analysis

All experimental data are shown as the Mean ± SD. Analysis was performed using SPSS software (version 12.0 for windows). Differ-

ences were set statistically significant when $p < 0.05$. If not otherwise stated, all experiments included three independent replications in triplicate.

3. Results

3.1. Ang II induced apoptosis in Caco-2 cells

Using the established *in vitro* model of Caco-2 apoptosis induced by Ang II, we investigated the effects of Ang II on cell apoptosis in different concentrations. All doses of Ang II tested significantly increased Caco-2 apoptosis compared with control treatment. Ang II (10^{-7} M and 10^{-8} M) induced about 15–20% apoptosis within 48 h, while 10^{-6} M induced approximately 40% apoptosis ($p < 0.05$) (Fig. 1). Higher concentrations of Ang II (10^{-5} M) did not induced more cell apoptosis when compared with 10^{-6} M of Ang II ($p > 0.05$) (Fig. 1). Based on these findings, Ang II (10^{-6} M) concentration was chosen in the following experiments.

3.2. Bax and Bcl-2 are involved in Ang II induced apoptosis through AT2 receptor

Ang II exerts its biological effects via AT1 and AT2 receptors. To investigate which receptor is related to apoptosis induced by Ang II, we used antagonists of AT1 and AT2. Pretreatment of Caco-2 cells with the AT2 receptor antagonist PD123319 inhibited Ang II-induced apoptosis, as determined by flow cytometry. In contrast, no remarkable inhibition of Ang II-induced apoptosis was observed when cells were pretreated with the AT1 receptor antagonist losartan (Fig. 2A). These data suggested that Ang II-induced apoptosis in Caco-2 cell is mediated by the AT2 receptor.

Bax is a pro-apoptotic protein whereas Bcl-2 is an anti-apoptotic protein. The ratio between Bax and Bcl-2 expression has been shown as an important marker of apoptotic probability in intestinal epithelium cell. We next investigated the effect of PD123319 on the expression of these two apoptosis regulators. The mRNA level of Bax showed a significant increase in Ang II treated cells. When cells were pretreated with PD123319 before adding Ang II, the mRNA level of Bax was significantly reduced, by nearly 30%, compared to cells treated with Ang II alone ($p < 0.05$) (Fig. 2B). The expression of Bcl-2 also increased in Ang II treated cells, however, pretreatment with PD123319 only reduced Bcl-2 mRNA by 10% compared to cells treated with Ang II alone (Fig. 2B). Similar results were obtained for Bax and Bcl-2 protein expression by western bolt analysis (Fig. 2C). Thus, the increased Bcl-2: Bax ratio when cells were pretreated with PD123319 may contribute to the protection of these cells from Ang II-induced apoptosis observed in Fig. 2A. Together, these results indicated the involvement of the AT2 receptor and Bax in Ang II-induced apoptosis in Caco-2 cells.

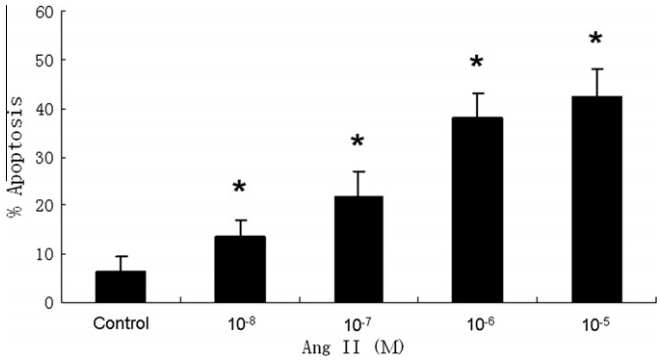

Fig. 1. Ang II-induced apoptosis in Caco-2 cells. Caco-2 cells were treated with the indicated concentrations of Ang II for 48 h before apoptosis detection using flow cytometry. Percentage of apoptotic cells increased in the presence of increasing doses of Ang II. Values are mean ± SD of triplicate samples. *Statistical significant difference versus control, $p < 0.05$.

