Biochemical and Biophysical Research Communications 430 (2013) 804–809

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

FoxP3 inhibits proliferation and induces apoptosis of gastric cancer cells by activating the apoptotic signaling pathway

Gui-Fen Ma [a], Shi-Yao Chen [a,b,*], Zhi-Rong Sun [c], Qing Miao [a], Yi-Mei Liu [a], Xiao-Qing Zeng [a], Tian-Cheng Luo [a], Li-Li Ma [b], Jing-Jing Lian [b], Dong-Li Song [d]

[a] Department of Gastroenterology, Zhongshan Hospital, Fudan University, Shanghai, China
[b] Endoscopy Center, Zhongshan Hospital, Fudan University, Shanghai, China
[c] Department of Anesthesiology, Cancer Center, Fudan University, Shanghai, China
[d] Biomedical Research Center, Zhongshan Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Article history:
Received 13 November 2012
Available online 29 November 2012

Keywords:
FoxP3
Gastric cancer
Cell proliferation
Cell apoptosis
Apoptotic pathway

ABSTRACT

Forkhead Box Protein 3 (FoxP3) was identified as a key transcription factor to the occurring and function of the regulatory T cells (Tregs). However, limited evidence indicated its function in tumor cells. To elucidate the precise roles and underlying molecular mechanism of FoxP3 in gastric cancer (GC), we examined the expression of FoxP3 and the consequences of interfering with FoxP3 gene in human GC cell lines, AGS and MKN45, by multiple cellular and molecular approaches, such as immunofluorescence, gene transfection, CCK-8 assay, clone formation assay, TUNEL assay, Flow cytometry, immunoassay and quantities polymerase chain reaction (PCR). As a result, FoxP3 was expressed both in nucleus and cytoplasm of GC cells. Up-regulation of FoxP3 inhibited cell proliferation and promoted cell apoptosis. Overexpression of FoxP3 increased the protein and mRNA levels of proapoptotic molecules, such as poly ADP-ribose polymerase1 (PARP), caspase-3 and caspase-9, and repressed the expression of antiapoptotic molecules, such as cellular inhibitor of apoptosis-1 (c-IAP1) and the long isoform of B cell leukemia/lymphoma-2 (Bcl-2). Furthermore, silencing of FoxP3 by siRNA in GC cells reduced the expression of proapoptotic genes, such as PARP, caspase-3 and caspase-9. Collectively, our findings identify the novel roles of FoxP3 in inhibiting proliferation and inducing apoptosis in GC cells by regulating apoptotic signaling, which could be a promising therapeutic approach for gastric cancer.

1. Introduction

FoxP3 was used to be defined as a transcription factor and be necessary and sufficient for induction of the immunosuppressive functions in regulatory T cells(Tregs) [1]. Its expression thus was expected in Treg and lymph nodes, and the prognosis prediction of Treg had been well documented [2–5]. Only recently has it been demonstrated that FoxP3 had also been expressed in many kinds of tumor cells [6,7] and associated with tumor progression and prognosis [8–10]. FoxP3 gene resided at Xp11.23 and was identified as a tumor suppressor gene for both prostate cancer and breast cancer [11,12]. FoxP3 expression in pancreatic carcinoma cells was thought to be an important mechanism of tumor escape [13]. Moreover, FoxP3 was an important regulator of the HER-2/ErbB2 oncogene [11], which was a biomarker for the clinic assessment of the targeted treatment by transtuzumab in breast cancer as well as in gastric cancer [14,15]. Therefore, depth study of the role and underlying mechanism of FoxP3 in GC cells is of great significance.

Previous study showed FoxP3 expression was reduced in the tissues of prostate cancer and breast cancer through somatic single hits to inactivate FoxP3 [16,17]; however, it was increased in most of other tumors, such as non-small cell lung cancer [8], tongue squamous cell carcinoma [18], liver cancer [9] and gastric cancer [19]. The function of this differential expression is not yet defined. Currently, limited evidence was presented in the FoxP3-mediated or FoxP3-regulating signaling pathway, and it was only demonstrated that FoxP3 inhibited tumor cell growth by repressing oncogenes (HER2 [11], c-Myc [20], Skp2 [12]) and increasing tumor suppressor gene p21 [21] in breast and prostate cancer. However, the precise function of FoxP3 gene in the process of apoptosis is poorly understood.

In the current study, we investigated the roles of FoxP3 in GC cell growth and apoptosis and explored its gene function in mediating apoptotic signaling pathway by the approaches of up-regulation and silencing of FoxP3 expression. Our results will show a newly functional relay of FoxP3 in apoptotic pathway and indicate that interference of FoxP3 could be a promising therapeutic approach for gastric cancer.

* Corresponding author at: Department of Gastroenterology, Zhongshan Hospital, Fudan University, No. 180, Fenglin Road, 200032 Shanghai, China. Fax: +86 21 64432583.
E-mail address: shiyao_chen@163.com (S.-Y. Chen).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.065

2. Materials and methods

2.1. Cells and cell culture

Human GC cell line AGS was purchased from Cell Bank of the Chinese academy of Sciences (Shanghai, China). Another human GC cell line MKN45 was obtained from Shanghai Key Laboratory of Gastric Neoplasm as a gift [22]. All cells were cultured in DMEM medium with 10% fetal bovine serum and 1% penicillin and streptomycin in 5% CO_2-humidified atmosphere at 37 °C.

