MicroRNA-146a promotes gastric cancer cell apoptosis by targeting transforming growth factor β-activated kinase 1

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Abstract. Accumulating evidence suggests that microRNA (miR)-146a functions as an oncogene or tumor suppressor in various cancers. However, the role of miR-146a in gastric cancer (GC) remains to be elucidated. The present study investigated the function of miR-146a in GC cells. The results of the present study revealed that miR-146a modulates GC cell apoptosis. Overexpression of miR-146a significantly increased apoptosis of SGC-7901 cells, whereas inhibition of miR-146a protected cells from apoptosis. miR-146a expression in GC cells was inversely correlated with transforming growth factor β-activated kinase 1 (TAK1) expression, at the mRNA and protein levels. Furthermore, small interfering RNA-mediated silencing of TAK1 enhanced GC cell apoptosis, whereas overexpression of TAK1 promoted survival of GC cells. Overexpression of miR-146a or knockdown of TAK1 led to a marked increase in inhibitor of κBα (IκBα) and a decrease in B-cell lymphoma 2 (Bcl-2) expression levels in SGC-7901 cells. By contrast, silencing of miR-146a or TAK1 overexpression downregulated IκBα and upregulated Bcl-2 expression levels. Therefore, the results of the present study demonstrated a novel negative feedback mechanism to promote GC cell apoptosis involving the miR-146a/TAK1/nuclear factor-κB axis.

Introduction

Gastric cancer (GC) is one of the most common human malignant diseases and the second leading cause of cancer-associated mortality worldwide. GC has a particularly high incidence and mortality rate in China (1,2), with 404,565 new cases of GC annually, and 312,432 patients succumbing to disease each year (3). During GC progression, GC cells become increasingly resistant to apoptosis, and therefore refractory to therapy (4,5). Despite improvements in systemic therapy, the prognosis of GC patients remains poor. Therefore, investigating the molecular mechanisms underlying GC progression may be beneficial for the development of novel targeted therapies.

MicroRNAs (miRNAs) are small (19-24 nucleotides in length), noncoding RNAs, that incompletely bind to the 3' untranslated region of target genes and subsequently negatively regulate expression of multiple genes by inducing translational silencing or degrading the mRNA (4). Accumulating evidence suggests that miRNAs act as oncogenes or tumor suppressors, and that the dysregulation of specific miRNAs is associated with the cancerous transformation of cells (6-8). miRNA (miR)-146a is involved in cell proliferation, differentiation and apoptosis (9). miR-146a dysregulation and dysfunction correlates with tumorigenesis and the development of various types of cancer (10,11). However, miR-146a exerts opposite effects in different cancers. It serves as an oncogene in anaplastic thyroid carcinoma and cervical cancer, and as a tumor suppressor in breast and prostate cancers (12-15). Although evidence has suggested that the expression of miR-146a is downregulated in GC, and that downregulation of miR-146a is associated with tumor size and poor prognosis (16), the underlying molecular mechanism of miR-146a and its effects remain largely unclear.

Cancer cells typically acquire a constitutively active nuclear factor (NF)-κB pathway to promote survival, proliferation and metastatic potential (17). In the majority of cell types, NF-κB complexes are retained in the cytoplasm in an inactive form by a family of inhibitory proteins known as inhibitors of κB (IκBs), which includes IκBα, IκBβ and IκBε (18,19). Degradation of IκB facilitates the release and nuclear translocation of NF-κB (18). Transforming growth factor β-activated kinase 1 (TAK1), a member of the mitogen-activated protein kinase kinase kinase family, has a prosurvival role in the activation of the NF-κB signaling pathway (20). Loss of TAK1 sensitizes cells for apoptosis or death in the majority of tissue types (21). These results indicate that TAK1 is an important upstream effector of the NF-κB signaling pathway and that altering the expression of TAK1 may affect apoptosis. In addition, previous studies have demonstrated that miR-146a serves as a negative regulator of constitutive NF-κB activity in breast

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cancer (12,17). However, whether miR-146a regulates GC cell apoptosis by negatively regulating TAK1 expression remains to be fully elucidated. The present study analyzed the function of miR-146a in GC and demonstrated that it inhibits GC cell apoptosis by targeting TAK1, with subsequent inhibition of the NF-κB signaling pathway.