3.3. Ang II up-regulated GATA-6 expression is an AT2 receptor-dependent manner

The signaling pathways activated by Ang II through its receptors have been demonstrated to be cell-type specific. Pharmacological inhibitors were used to identify signaling pathways originating from the AT2 receptor and leading to Bax up-regulation and apoptosis in Caco-2 cell. Since it was reported that Ang II induced apoptosis via GATA-6 in the Smooth Muscle Cell [21], we examined whether Ang II regulates GATA-6 expression in the Caco-2. Quantitative real-time PCR showed that GATA-6 mRNA level increased 50% when Caco-2 cell was exposed to Ang II. However, the up-regulation of GATA-6 mRNA reduced significantly when cells were pretreated with PD123319 ($p < 0.05$ relative to cells treated with Ang II alone), but not with losartan (Fig. 3A). GATA-6 protein level also increased in Ang II group, but again, the up-regulation reduced markedly in PD123319 group (Fig. 3B). Given the similar expression pattern of Bax and GATA-6, we next examine the role of GATA-6 in Ang II-induced apoptosis.

3.4. GATA-6 short hairpin RNA blocks Ang II inducible Bax expression and cell apoptosis

In order to correlate GATA-6 with Bax and Ang II inducible apoptosis, we used short hairpin RNA (shRNA) of GATA-6, which significantly reduced GATA-6 protein level in Caco-2 cell (Fig. 4A). Consequently, GATA-6 knockdown decreased Ang II-induced up-regulation of Bax protein, but not Bcl-2 protein (Fig. 4A). Importantly, GATA-6 knockdown also inhibited Ang II-induced apoptosis ($p < 0.05$ relative to cells transfected with a negative control shRNA) (Fig. 4B). Together, these results suggested that Ang II induces Bax expression and Caco-2 cell apoptosis via the AT2 receptor and GATA-6.

4. Discussion

In the present study, we found that Ang II induces significant apoptosis in human colonic epithelium Caco-2 cells. To determine which receptor relays the Ang II signal in intestinal epithelial cell apoptosis, we tested AT1 receptor antagonist, losartan and AT2 receptor antagonist, PD123319. We showed that apoptosis was abolished by pre-treatment with PD123319, but not losartan, suggesting a dominant role of AT2 in intestinal epithelial cell apoptosis. Others reported that AT2 also mediated Ang II-induced apoptosis in smooth muscle cells, HUVECs and PC12W cells [20–22]. However, a few other groups reported that Ang II could also induce apoptosis through the AT1 receptor [36,37]. Taken together, these studies provided evidence for the heterogeneous nature of Ang II-induced apoptosis in different tissues, cells, and different species.

Apoptosis is controlled partly by a complex interplay between pro-apoptotic and anti-apoptotic mediators, such as Bax and Bcl-2 [38], respectively. We used real-time PCR and western bolt analysis to detect Bax and Bcl-2 mRNA and protein expression in Ang II treated Caco-2 cells. Our finding of an increase in Bax and Bcl-2 level upon Ang II treatment is consistent with previous reports [2,39]. Interestingly, pretreatment of the cells with PD123319 resulted in a much more dramatic decrease in Bax induction by Ang II compared to Bcl-2 induction. This in turn resulted in a significant decrease in Bax: Bcl-2 ratio that inhibited the apoptosis of