2.2. Immunofluorescence assay

GC cell lines were fixed with 4% paraformaldehyde and then permeabilized with solution containing 0.1% Triton X-100, 0.1 mg/ml RNase A in phosphate-buffered saline (PBS). They were subsequently immunostained by incubating with primary antibodies against FoxP3 (1:250 dilution; Abcam, UK) at 4 °C for overnight. After incubated by Texas red-conjugated secondary antibodies (Molecular Probes, Eugene, OR) and DAPI at room temperature, the slides were then photographed using a fluorescence microscope (Olympus, Japan).

2.3. Selecting a population of cells that stably express FoxP3

The pEGFP-FoxP3 shRNA plasmid containing FoxP3 full-length cDNA was purchased from Genchem company (Shanghai, China), which confirmed by DNA sequencing technique. AGS and MKN45 cell lines were transfected with pEGFP-FoxP3 and pEGFP-vector plasmids, respectively, using Lipofectamine-2000 (Invitrogen, Carlsbad, CA, USA) for 24 h. After then, they were cultured in the medium containing 500 μg/mL G418 (Sigma, St. Louis, MO, USA) for antibiotic selection until all of the non-transfected cells were killed. Subsequently, the antibiotic-resistant cells were seeded in 96-well plates and then chosen for 8 monoclones with green fluorescent protein (GFP). Real-time PCR and Immunoblot techniques were used to select the clones with the highest stable FoxP3 expression. The selected clones were widely reproduced for further use, which were named as AGS/FoxP3 and MKN45/FoxP3, and the empty vector-transfected cell were named as AGS/vector and MKN45/vector, respectively.

2.4. Cell proliferation assay

Cell proliferation was detected by a Cell Counting Kit-8 (CCK-8) assay (Beyotime, Jiangsu, China). Cells were suspended in a complete DMEM medium and subsequently seeded in 96-well plates and incubated for 1 d, 2 d, 3 d and 4 d, respectively. The cultures were added CCK-8 solution and incubated at 37 °C for another 4 h. Absorbance at 450 nm was measured using an immunoreader. The results were plotted as means ± SD of two independent experiments having three determinations per sample for each experiment.

2.5. Clone formation assay

Cells were seeded at 3×10^3 cells/well in 6-well plates and left to form clones in 2–3 weeks. Cultures were stained with 0.1% crystal violet and the number of clones were scored on a 2×2 cm grid to determine the clone-forming ability of the cells. Clones containing over 50 cells were counted. Clone-forming efficiency was calculated as clone counts in 1000 cells. This experiment was conducted twice.

2.6. Apoptosis assay

AGS/FoxP3 and AGS/vector cells were seeded in 6-well plates (4×10^5/well). Flow cytometry was used to detect cell apoptosis. Unstained cells, cells stained with AnnexinV alone, cells stained with propidium iodide (PI) alone were used as controls. The early apoptosis was indicated by the relative amount of AnnexinV-positive–PI-negative cells. Singly stained cells were used to adjust electronic compensation on FL1 and FL2 channels. This experiment was conducted three times, each sample was assessed in triplicate, and data were averaged.

2.7. Terminal transferase dUTP nick end labeling (TUNEL) assay

TUNEL assay was applied to detect DNA degradation of nuclear chromatin *in situ* in apoptotic cells according to the manufacturer's instructions (Roche, Switzerland). Briefly, apoptotic cells were identify by labeling the DNA breaks with fluorescent-tagged deoxyuridine triphosphate nucleotides (F-dUTP). The enzyme terminal deoxynucleotidyl transferase catalyzes a template-independent addition of deoxyribonucleoside triphosphates to the 3′-hydroxyl ends of double-or single-stranded DNA strands with exposed 3′-hydroxyl ends. Finally, substrate solution DAB was added to present the positive particles and then viewed by an optical microscope. The experiment was repeated twice. During the process, positive and negative controls were included in each experiment. The number of apoptotic cells was counted under a microscope (100×) and expressed as the apoptotic index (AI) (AI = the number of apoptotic bodies/1000 cells). This experiment was conducted three times, each group was assessed in triplicate, and data was averaged.

2.8. Design and transfection of FoxP3 small interfering RNA (siRNA)

Specific siRNA oligo for FoxP3 was made from Genephama (Shanghai, China). Two human FoxP3-specific sequences were used, siRNA1 (5′-GUCUGCACAAGUGCUUUGUTT-3′, 3′-ACAAAGCACUUGUGCAGACTT) and siRNA2 (5′-CUGCCUCAGUACACUCAAATT-3′, 3′-UUUGAGUGUACUG AGGCAGTT), according to nucleotides 1355–1375 and 1896–1916, respectively (Genbank accession number BC113401). AGS cells were seeded at 60% density in 6-well plates. After 24 h, transfection was done using Lipofectamine-2000 (Invitrogen). After transfection of 48 h, the transfected cells were collected for extracting total RNA. This part of experiment was also confirmed by 293ET cells. Each experiment was conducted three times, each sample point was assessed in triplicate, and data were averaged.