Materials and methods

Cell culture and transfection. SGC-7901 human GC cells (Shanghai Institute of Cell Biology, Shanghai, China) were cultured in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) in a humidified 5% CO_2 incubator at 37˚C. The miR-146a mimic and inhibitor were obtained from Biotend Biological Technology (Shanghai, China). Scrambled negative control mimic or inhibitor (Biotend Biological Technology) were transfected to serve as matched controls. TAK1-Flag plasmid was obtained from Addgene, Inc. (Cambridge, MA, USA). *TAK1* siRNA and the scrambled negative control were purchased from Shanghai GenePharma, Co., Ltd. (Shanghai, China). Oligomers used are listed in Table I. Cells ($5x10^5$/well) were seeded into 6-well plates the day prior to transfection to ensure a suitable cell confluence (70%) on the day of transfection. Cells were subsequently transfected using Lipofectamine® RNAiMax (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h according to the manufacturer's protocol. The miR-146a mimic, inhibitor and respective controls were used at a final concentration of 10 nM. si*TAK1* and control were used at a final concentration of 20 nM. A total of 3 μg TAK1-Flag plasmid was used for overexpression of TAK1.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Single-stranded cDNA was synthesized from total RNA using Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) and oligo (dT) 18. qPCR was performed in a 96-well plate on a Bio-Rad CFX Real-Time system using iQ™ SYBR®-Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA). For miR-146a detection, the following thermocycling conditions were used: An initial predenaturation step at 50˚C for 2 min, followed by 40 cycles of denaturation at 95˚C for 10 min and annealing at 60˚C for 1 min. For TAK1, the thermocycling conditions were as follows: An initial predenaturation step at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 20 sec. Each run was performed in triplicate. The primer sequences used are listed in Table I. The data were expressed as fold change. The comparative quantitation cycle method was used to quantify the expression levels of target genes relative to endogenous controls (22). To normalize the relative abundance of miR-146a and TAK1, U6 and GAPDH served as endogenous controls, respectively (23). For each individual analysis, the control group was used as the calibrator and given a relative value of 1.0. All quantities were expressed as n-fold relative to the calibrator.

Table I. Oligomers and primers sequences and used in the present study.

Gene (*Homo sapiens*)	Sequence (5'-3')
miR-146a mimic	UGAGAACUGAAUUCCAUGGGUU
Mimic NC	UUCUCCGAACGUGUCACGUTT
miR-146a inhibitor	AACCCAUGGAAUUCAGUUCUCA
Inhibitor NC	ACGUGACACGUUCGGAGAATT
si*TAK1*	AAAGCGTTTATTGTAGAGCTT
si*TAK1* NC	ACGUGACACGUUCGGAGAATT
miR-146a-forward	CAGTGCGTGTCGTGGAGT
miR-146a-reverse	GGGTGAGAACTGAATTCCA
U6-forward	CTCGCTTCGGCAGCACA
U6-reverse	AACGCTTCACGAATTTGCGT
TAK1-forward	ATCAGCAGAGTAGCTGCGGT
TAK1-reverse	GAGGAGCTTGCTGCAGAGT
GAPDH-forward	AGGGCTGCTTTTAACTCTGGT
GAPDH-reverse	CCCCACTTGATTTTGGAGGGA

miR, microRNA; NC, negative control; TAK1, transforming growth factor β-activated kinase 1; si, small interfering.

Flow cytometry. An Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Staining kit (BD Biosciences, Franklin Lakes, NJ, USA) was used to detect apoptotic cells. Cells were harvested 48 h following transfection and resuspended in 1X binding buffer at a concentration of $1x10^6$ cells/ml. Subsequently, cells were stained with 5 μl Annexin V-FITC and 5 μl PI for 15 min at room temperature in the dark. Cells were acquired using a BD Accurri™ C6 flow cytometer (BD Biosciences) and data was analyzed using FlowJo software version 7.6.2 (Tree Star, Inc., Ashland, OR USA).

Caspase-3 activity assay. Following transfection with miR-146a mimic or inhibitor, SGC-7901 cells were seeded into 96-well plates at a density of $1x10^5$ cells/well, incubated overnight and processed using a Caspase-3 Activity assay kit (Cell Signaling Technology, Inc., Danvers, MA, USA). Cells were lysed in 30 μl 1X PathScan® Sandwich ELISA Lysis Buffer. The cell lysate was mixed with the substrate solution and incubated at 37˚C in the dark for 2 h, following which the relative fluorescent was measured using a fluorescence plate reader (BioTek Instruments, Inc., Winooski, VT, USA) with an excitation wavelength of 380 nm and an emission wavelength of 420 nm.

Cell proliferation assay. SGC-7901 cell proliferation was detected using Cell Counting kit-8 (CCK8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Following transfection, SGC-7901 cells were seeded into 96-well plates at a density of $4x10^3$ cells/well. CCK8 solution (10 μl) was added to each well and cells were incubated for 2 h at 37˚C. Optical density (OD) values were obtained at a wavelength of 450 nm using a microplate reader (BioTek Instruments, Inc.). The growth curve was constructed using the mean OD value every 24 h for 3 days.