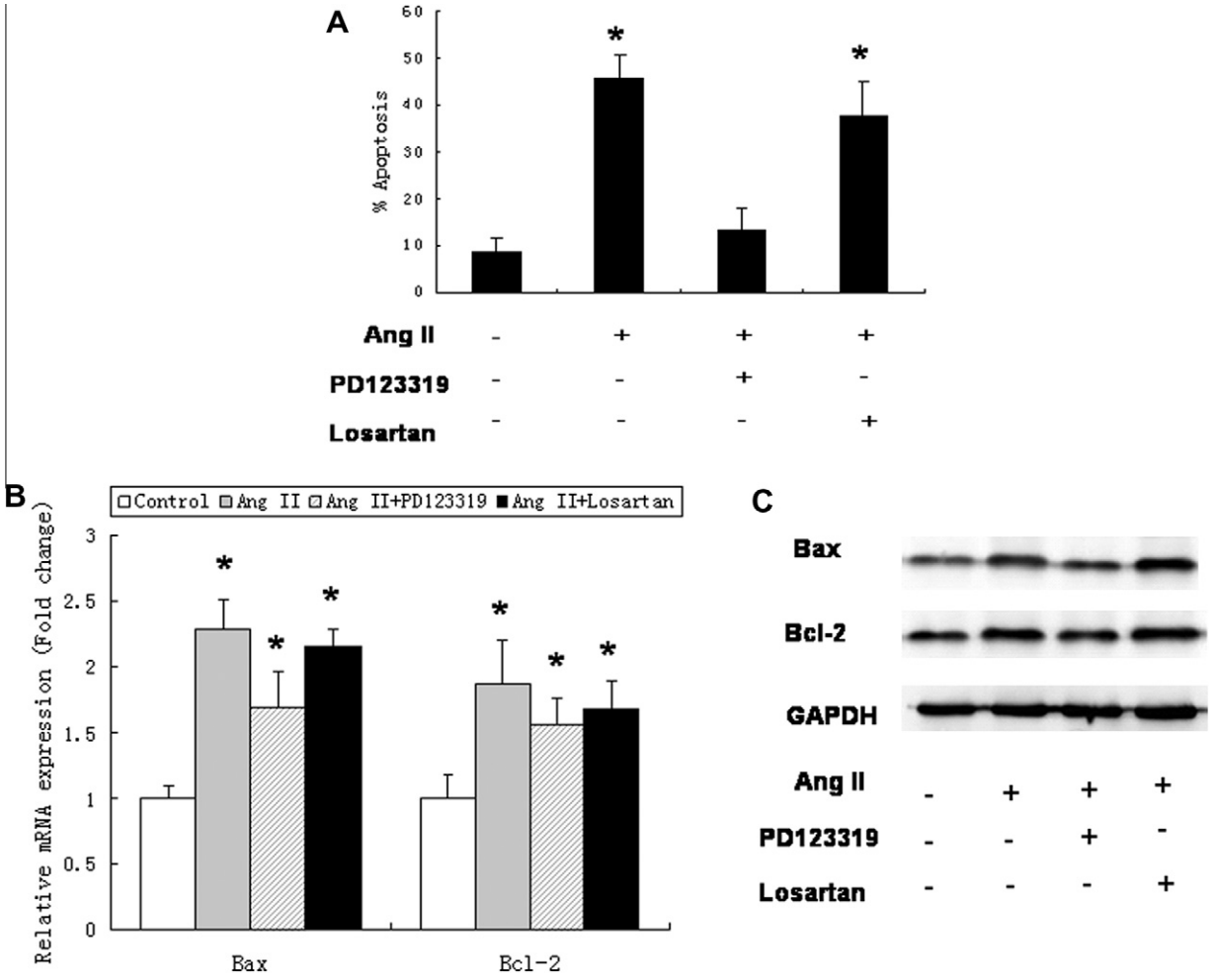

Fig. 2. Effects of PD123319 and losartan on Ang II-induced apoptosis. Caco-2 cells were treated with AT2 antagonist PD123319 (10^{-6} M) or AT1 antagonist losartan (10^{-6} M) for 1 h prior to the addition of Ang II (10^{-6} M). After 48 h, apoptosis was determined using flow cytometry. (A) Ang II-induced apoptosis was blocked by PD123319, but not losartan. (B and C) Ang II-induced expression of Bax and Bcl-2 in Caco-2 cells. mRNA expression by real-time PCR (B) and protein expression by western blot analysis (C) of both Bax and Bcl-2 were significantly induced by Ang II, and the induction was reduced when cells were pretreated with PD123319. However, the reduction of Bax was more than that of Bcl-2. β-Actin mRNA and GAPDH protein expression were used as control in (B) and (C), respectively. Values are mean ± SD of triplicate samples. *Statistically significant difference versus cells treated with Ang II alone (A) or control gene expression (B), $p < 0.05$.