2.9. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the cultured cells, using Trizol reagent (Invitrogen). cDNA was prepared using oligo^{dT} primers according to the protocol supplied with the Primer Script™ RT Reagent (TaKaRa, Tokyo, Japan). Expression levels of PARP 1, caspase-3 and caspase-9 mRNAs were detected by SYBR® Green II qRT-PCR using Mastercycler ep realplex (Eppendorf, Hamburg,Germany). Aliquots of the PCR products were analyzed by melting curves. All the mRNA levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels. All qRT-PCR experiments were performed by the same investigator with no knowledge of the corresponding grouped data.

2.10. Western blotting

All transfected cells were plated in 6-well plates for 48 h and then harvested. The total protein were extracted and then separated on 10% SDS-PAGE gels and transferred to PVDF membranes

(Millipore, Bedford, MA). After blocked with 5% non-fat dry milk in TBS, the membrane was probed with primary monoclonal antibody specific to PARP, caspase-3, caspase-9, Bcl-2, c-IAP (all 1:1000, Cell signal technology, USA) or GAPDH (1:2000, Beyotime) as an internal control for protein. The membrane was subsequently probed with horseradish peroxidase (HRP)-conjugated secondary antibody (1:4000, Santa Cruz, USA) and the protein bands were visualized by enhanced chemiluminescence (Millipore). The relative intensity was determined using Quantity One Image software (Bio-Rad, USA). Each experiment was conducted three times, and data were averaged.

2.11. Statistical analysis

Quantitative results were expressed as mean ± SD. Statistical analysis was assessed by Student-*t* test (between two groups) or Student–Newman–Keuls test (among three or more groups), with SPSS 16.0 software (SPSS, Chicago, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cellular localization of FoxP3 protein

FoxP3 protein mainly expressed in the nucleus of AGS cells using immunofluorescence technique, and also expressed in the perinuclear cytoplasm (Fig. 1), where is the area of endoplasmic reticulum. This special localization might correlate to the process and sythsis of FoxP3 protein. FoxP3 as a transcript factor was used to be expressed only in the nucleus of lymphocytes and tumor cells [8,23]. Our finding presented the novel distribution of FoxP3 protein, which might contribute to its role and function in GC cells.

3.2. Up-regulation of FoxP3 inhibits cell proliferation

To explore FoxP3 gene's function, we firstly transfected FoxP3-shRNA plasmid and the empty vector into AGS and MKN45 GC cell lines. After drug-selecting and monoclone-chosen, the stable FoxP3-overexpressed cells were distinguished out and their protein levels were confirmed by western blotting, named as AGS/FoxP3 and MKN45/FoxP3 cells. The transfection efficiency of FoxP3 is approaching 100% confirmed by Flow cytometry (Supplementary Fig. S1). Our results showed that up-regulation of FoxP3 elicited a significant inhibit effect on the proliferation of the FoxP3-transfected cells compared with vector-transfect cells at 96 h by CCK-8 assay ($P < 0.05$) (Fig. 2A). To further evaluate the effect of FoxP3 on cell growth, we performed clone formation experiment *in vitro*, and found that the FoxP3-transfected cells grew slowly and formed a few small clones and the empty vector-transfected cells grew quickly and formed many large clones on soft agar after 2 weeks (clone-forming efficiency: 76 ± 10‰ vs. 195 ± 22‰, $P < 0.01$; Fig. 2B).

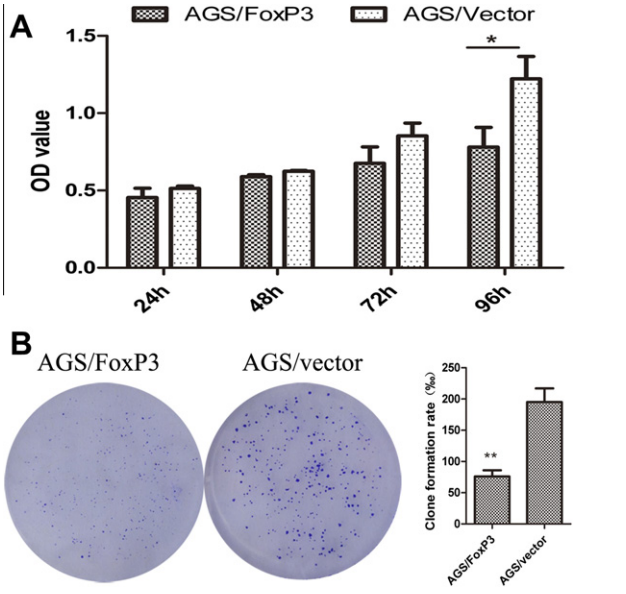

Fig. 2. Up-regulated FoxP3 inhibits the cell growth. (A) The cell proliferation of AGS/FoxP3 was significantly inhibited compared with AGS/vector cells at 96 h by CCK-8 assay. (B) AGS/FoxP3 and AGS/vector cells were cultured on soft agar in 6-well plates. After 2 weeks, the formed cell clones in AGS/FoxP3 cells significantly fewer and smaller compared with the AGS/vector cells by soft agar clone formation experiment *in vitro* (clone-forming efficiency: 76 ± 10‰ vs. 195 ± 22‰, $P < 0.01$). All data are shown as means ± SD of triplicates. Each experiment was conducted twice. $^{*}P < 0.05$; $^{**}P < 0.01$.