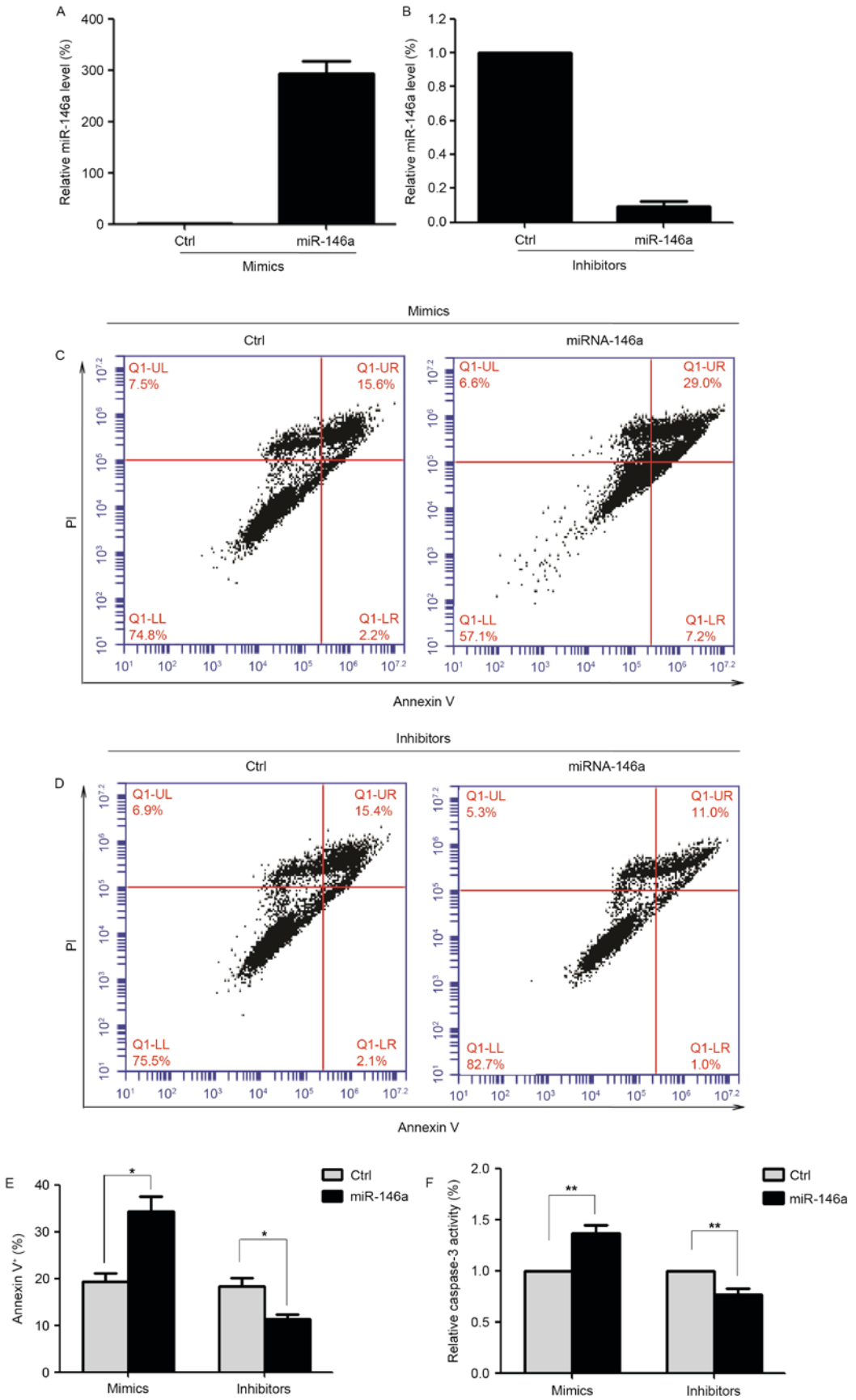

Figure 1. miR-146a regulates SGC-7901 cell apoptosis and proliferation. SGC-7901 cells were transfected with an miR-146a mimic or inhibitor, or scrambled controls, at a final concentration of 10 nM for 48 h. Reverse transcription-quantitative polymerase chain reaction confirmed (A) upregulation of miR-146a levels in mimic-transfected cells and (B) downregulation of miR-146a levels in inhibitor-transfected cells, compared with controls. Apoptosis detection by Annexin V/PI staining followed by flow cytometry in (C) miR-146a overexpressing and (D) miR-146a inhibited cells. (E) The percentage of Annexin V^+ cells (Annexin V^+/PI^- and Annexin V^+/PI^+ cells). (F) Caspase-3 activity was measured with the Caspase-3 Activity assay kit. Data are expressed as the mean ± standard deviation of six (E), four (A and B) or two (F) independent experiments. $^*P<0.05$; $^{**}P<0.01$. miR, microRNA; PI, propidium iodide; Ctrl, control.

Western blotting. After 48 h transfection, cultured SGC-7901 cells were washed with cold PBS once and lysed with lysis buffer (0.5% NP40). Equal quantities of protein (30 μg) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% skimmed milk and incubated with the following primary antibodies, diluted 1:1,000, overnight at 4˚C: Rabbit anti-TAK1 (catalog no. 4505), rabbit anti-IκBα (catalog no. 4812), anti-rabbit B-cell lymphoma 2 (Bcl-2; catalog no. 4223), rabbit anti-β-actin (catalog no. 4970) and rabbit anti-Flag (catalog no. 14793), all purchased from Cell Signaling Technology, Inc. Blots were washed with TBS containing Tween 20 (TBST) three times for 10 min each time. Subsequently, membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (1:3,000; catalog no. A00098; GenScript, Piscataway, NJ, USA) for 2 h at room temperature and washed with TBST three times for 10 min each time. Protein bands were visualized using Enhanced Chemiluminescence Plus (Thermo Fisher Scientific, Inc.) and scanned by Typhoon™ FLA 9500 (GE Healthcare Life Sciences, Chalfont, UK). Densitometry was quantified using Image J software (National Institutes of Health, Bethesda, MD, USA) and normalized to β-actin.