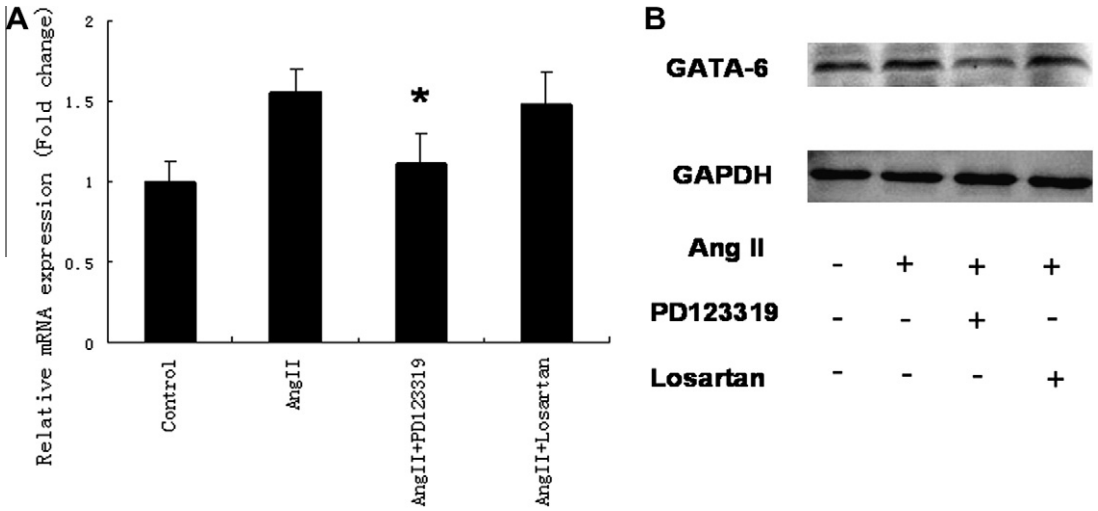

Fig. 3. Effects of PD123319 and losartan on Ang II-induced GATA-6 expression in Caco-2 cells. Cells were treated, GATA-6 mRNA (A) and protein (B) expression were measured as in Fig. 2. Ang II induced GATA-6 mRNA and protein expression, and the induction was blocked by pretreatment of cells with PD123319. Values are mean ± SD of triplicate samples. *Statistical significant difference versus cells treated with Ang II alone, $p < 0.05$.

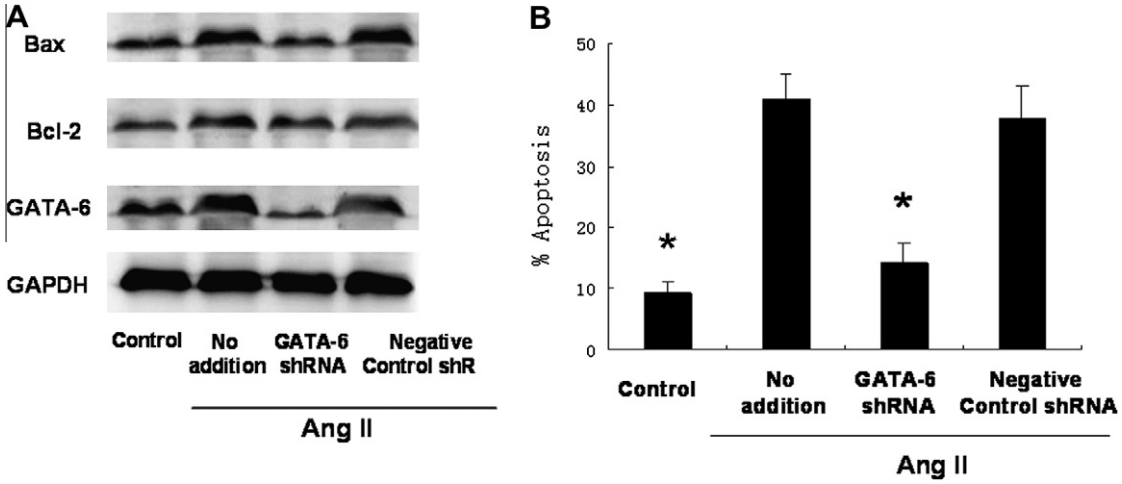

Fig. 4. GATA-6 mediates Ang II-induced Bax expression and Caco -2 cell apoptosis. Caco-2 cells were transfected with GATA-6 or control shRNA-expressing plasmid for 6 h, and then cultured in Ang II (10^{-6} M) for 48 h before harvested for western blot analysis (A) or apoptosis detection by flow cytometry (B). (A) As expected, GATA-6 shRNA significantly knocked down GATA-6 protein expression, which led to significant decrease in Ang II-induced expression of Bax, but not Bcl-2. (B) GATA-6 shRNA blocked Ang II-induced apoptosis. Values are mean ± SD of triplicate samples. *Statistically significant difference versus cells treated with Ang II alone, $p < 0.05$.

Ang II treated Caco-2 cells. This finding is supported by other studies that showed the Bax: Bcl-2 ratio as a valuable indicator for modulating apoptosis [38,40]. The results suggested that Ang II induced intestinal epithelial cell apoptosis is linked to the Bax/Bcl-2 intrinsic pathway.