3.3. Up-regulation of FoxP3 promotes cell apoptosis and regulates mitochondria-associated proteins

To further understand the mechanism to cell proliferation inhibition, we explored whether it was due to the apoptosis-induced function. The result showed that up-regulation of FoxP3 elicited a significant accumulation of early apoptotic cells compared with the control (8.53 ± 2.51% vs. 3.12 ± 1.43%, $P < 0.01$) by Flow cytometry (Fig. 3A). To further confirm the role of FoxP3 in apoptosis, TUNEL assay was applied to detect cell death *in situ*. The result demonstrated that the apoptosis index (AI) in FoxP3-upregulated cells was significantly increased compared with that in the control (75 ± 10‰ vs. 10 ± 2‰, $P < 0.01$) (Fig. 3B), indicating that the late stage apoptosis through the fragmentation of nuclear chromatin was also increased.

To understand the mechanism by which FoxP3 induces apoptosis, some proteins involved in apoptosis were examined. Immunoblot analysis revealed that up-regulation of FoxP3 significantly increased proapoptotic proteins (PARP, caspase-3 and caspase-9) and significantly decreased antiapoptotic proteins (c-IAP1 and Bcl-2) (Fig. 4A). PARP (116 kDa) is a key molecular involved in DNA repair [24], through separating the PARP amino-terminal

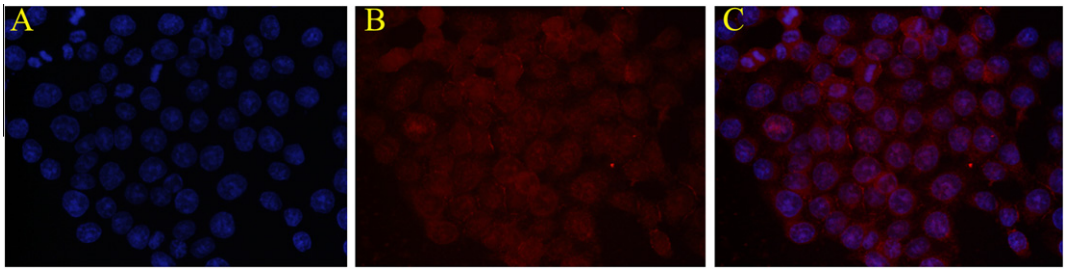

Fig. 1. FoxP3 cellular localization. (A) DAPI stains the nucleus. (B) FoxP3 protein was red staining. (C) Merged image demonstrated that FoxP3 protein in AGS cell mainly expressed in nucleus and in the cytoplasm (photographed at 200×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