Statistical analysis. Statistical analysis was performed using SPSS software version 19 (IBM SPSS, Armonk, NY, USA). Data are expressed as the mean ± standard deviation. Paired Student's t-tests or one-way analyses of variance followed by the Bonferroni post hoc test were used to determine statistical significance. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-146a serves as an apoptotic regulator. Abnormal apoptosis of tumor cells is critical for cancer progression (5). Previous studies have suggested that miR-146a provides negative feedback inhibition of tumor progression (8,24,25). Therefore, the present study evaluated the effect of altering miR-146a expression levels on SGC-7901 cell apoptosis. SGC-7901 cells were transfected with an miR-146a mimic or scrambled control for 48 h. Cells transfected with an miR-146a mimic demonstrated a ~300-fold increase in expression levels of miR-146a compared with control cells, as assessed by RT-qPCR (Fig. 1A). In addition, SGC-7901 cells were transfected with an miR-146a inhibitor or scrambled control for 48 h. Cells transfected with an miR-146a inhibitor exhibited a decrease in miR-146a expression levels to ~10% of the levels of control cells (Fig. 1B). Apoptosis was assessed in transfected cells using Annexin V/PI staining (Fig. 1C and D). Compared with the scrambled control-transfected cells, overexpression of miR-146a significantly increased the proportion of apoptotic cells (34.4±7.9 vs. 19.4±4.3%; n=6; P<0.05; Fig. 1E). The proportion of apoptotic cells following transfection with an miR-146a inhibitor was significantly decreased compared with scrambled control-transfected cells (11.3±2.6 vs. 18.4±4.1%; n=6; P<0.05; Fig. 1E).

In addition, the effect of miR-146a on apoptosis of SGC-7901 cells was assessed by measuring caspase-3 activity, a feature of apoptosis. Overexpression of miR-146a significantly increased caspase-3 activity, whereas inhibition of miR-146a decreased caspase-3 activity (Fig. 1F). These results demonstrated that miR-146a may serve a pro-apoptotic role in GC cells.

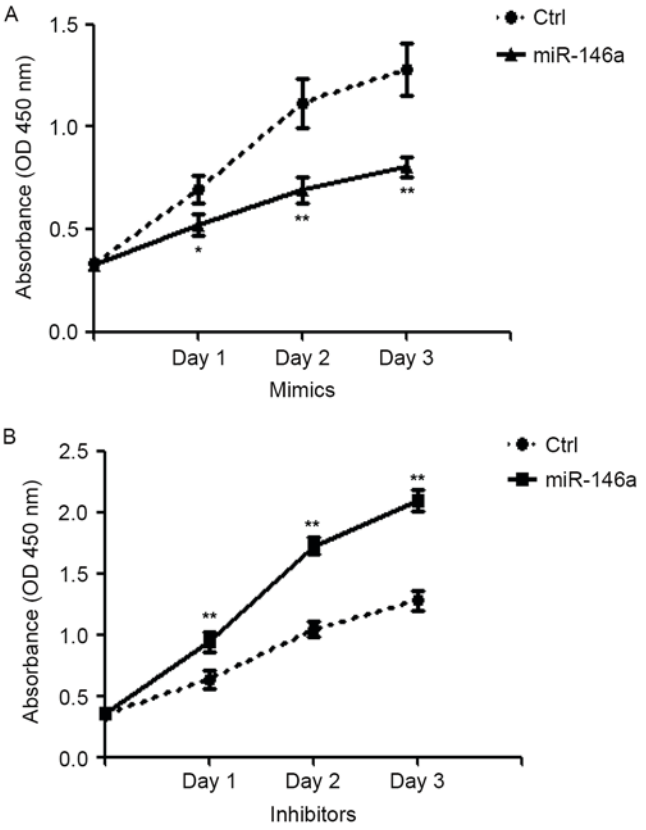

Figure 2. miR-146a expression modulates cell proliferation in gastric cancer cells. SGC-7901 cells were transfected with (A) an miR-146a mimic or (B) an miR-146a inhibitor, or scrambled controls, at a final concentration of 10 nM for 48 h. Proliferation of cells was evaluated using a Cell Counting kit-8 assay. miR-146a overexpression significantly inhibited proliferation of SGC-7901 cells. By contrast, the miR-146a inhibitor significantly promoted the proliferation of SGC-7901 cells compared with the scrambled control. Data are expressed as the mean ± standard deviation of three independent experiments. *P<0.05 and **P<0.01 vs. scrambled control at the same time point. miR, microRNA; Ctrl, control; OD, optical density.

miR-146a modulates GC cell proliferation. As the rapid proliferation of tumor cells serves a pivotal role in tumor growth and development (5), the present study assessed the effects of altering miR-146a expression levels on the proliferation of SGC-7901 cells using a CCK8 assay. Compared with scrambled control-transfected cells, miR-146a overexpression caused a significant inhibition of cell proliferation (day 1, P<0.05; days 2 and 3, P<0.01; Fig. 2A). By contrast, knockdown of miR-146a led to a significant increase in cell proliferation compared with the scrambled control (P<0.01; Fig. 2B). These data suggested that miR-146a expression is inversely correlated with cell proliferation.