Several lines of evidence demonstrated that GATA family members are involved in the regulation of apoptosis in multiple tissues [35,41–43]. GATA-1 regulates the apoptosis-related proteins Bcl-xl and Bcl-2 [41,42] whereas GATA-4 modulates Bcl-2 level in human ovary cells [43]. In human fetal testis, GATA-6 is linked to the expression level of Bax and Bcl-2 [35]. The regulation of apoptosis-related proteins by GATA factors suggests a role of these factors in apoptosis. We focused on GATA-6 in this study because our preliminary results showed that GATA-6 is highly expressed in Caco-2 cells whereas GATA-4 is not (data not shown).

Our data show that GATA-6 protein level is increased in Ang II-induced Caco-2 cells, and this increase is abolished when cells were pretreated with PD123319. It suggests that Ang II-induced GATA-6 up-regulation is dependent on AT2 receptor. This finding is consistent with previous studies [21]. Furthermore, the expression of GATA-6 and Bax are regulated similarly by Ang II and PD123319, indicating that GATA-6 might stimulate Bax expression and thus mediate Ang II-induced apoptosis in Caco-2 cells. To test this hypothesis, we used shRNA to knock down GATA-6 expression. As expected, GATA-6 shRNA significantly inhibited Bax induction, but not Bcl-2, by Ang II and thus prevented apoptosis. These results emphasize the key role of the Bax pathway in Ang II-induced apoptosis in intestinal epithelial cells. Interestingly, an earlier study showed that GATA-6 mediates Ang II-induced apoptosis through Fasl in smooth muscle cells [21], suggesting that different pro-apoptotic proteins facilitate Ang II-induced apoptosis in different cell types. More complicated, GATA-6 has also been reported to prevent apoptosis in Bovine Corpus Luteum [44]. The opposite effects of GATA-6 suggest that GATA-6 can acts as a transcriptional activator or repressor of pro-apoptotic proteins in different cell types, depending on the context of the regulatory regions and other regulatory factors/co-factors of the target genes [35]. In light of these findings, our future studies will focus on how GATA-6 controls the expression of Bax at transcription level in intestinal epithelial cells.

In conclusion, our results suggest the following model. In intestinal epithelial cells, Ang II signals through the AT2 receptor to up-regulate GATA-6 expression, which in turn up-regulates the expression of Bax, eventually leading to apoptosis in these cells. These results provide insight into the molecular mechanism of intestinal adaptation after the development of short bowel syndrome (SBS). Future studies will identify more components along this pathway and lead to a more complete understanding of this important adaptive process.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (NSFC 30973113 to H.Y., NSFC 81020108023 to H.Y.); and Chongqing Science & Technology Commission International Key Collaboration Project (CSTC2008BA5006 to H.Y.).

References

[1] K.A. Tappenden, Mechanisms of enteral nutrient-enhanced intestinal adaptation, Gastroenterology 130 (2 Suppl 1) (2006) S93–S99.
[2] H. Koga, H. Yang, E.Q. Haxhija, et al., The role of angiotensin II type 1a receptor on intestinal epithelial cells following small bowel resection in a mouse model, Pediatr. Surg. Int. 24 (12) (2008) 1279–1286.
[3] E.Q. Haxhija, H. Yang, A.U. Spencer, et al., Influence of the site of small bowel resection on intestinal epithelial cell apoptosis, Pediatr. Surg. Int. 22 (1) (2006) 37–42.
[4] C.S. Potten, C. Booth, D.M. Pritchard, The intestinal epithelial stem cell: the mucosal governor, Int. J. Exp. Pathol. 78 (4) (1997) 219–243.
[5] M.A. Helmrath, C.R. Erwin, C.E. Shin, et al., Enterocyte apoptosis is increased following small bowel resection, J. Gastrointest. Surg. 2 (1) (1998) 44–49.
[6] H. Yang, P.A. Antony, B.E. Wildhaber, et al., Intestinal intraepithelial lymphocyte gamma delta-T cell-derived keratinocyte growth factor modulates epithelial growth in the mouse, J. Immunol. 172 (7) (2004) 4151–4158.
[7] M. Koopmann, D. Nelson, S. Murali, et al., Exogenous glucagon-like peptide-2 (GLP-2) augments GLP-2 receptor mRNA and maintains proglucagon mRNA levels in resected rats, JPEN J. Parenter. Enteral. Nutr. 32 (3) (2008). 254–26.
[8] M.A. Helmrath, C.E. Shin, C.R. Erwin, et al., The EGF/EGF-receptor axis modulates enterocyte apoptosis during intestinal adaptation, J. Surg. Res. 77 (1) (1998) 17–22.
[9] I. Sukhotnik, E. Yakirevich, A.G. Coran, et al., Effect of transforming growth factor-alpha on intestinal adaptation in a rat model of short bowel syndrome, J. Surg. Res. 108 (2) (2002) 235–242.
[10] B.E. Wildhaber, H. Yang, E.Q. Haxhija, et al., Intestinal intraepithelial lymphocyte derived angiotensin converting enzyme modulates epithelial cell apoptosis, Apoptosis 10 (6) (2005) 1305–1315.
[11] C. Röcken, F.W. Röhl, E. Diebler, et al., The angiotensin II/angiotensin II receptor system correlates with nodal spread in intestinal type gastric cancer, Cancer Epidemiol Biomarkers Prev. 16 (6) (2007) 1206–1212.
[12] U.V. Shenoy, E.M. Richards, X.-C. Huang, et al., Angiotensin II type 2 receptor–mediated apoptosis of cultured neurons from newborn rat brain, Endocrinology 140 (1999) 500–509.