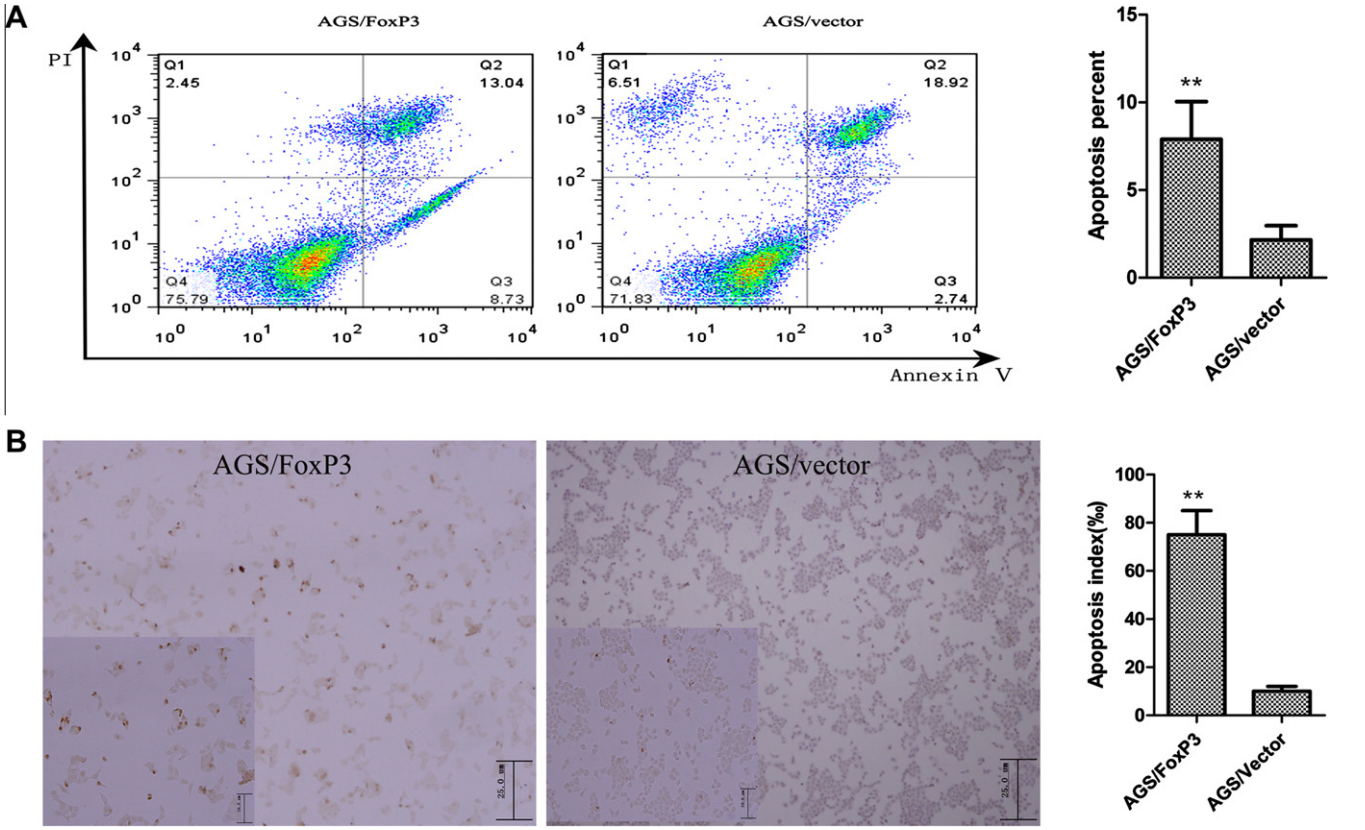

Fig. 3. Up-regulated FoxP3 induces cell apoptosis. (A) Detected by Flow cytometry, the Annexin v-positive-PI-negative cells was compared. The apoptotic percentage was increased in the AGS/FoxP3 cells compared with the AGS/vector cells (8.53 ± 2.51% vs. 3.12 ± 1.43%, $P < 0.05$). (B) The apoptotic cells were stained in brown in nucleus by Terminal Transferase dUTP Nick End Labeling (TUNEL) assay. Apoptosis index (AI) was calculated by counting apoptotic cell counts per 1000 cells at 10 random high power fields. Analysis showed AI was significantly increased in the AGS/FoxP3 cells compared with the AGS/vector cells (AI: 75 ± 10‰ vs. 10 ± 2‰, $P < 0.01$). Results are shown as means ± SD, and were measured three times. PI, propidium iodide; $^{**}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) to induce apoptosis [25]. Caspase-3 is the upstream molecular of PARP and mediated by caspase-9, all of them take parts in the process of the mitochondria-mediated apoptosis. Bcl-2 and c-IAP1 can block the process of apoptosis [26]. Our findings suggest that FoxP3-inducing apoptosis might correlate to the releasing of a series of apoptosis-associated proteins.

3.4. FoxP3 gene is sufficient and necessary to caspase genes

We next determined whether FoxP3 altered caspase-associated genes. Our results showed that PARP and caspase-3 were almost 3-fold increased in the FoxP3-overexpressed cells compared with the control. There was no significant difference in the caspase-9 levels between the two groups (Fig. 4B). To further test whether FoxP3 is sufficient and necessary to increase caspase genes, we used specific siRNA to silence FoxP3 expression and found that down-regulation of FoxP3 significantly decreased PARP, caspase-3 and caspase-9 at 48 h in the FoxP3-siRNA cells compared with the scrambled control (Sc) (Fig. 4C). This section of experiment was also confirmed by 293ET cells (date not shown). We therefore conclude that FoxP3 is critical for inducing caspase-associated apoptosis.

4. Discussion

Resistance to death signal of tumor cell is the most important obstacles in treatment of many cancers [26]. It will be helpful for searching the molecular targets to find out some promising therapeutic approaches. In this study, we had showed the novel roles of FoxP3 in inhibiting proliferation and promoting apoptosis. We also firstly revealed that FoxP3 was sufficient and necessary for activating the apoptotic signaling pathway and silencing of FoxP3 in GC cells decreased proapoptotic genes expression, suggesting that endogenous FoxP3 might act as a positive modulator in apoptotic pathway. However, how FoxP3 interacted with these genes and whether it is acted by a direct or indirect way need further investigation.

Cellular localization of FoxP3 characterizes with various sources of tumor cells. Immunohistochemical staining showed cytoplasmic expression of FoxP3 predominantly in cell lines of melanoma, colon, and breast cancer; however, both cytoplasmic and nuclear expression in cell lines of lung cancer, T lymphoblastic leukemia and hepatocellular carcinoma [7,9,27]. Meanwhile, the locations of FoxP3 in various tissues were also of great difference. For example, FoxP3 protein was stained in the nucleus of non-small lung cancer tissues [8,23], and in the cytoplasm of pancreatic cancers tissues [13] and HER2-positive breast cancer samples [16]. Whereas we indicated that FoxP3 expressed both in cytoplasm and in nucleus of GC cell lines, using the recommended FoxP3 monoclone antibody 236A/E7 which was confirmed to be a highest specificity [28]. The sources and functions of the protein expression difference were not yet to be defined. Someone believed it was due that alternative splicing forms disrupted the localization of FoxP3 [7]. Others considered it was due to the specificity and sensitivity of different anti-FoxP3 monoclonal antibodies [28]. This variation might also imply a different role of FoxP3 in diverse tumor cells, which needs further investigation.