TAK1 expression is inversely correlated with miR-146a expression. To determine whether miR-146a regulates TAK1 in GC, the present study assessed the expression of TAK1 following inhibition or overexpression of miR-146a in SGC-7901 cells, using RT-qPCR and western blotting. Compared with scrambled mimic-transfected cells, TAK1 expression at the mRNA (Fig. 3A) and protein (Fig. 3B and C) levels was decreased

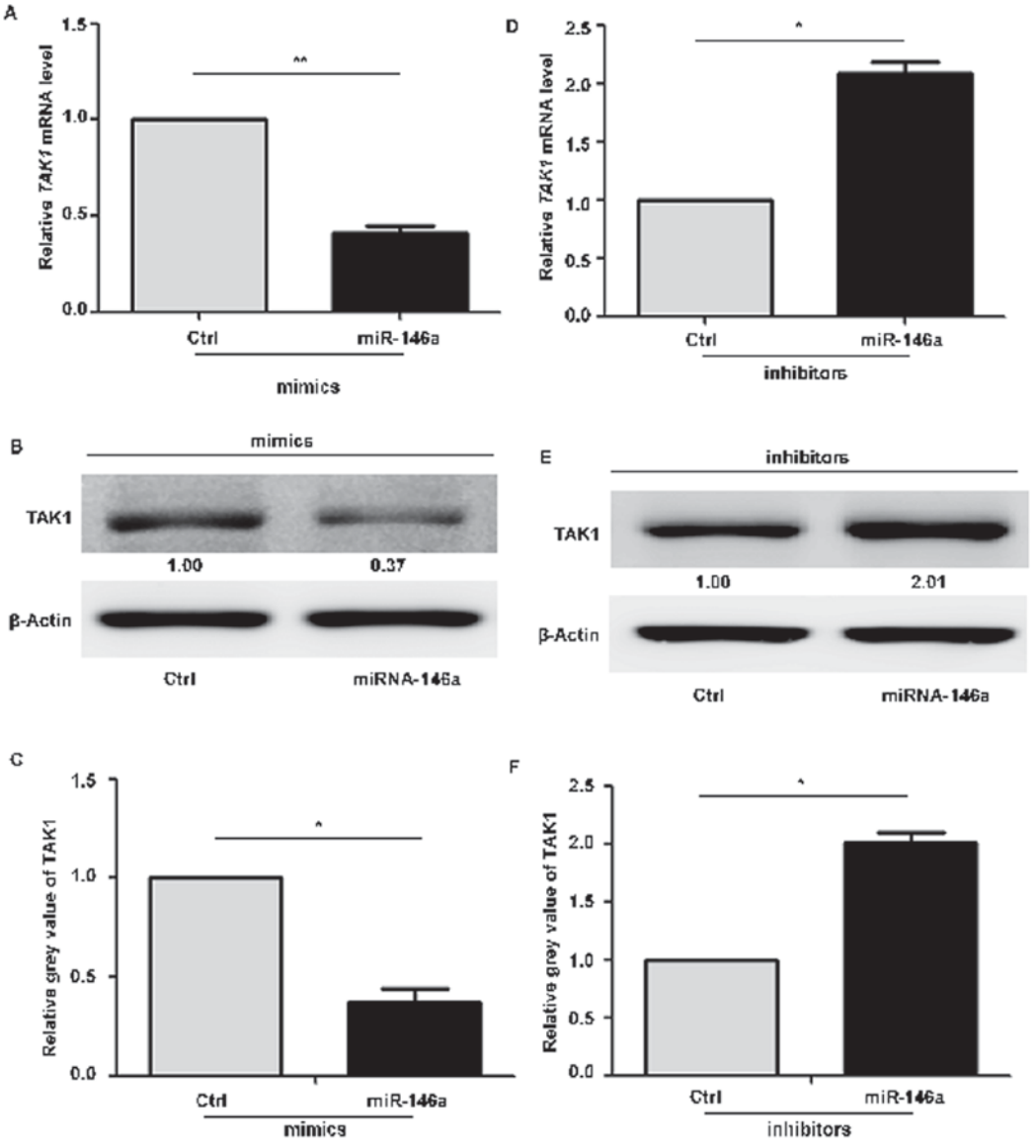

Figure 3. miR-146a expression regulates TAK1 expression levels. SGC-7901 cells were transfected with an miR-146a mimic or inhibitor, or scrambled controls, at a final concentration of 10 nM for 48 h. TAK1 (A) mRNA and (B and C) protein expression levels were analyzed in cells transfected with an miR-146a mimic or scrambled control. TAK1 (D) mRNA and (E and F) protein expression levels were analyzed in cells transfected with an miR-146a inhibitor or scrambled control. Data are expressed as the mean ± standard deviation and represent six (A and D) or three (B, C, E and F) independent experiments. *P<0.05; **P<0.01. miR, microRNA; TAK1, transforming growth factor β-activated kinase 1; Ctrl, control.

following transfection with an miR-146a mimic. By contrast, inhibition of miR-146a resulted in significant increases in TAK1 mRNA (Fig. 3D) and protein (Fig. 3E and F) expression levels compared with scrambled controls. These data indicated that miR-146a expression is inversely correlated with TAK1, which may be a potential target molecule of miR-146a in GC cell apoptosis.