[13] M. Tunçdemir, O. Demirkesen, M. Oztürk, et al., Antiapoptotic effect of angiotensin-II type-1 receptor blockade in renal tubular cells of hyperoxaluric rats, Urol. Res. 38 (2) (2010) 71–80.
[14] Y.H. Lee, O. Mungunsukh, R.L. Tutino, et al., Angiotensin-II-induced apoptosis requires regulation of nucleolin and Bcl-xL by SHP-2 in primary lung endothelial cells, J. Cell Sci. 123 (Pt 10) (2010) 1634–1643.
[15] J. Suzuki, M. Iwai, H. Nakagami, et al., Role of angiotensin II – regulated apoptosis through distinct AT1 and AT2 receptors in neointimal formation, Circulation 106 (7) (2002) 847–853.
[16] L. Guo, M. Wang, Z.Y. Zhang, et al., Angiotensin II induces interleukin-6 synthesis in osteoblasts through ERK1/2 pathway via AT1 receptor, Arch. Oral Biol. 56 (3) (2011) 205–211.
[17] J. Xu, X.W. Lü, Y. Huang, et al., Synergism of simvastatin with losartan prevents angiotensin II-induced cardiomyocyte apoptosis in vitro, J. Pharm. Pharmacol. 61 (4) (2009) 503–510.
[18] D. Li, B. Yang, M.I. Philips, et al., Proapoptotic effects of ANG II in human coronary artery endothelial cells: role of AT1 receptor and PKC activation, Am. J. Physiol. 276 (1999) H786–H792.
[19] X. Feng, L. Wang, Y. Li, Change of telomere length in angiotensin II-induced human glomerular mesangial cell senescence and the protective role of losartan, Mol. Med. Rep. 4 (2) (2011) 255–260.
[20] Y.F. Bian, H.Y. Yang, Z.M. Yang, et al., Amlodipine treatment prevents angiotensin II-induced human umbilical vein endothelial cell apoptosis, Arch. Med. Res. 42 (1) (2011) 22–27.
[21] N.Y. Tan, J.M. Li, R. Stocker, et al., Angiotensin II – inducible smooth muscle cell apoptosis involves the angiotensin II type 2 receptor, GATA-6, Circ. Res. 105 (5) (2009) 422–430.
[22] T. Yamada, M. Horiuchi, V.J. Dzau, Angiotensin II type 2 receptor mediates programmed cell death, Proc. Natl. Acad. Sci. USA 93 (1) (1996) 156–160.
[23] J.J. Tremblay, R.S. Viger, GATA factors differentially activate multiple gonadal promoters through conserved GATA regulatory elements, Endocrinology 142 (3) (2001) 977–986.
[24] J.B. Burch, Regulation of GATA gene expression during vertebrate development, Semin. Cell Dev. Biol. 16 (2005) 71–81.
[25] H. Haveri, M. Westerholm-Ormio, K. Lindfors, et al., Transcription factors GATA-4 and GATA-6 in normal and neoplastic human gastrointestinal mucosa, BMC Gastroenterol. 8 (2008) 9.
[26] S.H. Orkin, GATA-binding transcription factors in hematopoietic cells, Blood 80 (1992) 575–581.
[27] T. Evans, Regulation of cardiac gene expression by GATA-4/5/6, Trends Cardiovasc. Med. 7 (1997) 75–83.
[28] N.S. Belaguli, M. Aftab, M. Rigi, et al., GATA6 promotes colon cancer cell invasion by regulating urokinase plasminogen activator gene expression, Neoplasia 12 (11) (2010) 856–865.
[29] R. Vähätalo, T.M. Asikainen, R. Karikoski, et al., Expression of transcription factor GATA-6 in alveolar epithelial cells is linked to neonatal lung disease, Neonatology 99 (231) (2011) 231–240.