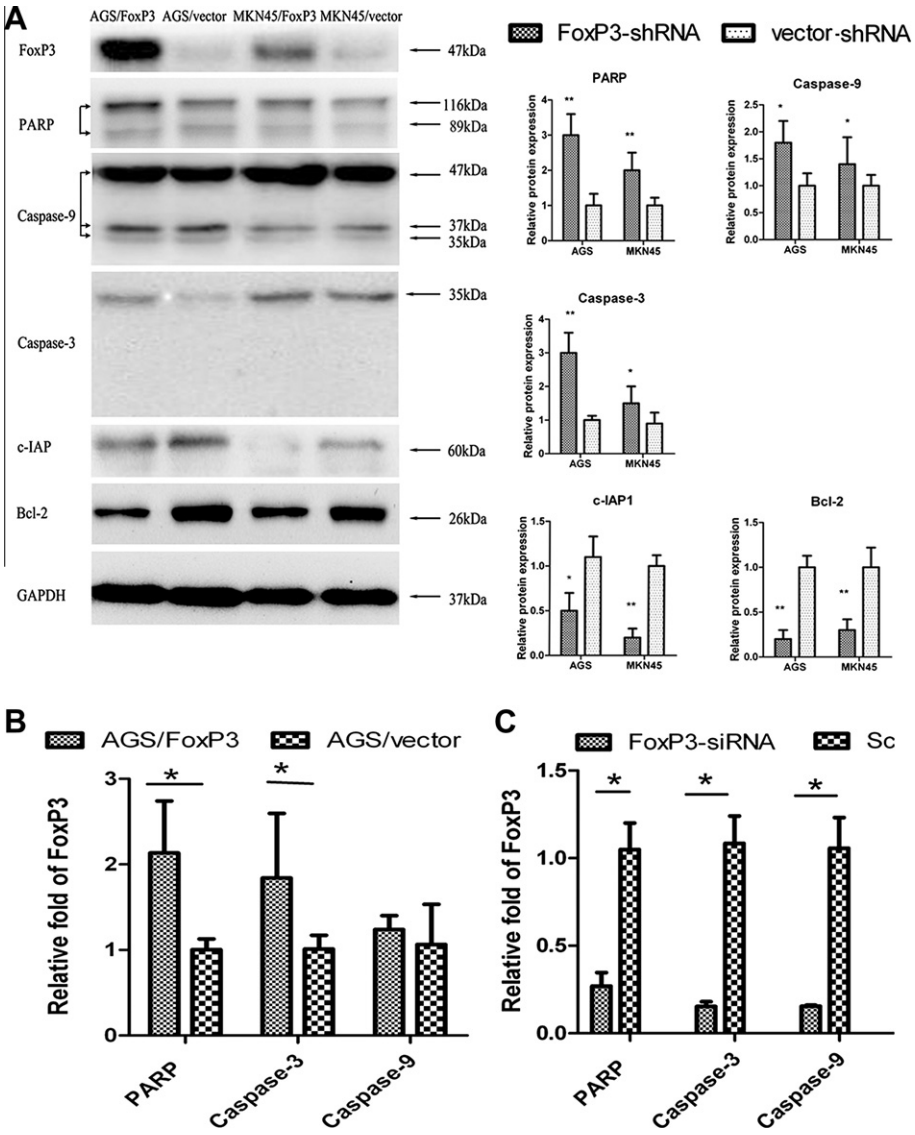

Fig. 4. FoxP3 induces the expression of proapoptotic genes and reduces antiapoptotic proteins. (A) FoxP3- or vector-shRNA transfected cells were cultured for 48 h. Cell lysates were prepared and Western blot was done. The relative intensity was determined using Quantity One Image software. Up-regulation of FoxP3 expression significantly increased proapoptotic proteins, such as PARP, caspase-3 and caspase-9, as well as significantly decreased antiapoptotic proteins, such as c-IAP1 and the Bcl-2. The relative intensity was determined using Quantity One Image software. Protein expression was normalized to GAPDH, and levels in the vector-transfected cells were defined as 1.0. All data are shown as means ± SD. (B), Total RNA was extracted from AGS/FoxP3 and AGS/vector cells and qRT-PCR was done using specific primers for PARP, caspase-3 and caspase-9. Data are given as means ± SD of transcript levels normalized to GAPDH. Proapoptotic genes (PARP, caspase-3 and caspase-9) mRNA levels were increased in AGS/FoxP3 compared with the vector at 48 h. (C) Silencing of FoxP3 reduced the levels proapoptotic genes mRNA in FoxP3-siRNA cells compared with the scrambled control at 48 h. All qRT-PCR experiments were performed by the same investigator with no knowledge of the corresponding grouped data. Data shown are mean ± SD of three independent experiments. Sc, scrambled control; $^{*}P < 0.05$; $^{**}P < 0.01$.