Knockdown of TAK1 induces GC cell apoptosis. To investigate whether TAK1 is involved in regulating miR-146a-mediated SGC-7901 apoptosis, the present study knocked down *TAK1* using siRNA (si*TAK1*) to determine the effect on GC cell apoptosis. *TAK1* mRNA expression levels were significantly decreased following siRNA transfection in SGC-7901 cells compared with scrambled controls (Fig. 4A). Overexpression of TAK1 using a TAK1-Flag plasmid increased protein expression levels of TAK1 compared with control cells (Fig. 4B and C). Apoptosis was assessed in transfected cells using Annexin V/PI staining (Fig. 4D and E). Compared with scrambled control siRNA, the percentage of apoptotic SGC-7901 cells following transfection with si*TAK1* was significantly increased (37.33±8.87 vs. 19.18±6.30%; n=6; P<0.05; Fig. 4F), in accordance with the results obtained with miR-146a overexpression. By contrast, overexpression of TAK1 led to increased SGC-7901 cell survival compared with control cells (Fig. 4G). These data indicated that miRNA-146a mediates apoptosis in human GC via targeting *TAK1*.

miR-146a induces apoptosis via the NF-κB pathway in GC. To confirm that miR-146a-induced GC cell apoptosis involves

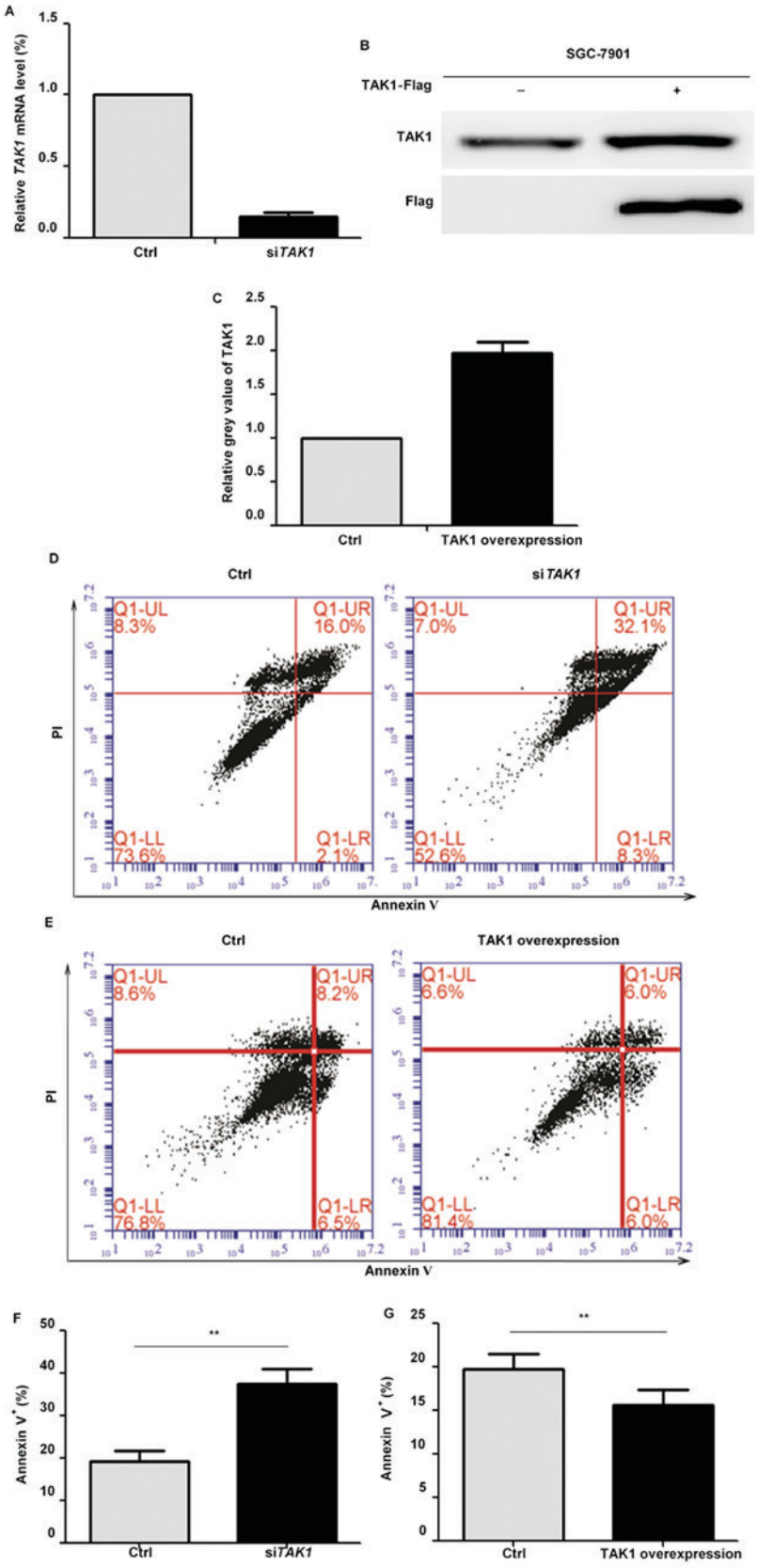

Figure 4. TAK1 regulates gastric cancer cell apoptosis. SGC-7901 cells were transfected with *TAK1* siRNA or scrambled control at a final concentration of 20 nM, or TAK1-Flag plasmid, for 48 h. (A) Reverse transcription-quantitative polymerase chain reaction confirmed decreased TAK1 mRNA expression levels in si*TAK1*-transfected cells and (B and C) western blotting confirmed increased TAK1 protein expression levels in TAK1-Flag-transfected cells, compared with controls. Apoptosis detection by Annexin V/PI staining followed by flow cytometry in (D) TAK1 knockdown and (E) TAK1 overexpressing cells. The percentage of Annexin V^+ cells (Annexin V^+/PI^- and Annexin V^+/PI^+ cells) in (F) TAK1 knockdown and (G) TAK1 overexpressing cells. Data are expressed as the mean ± standard deviation and represent six independent experiments. $^*P<0.05$; $^{**}P<0.01$. TAK1, transforming growth factor β-activated kinase 1; si, small interfering; PI, propidium iodide; Ctrl, control.