[30] K.Q. Cai, C. Caslini, C.D. Capo-chichi, et al., Loss of GATA4 and GATA6 expression specifies ovarian cancer histological subtypes and precedes neoplastic transformation of ovarian surface epithelia, PLoS One 4 (7) (2009) e6454.
[31] M. Maitra, M.K. Schluterman, H.A. Nichols, et al., Interaction of Gata4 and Gata6 with Tbx5 is critical for normal cardiac development, Dev. Biol. 326 (2009) 368–377.
[32] X. Gao, T. Sedgwick, Y.B. Shi, et al., Distinct functions are implicated for the GATA-4, -5, and -6 transcription factors in the regulation of intestine epithelial cell differentiation, Mol. Cell. Biol. 18 (1998) 2901–2911.
[33] I. Shureiqi, W. Jiang, S.M. Fischer, et al., GATA-6 transcriptional regulation of 15-lipoxygenase-1 during NSAID-induced apoptosis in colorectal cancer cells, Cancer Res. 62 (4) (2002) 1178–1183.
[34] F. Boudreau, E.H. Rings, H.M. van Wering, et al., Hepatocyte nuclear factor-1α, GATA-4, and caudal related homeodomain protein Cdx2 interact functionally to modulate intestinal gene transcription. Implication for the developmental regulation of the sucrase–isomaltase gene, J. Biol. Chem. 277 (2002) 31909–31917.
[35] I. Ketola, J. Toppari, T. Vaskivuo, et al., Transcription factor GATA-6, cell proliferation, apoptosis, and apoptosis-related proteins Bcl-2 and Bax in human fetal testis, J. Clin. Endocrinol. Metab. 88 (4) (2003) 1858–1865.
[36] E. Cigola, J. Kajstura, B. Li, et al., Angiotensin II activates programmed cell death in vitro, Exp. Cell Res. 231 (1997) 363–371.
[37] S. Dimmeler, V. Rippmann, U. Weiland, et al., Angiotensin II induces apoptosis of human endothelial cells: protective effect of nitric oxide, Circ. Res. 81 (1997) 970–976.
[38] J. Pawlowski, A.S. Kraft, Bax-induced apoptotic cell death, Proc. Natl. Acad. Sci. USA 97 (2) (2000) 529–531.
[39] L.E. Stern, F. Huang, C.J. Kemp, et al., Bax is required for increased enterocyte apoptosis after massive small bowel resection, Surgery 128 (2) (2000) 165–170.
[40] Y. Tang, D.A. Swartz-Basile, E.A. Swietlicki, et al., Bax is required for resection-induced changes in apoptosis, proliferation, and members of the extrinsic cell death pathways, Gastroenterology 126 (1) (2004) 220–230.
[41] T. Gregory, C. Yu, A. Ma, et al., GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating Bcl-xL expression, Blood 94 (1999) 87–96.
[42] H. Tanaka, I. Matsumura, K. Nakajima, et al., GATA-1 blocks IL-6-induced macrophage differentiation and apoptosis through the sustained expression of cyclin D1 and Bcl-2 in a murine myeloid cell line M1, Blood 95 (2000) 1264–1273.
[43] T.E. Vaskivuo, M. Anttonen, R. Herva, et al., Survival of human ovarian follicles from fetal to adult life: apoptosis, apoptosis-related proteins, and transcription factor GATA-4, J. Clin. Endocrinol. Metab. 86 (2001) 3421–3429.
[44] H. Taniguchi, J. Komiyama, R.S. Viger, et al., The expression of the nuclear receptors NR5A1 and NR5A2 and transcription factor GATA6 correlates with steroidogenic gene expression in the Bovine Corpus Luteum, Mol. Reprod. Dev. 76 (9) (2009) 873–880.