We herein showed that up-regulation of FoxP3 can significantly inhibit cell growth by cell proliferation assay and clone formation assay. This result was consistent with the previous documents that showed FoxP3 inhibited cell growth of breast cancer and prostate cancer *in vitro* and *in vivo* [16,17,29] and up-regulation of Foxp3 inhibited cell proliferation, migration and invasion in epithelial ovarian cancer [30]. There are many reasons contributing to cell growth, such as growth-associated protein and cell cycle and cytokines. We herein explored whether this inhibition effect of cell growth correlated with the alternation of apoptosis ability.

Mitochondrial and death receptor pathways are two major signaling pathways involved in apoptosis. Many studies have shown that collapse of mitochondrial membrane potential is the early step in the apoptotic cascade [31,32] The current result showed that up-regulation of FoxP3 elicited an accumulation of apoptotic cells by Flow cytometry and an increase of DNA damage by TUNEL staining, indicating that FoxP3 can promote apoptosis in both the earlier and the later process. Previous studies demonstrated that FoxP3 inhibited the growth of breast cancer and prostate cancer by transcriptional repressing oncogenes, such as HER2 [11], c-Myc [20], Skp2 [12]. However, they did not explore the apoptosis-induced roles in the process. We herein present its novel role in promoting apoptosis in GC cells and showed FoxP3 can affect the releasing of a series of caspase-associated proteins.

PARP, as one of the main cleavage targets of caspase-3, was thought to be involved in DNA repair and play key roles in apoptosis [24]. We herein firstly showed that up-regulation of FoxP3 expression significantly increased the mRNA levels of proapoptotic genes, such as PARP, caspase-3 and caspase-9, leading to efficient induction of apoptosis in these cells. Meanwhile, silencing of FoxP3

was sufficient and necessary for inhibiting the expression of apoptosis-associated proteins. Jung et al. [33] showed that up-regulation of FoxP3 promoted the function of tumor suppressor gene p53, indicating that FoxP3 was a key determinant of cell fate via p53-dependent DNA damage responses. Other studies documented that higher proapoptotic genes and lower antiapoptotic genes were found in the comparison of natural Treg with conventional T cells [34], the great difference of which is the expression of FoxP3, FoxP3 was critical in the anisomycin-induced apoptosis of breast cancer cells [29], and that prostate-specific deletion of FoxP3 caused precancerous lesions [20]. Based on these findings, our study is the first time to reveal that FoxP3 is necessary for activating apoptotic signaling pathway**.** However, whether FoxP3 binds directly to caspase gene or cooperates with other genes needs further studies.

In summary, the current study presented the first evidences about the roles of FoxP3 in inhibiting proliferation and promoting apoptosis in GC cells and its mechanisms by regulating apoptotic signaling pathway. From these data, we could expect that up-regulation of FoxP3 might potentially become an effective therapeutic approach for cancer. The biological significance of these findings warrants further investigation about FoxP3 roles in the context of interaction with Tregs and co-contribution in tumor development, especially under the light of current anti-cancer efforts by interfering with FoxP3 expression.

Grant support

Xinjiang Uygur Autonomous Region Science and Technology Support Project (No. 200991127).

Competing interests

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Mei-Yu Hu and Jian-Jun Jin from Biomedical Research Center for technical support; thank Shanghai Key Laboratory of Gastric Neoplasm of Ruijin Hospital for MKN45 cell line and thank Biomedical Research Center of Zhongshan Hospital for experiment equipments supply.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.11.065.