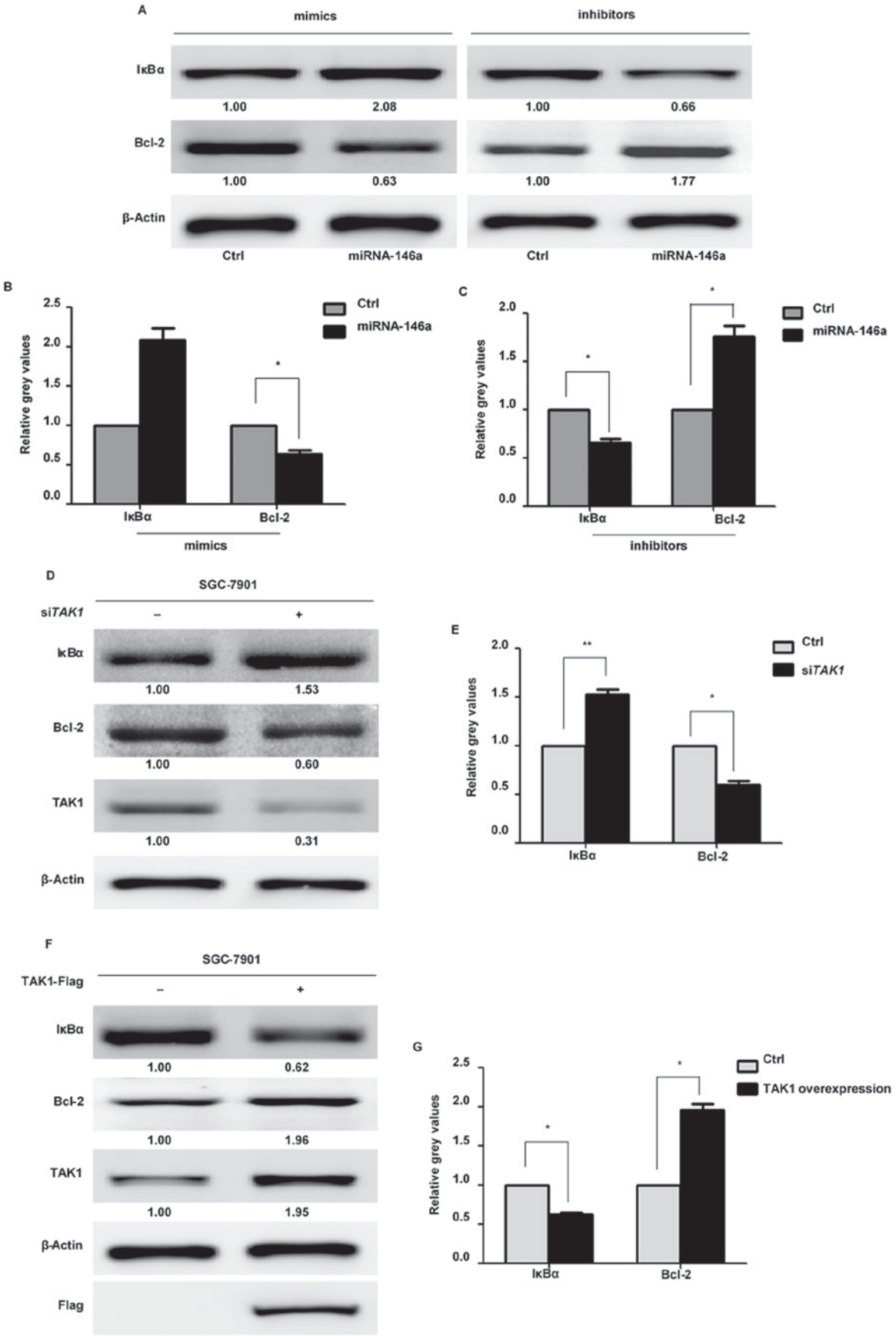

Figure 5. miR-146a modulates apoptosis via the nuclear factor-κB signaling pathway. (A-C) SGC-7901 cells were transfected with an miR-146a mimic or inhibitor, or scrambled controls, at a final concentration of 10 nM. TAK1 was (D and E) knocked down or (F and G) overexpressed in SGC-7901 cells. Cells were subsequently analyzed for IκBα and Bcl-2 protein expression levels by western blotting. Blots were quantified by densitometry and normalized to β-actin. Data are representative of three independent experiments. *P<0.05; **P<0.01. miR, microRNA; TAK1, transforming growth factor β-activated kinase 1; IκBα, inhibitor of κBα; Bcl-2, B-cell lymphoma 2; si, small interfering; Ctrl, control.

inhibition of the NF-κB signaling pathway, at least partially via downregulation of the NF-κB pathway mediator TAK1, the present study detected IκBα protein expression levels by western blotting. Following transfection with an miR-146a mimic, IκBα protein expression levels were significantly upregulated, whereas these levels were downregulated following transfection of an miR-146a inhibitor (Fig. 5A-C). Knockdown of *TAK1* by siRNA additionally led to increased IκBα protein expression levels (Fig. 5D and E). By contrast, overexpression of TAK1 resulted in decreased expression levels of IκBα (Fig. 5F and G). These results indicated that miR-146a inhibits the NF-κB signaling pathway. The protein expression levels of Bcl-2, a known anti-apoptotic downstream effecter of NF-κB, were also assessed. Transfection with an miR-146a mimic or si*TAK1* induced a decrease in Bcl-2 expression, whereas transfection of an miR-146a inhibitor led to an increase in Bcl-2 expression in SGC-7901 cells. Increased expression levels of Bcl-2 were additionally observed following overexpression of TAK1. Taken together, these data suggested that miR-146a modulates SGC-7901 cell apoptosis through suppression of the NF-κB signaling pathway via targeting TAK1.

Discussion

The present study revealed three important findings concerning the function of miR-146a in human GC progression. Firstly, miR-146a may be a critical regulator of GC cell proliferation and apoptosis. Overexpression of miR-146a significantly enhanced apoptosis and inhibited proliferation of SGC-7901 cells, whereas inhibition of miR-146a resulted in reduced apoptosis and increased survival. Secondly, miR-146a inversely affected the expression of TAK1 in GC cells. Finally, miR-146a targeted TAK1, leading to inhibition of NF-κB and reduced expression of Bcl-2. Therefore, miR-146a may regulate GC cell apoptosis and proliferation by inhibiting the NF-κB signaling pathway via targeting TAK1, suggesting a novel negative feedback mechanism for miR-146a in the regulation of GC development.

The capacity of tumor cell populations to expand in number is attributed to the rate of cell growth and apoptosis. Rapid proliferation and evasion of apoptosis, two of the primary features of cancer cells, are the leading causes of GC progression (5,26). Hou *et al* (25) revealed that miR-146a enhances apoptosis and inhibits survival in MKN-45 cells, a poorly differentiated GC cell line. Consistent with these results, the present study demonstrated that overexpression of miR-146a promoted apoptosis and inhibited proliferation in SGC-7901 cells, a moderately differentiated GC cell line, whereas inhibition of miR-146a decreased apoptosis and increased proliferation. These data suggested that miR-146a is an important regulator of GC cell survival. Previously, up- and down-regulated expression of miR-146a has been reported in GC tissues (16,25,27). miR-146a expression was revealed to be high in noncancerous prostatic epithelium and gradually decreased with cancer progression (28). Reduced levels of miR-146a have been associated with lymph node metastasis and venous invasion (16). Furthermore, the loss of miR-146a may be a late event in the progression of GC (10). Therefore, the conflicting data regarding the expression of miR-146a may be attributed to different tumor progression status and clinical stages. of GC. Although the expression of miR-146a in GC remains controversial, there is a consensus that miR-146a acts as a tumor suppressor in GC progression by inhibiting proliferation and promoting apoptosis.

In GC, the NF-κB signaling pathway serves a pivotal role in modulating cell survival, apoptosis, immunity and inflammation, and NF-κB activation is associated with poor prognosis (27,29). Various regulatory proteins, including IκBα and Bcl-2, are direct transcriptional targets of NF-κB, thus forming a negative feedback loop (30). Recently, evidence has demonstrated that phosphorylation of IκBα at Ser-32 is necessary for its degradation, and that this phosphorylation is decreased by overexpression of miR-146a in breast cancer cells (12). In addition, upregulation of IκBα by overexpression of miR-146a has been revealed in non-small cell lung cancer cells (31). Consistent with this, the present study demonstrated that overexpression of miR-146a increased IκBα expression and decreased Bcl-2 expression in SGC-7901 cells. By contrast, inhibition of miR-146a downregulated expression levels of IκBα and upregulated those of Bcl-2. Therefore, the results of the present study supported published data suggesting that miR-146a modulates SGC-7901 apoptosis by inhibition of the NF-κB signaling pathway.

TAK1, a key molecular component in the determination of cell fate, is an upstream kinase of NF-κB (20,21). Accumulating evidence suggests the existence of a TAK1-NF-κB loop in various diseases (21,30). The present study identified the mechanism underlying miR-146a regulation of GC cell apoptosis through the miR-146a/TAK1/NF-κB axis. Expression of TAK1 was inversely modulated by miR-146a. Furthermore, overexpression of miR-146a or silencing of TAK1 led to increased expression levels of IκBα in SGC-7901 cells. By contrast, inhibition of miR-146a or overexpression of TAK1 decreased the protein expression levels of IκBα. Previous studies have suggested that TAK1 may phosphorylate the IκB kinase complex for degradation and release NF-κB (20,32), which may subsequently translocate to the nucleus and activate a range of genes involved in inhibition of apoptosis and promotion of proliferation (20,21). Therefore, miR-146a may act as an NF-κB signaling pathway negative regulator via repression of TAK1 in GC cells.

In conclusion, the results of the present study suggested that miR-146a, serving as a tumor suppressor, may significantly promote GC cell apoptosis by inhibition of the NF-κB signaling pathway via targeting TAK1. The newly identified miR-146a/TAK1/NF-κB axis provides a novel insight into GC progression. These findings suggested that miR-146a may be a potential therapeutic target for the treatment of GC.

Acknowledgements

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