References

[1] S. Hori, S. Sakaguchi, Foxp3: a critical regulator of the development and function of regulatory T cells, Microbes Infect. 6 (2004) 745–751.
[2] P. Salama, M. Phillips, F. Grieu, et al., Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer, J. Clin. Oncol. 27 (2009) 186–192.
[3] W.Y. Kim, Y.K. Jeon, T.M. Kim, et al., Increased quantity of tumor-infiltrating FOXP3-positive regulatory T cells is an independent predictor for improved clinical outcome in extranodal NK/T-cell lymphoma, Ann. Oncol. 20 (2009) 1688–1696.
[4] X.L. Yuan, L. Chen, M.X. Li, et al., Elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner, Clin. Immunol. 134 (2010) 277–288.
[5] Z. Shen, S. Zhou, Y. Wang, et al., Higher intratumoral infiltrated Foxp3+ Treg numbers and Foxp3+/CD8+ ratio are associated with adverse prognosis in resectable gastric cancer, J. Cancer Res. Clin. Oncol. 136 (2010) 1585–1595.
[6] L.M. Ebert, B.S. Tan, J. Browning, et al., The regulatory T cell-associated transcription factor FoxP3 is expressed by tumor cells, Cancer Res. 68 (2008) 3001–3009.
[7] V. Karanikas, M. Speletas, M. Zamanakou, et al., Foxp3 expression in human cancer cells, J. Trans. Med. (2008) 6.
[8] F.I. Dimitrakopoulos, H. Papadaki, A.G. Antonacopoulou, et al., Association of FOXP3 expression with non-small cell lung cancer, Anticancer Res. 31 (2011) 1677–1683.
[9] W.H. Wang, C.L. Jiang, W. Yan, et al., FOXP3 expression and clinical characteristics of hepatocellular carcinoma, World J. Gastroenterol. 16 (2010) 5502–5509.
[10] F. Martin, S. Ladoire, G. Mignot, L. Apetoh, F. Ghiringhelli, Human FOXP3 and cancer, Oncogene 29 (2010) 4121–4129.
[11] T. Zuo, L.Z. Wang, C. Morrison, FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene, Cell 129 (2007) 1275–1286.
[12] T. Zuo, R.H. Liu, H.M. Zhang, et al., FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2, J. Clin. Invest. 117 (2007) 3765–3773.
[13] S. Hinz, L. Pagerols-Raluy, H.H. Oberg, et al., Foxp3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer, Cancer Res. 67 (2007) 8344–8350.
[14] Y.J. Bang, E. Van Cutsem, A. Feyereislova, et al., Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial, Lancet 376 (2010) 687–697.
[15] J. Vanderhaegen, R. Paridaens, P. Neven, Adjuvant trastuzumab in HER2-positive breast cancer, N. Engl. J. Med. 366: 664 (2012) 664–666.
[16] S. Ladoire, L. Arnould, G. Mignot, et al., Presence of Foxp3 expression in tumor cells predicts better survival in HER2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy, Breast Cancer Res. Treat. 125 (2011) 65–72.
[17] L.Z. Wang, R.H. Liu, W.Q. Li, Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate, Cancer Cell 16 (2009) 336–346.
[18] Y.J. Liang, H.C. Liu, Y.X. Su, et al., Foxp3 expressed by tongue squamous cell carcinoma cells correlates with clinicopathologic features and overall survival in tongue squamous cell carcinoma patients, Oral Oncol. 47 (2011) 566–570.
[19] M. Yoshii, H. Tanaka, M. Ohira, et al., Expression of forkhead box P3 in tumour cells causes immunoregulatory function of signet ring cell carcinoma of the stomach, Br. J. Cancer 106 (2012) 1668–1674.
[20] L. Wang, R. Liu, W. Li, et al., Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate, Cancer Cell 16 (2009) 336–346.
[21] R. Liu, L. Wang, G. Chen, et al., FOXP3 up-regulates p21 expression by site-specific inhibition of histone deacetylase 2/histone deacetylase 4 association to the locus, Cancer Res. 69 (2009) 2252–2259.
[22] L. Chen, B. Lan, X.H. Chen, et al., Cell cycle dependent genes from the gastric cancer cell MKN45 can affect tumorigenesis, Hepatogastroenterology 58 (2011) 674–681.
[23] K. Karube, R. Aoki, Y. Sugita, et al., The relationship of FOXP3 expression and clinicopathological characteristics in adult T-cell leukemia/lymphoma, Modern Pathol. 21 (2008) 617–625.
[24] M.S. Satoh, T. Lindahl, Role of poly (ADP-ribose) formation in DNA repair, Nature 356 (1992) 356–358.
[25] Y.A. Lazebnik, S.H. Kaufmann, S. Desnoyers, G.G. Poirier, W.C. Earnshaw, Cleavage of poly (ADP-ribose) polymerase by a proteinase with properties like ICE, Nature 371 (1994) 346–347.
[26] R. Nimmanapalli, K. Bhalla, Targets in apoptosis signaling: promise of selective anticancer therapy, Meth. Mol. Biol. 223 (2003) 465–483.
[27] L.M. Ebert, B.S. Tan, J. Browning, et al., The regulatory T cell-associated transcription factor FoxP3 is expressed by tumor cells, Cancer Res 68 (2008) 3001–3009.
[28] L. Wang, R. Liu, M. Ribick, P. Zheng, Y. Liu, FOXP3 as an X-linked tumor suppressor, Discov. Med. 10 (2010) 322–328.
[29] Y. Liu, Y. Wang, W. Li, et al., Activating transcription factor 2 and c-Jun-mediated induction of FoxP3 for experimental therapy of mammary tumor in the mouse, Cancer Res 69 (2009) 5954–5960.
[30] H.Y. Zhang, H. Sun, Up-regulation of Foxp3 inhibits cell proliferation, migration and invasion in epithelial ovarian cancer, Cancer Lett 287 (2010) 91–97.
[31] X. Wang, The expanding role of mitochondria in apoptosis, Genes Dev. 15 (2001) 2922–2933.
[32] K.C. Pramanik, S.R. Boreddy, S.K. Srivastava, Role of mitochondrial electron transport chain complexes in capsaicin mediated oxidative stress leading to apoptosis in pancreatic cancer cells, PLoS One 6 (2011) e20151.
[33] D.J. Jung, D.H. Jin, S.W. Hong, et al., Foxp3 expression in p53-dependent DNA damage responses, J. Biol. Chem. 285 (2010) 7995–8002.
[34] D. Tischner, I. Gaggl, I. Peschel, et al., Defective cell death signalling along the Bcl-2 regulated apoptosis pathway compromises Treg cell development and limits their functionality in mice, J. Autoimmun. 38 (2012) 59–69.