Role of REG Iα in gastric carcinogenesis: Gastrin-associated proliferative and anti-apoptotic activities

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Abstract. Gastric carcinogenesis is a multiple-stage process. It is believed that a premalignant lesion often precedes or accompanies gastric cancer, although the underlying mechanisms have not been fully elucidated. Here, we revealed that REG Iα was frequently overexpressed not only in gastric cancer tissues, but also in the intestinal metaplastic and atypical dysplasia gland, which are considered precancerous lesions, in 102 patients. To investigate the role of REG Iα in gastric cancer, we employed siRNA-mediated silencing techniques and found that the downregulation of REG Iα significantly inhibited gastric cancer cell proliferation, whereas overexpression of REG Iα promoted proliferation. In addition, REG Iα appeared to have an anti-apoptotic effect in gastric cancer cells, which was associated with the Bad/Bcl-xL/caspase-3 pathway. Furthermore, gastrin was found to activate REG Iα expression and nuclear translocation of β-catenin in gastric cancer cells. Thus, these data suggest that REG Iα, a potential downstream of gastrin, may be involved in gastric carcinogenesis.

Introduction

Despite significant advances in basic and clinical research on gastric cancer, it remains the second leading cause of cancer-related death in the world (1,2). According to Correa's hypothesis, the development of gastric cancer is a multiple-stage process. Atrophic gastritis, intestinal metaplasia (IM) and dysplasia are considered precancerous lesions associated with a considerable risk of developing into gastric carcinoma (3). It is believed that gastric premalignant lesions often precede or accompany gastric cancer, although the underlying mechanisms have not been fully elucidated (4).

A pathogenic link to gastric cancer may be related to growth factors and inflammatory mediators of *Helicobactor pylori* (*H. pylori*) infection in gastric glands (5). Indeed, studies have shown that growth factors, including epidermal growth factor (EGF), transforming growth factor-α (TGF-α) and vascular endothelial growth factor (VEGF), appear to be responsible for epithelial cell regeneration and gland restoration in gastritis; furthermore, overexpression of these growth factors promotes proliferation, differentiation, invasion and metastasis of gastric cancer cells (6,7). Thus, growth factors may play an important role in the progression from chronic gastritis to gastric cancer. Although growth factors are predicted to play a key role in the progression from chronic gastritis to gastric cancer, the underlying molecular mechanisms involved in this transition are poorly understood.

The regenerating gene (REG) Iα was first isolated as a trophic or growth factor in regenerating pancreatic islet cells (8). REG Iα protein is a member of the REG family that is predominantly expressed in the normal pancreas and at low levels in the stomach and colon, suggesting physiological roles for REG Iα in these organs (9). Previous studies have reported that REG Iα was overexpressed in *H. pylori*-induced gastritis and gastric ulcer lesions (10-12). Notably, studies have also shown that REG Iα promoted gastric cell growth and differentiation in the neck zone, suggesting a role as a potent trophic agent of progenitor cells of the gastric fundic mucosa (13,14). In addition to the important role of REG Iα in the pathogenesis of gastric inflammatory diseases, REG Iα has been proposed to function in the carcinogenesis of gastrointestinal tissues, including the pancreas (15), colon (16) and liver (17). However, the role of REG Iα in gastric precancerous lesions and in the development of gastric cancer remains poorly understood.

REG Iα is produced primarily by enterochromaffin-like (ECL) cells and chief cells in human gastric mucosa. Gastrin

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Table I. Primers used for PCR amplification of REG Iα.

Gene	Primer sequence	Product size (bp)
REG Iα (RT-PCR) NM_002909.4	Forward: 5'-GATTGTTGATTTGCCTCTTA-3' Reverse: 5'-TCCAGCTGCCTCTAGTTTTTGAA-3'	557
REG Iα (Q-PCR) NM_002909.4	Forward: 5'-TGCCTATCGCTCCTACTGCT-3' Reverse: 5'-CTTGAGGTCAGGCTCACACA-3'	306
GAPDH NM_002024.3	Forward: 5'-GAAGGTGAAGGTCGGAGT-3' Reverse: 5'-GAAGATGGTGATGG'GATTTC-3'	226

was previously shown to stimulate REG Iα production by ECL cells (18-20). As gastrin does not directly promote proliferation of cultured rat gastric epithelial cells, it was proposed that REG Iα indirectly regulates proliferation via gastrin; however, the gastrin-associated pathway involved in REG Iα stimulation remains unclear. Previous studies found that REG Iα was overexpressed in human primary liver tumors and that REG Iα may act as a downstream target of the β-catenin pathway during liver tumorigenesis (21).

In the present study, we provide evidence that REG Iα expression is involved in the progression from active gastritis and precancerous lesions to gastric cancer. We report that REG Iα promotes cell proliferation and appears to have an anti-apoptotic effect via the Bad/Bcl-xL/caspase-3 pathway in gastric cancer cells. Finally, gastrin activated REG Iα expression and induced β-catenin nuclear translocation in gastric cancer cells.

Materials and methods

Clinical samples. Gastric biopsy specimens were obtained from the Endoscopy Centre of Sir Run Run Show Hospital and the Second Affiliated Hospital, Zhejiang University, School of Medicine. A total number of 102 patients were enrolled in the study, and gastric biopsy tissue samples were grouped as follows: normal gastric tissue without *H. pylori* infection (control, n=25); active gastritis with *H. pylori* infection (AG, n=20); intestinal metaplasia (IM, n=40); and dysplasia (DYS, n=17). Thirty surgically resected gastric cancer (GC, n=30) specimens were also obtained from Sir Run Run Shaw Hospital. Gastric carcinoma was classified according to the WHO criteria and staged according to the tumor-node-metastasis (TNM) system. The IM and DYS samples were evaluated according to the updated Sydney system. All specimens were immediately snap-frozen in liquid nitrogen and stored at -80˚C until further processing. Total RNA was extracted using Trizol reagent.

Cell culture. Human gastric cancer cell lines, AGS, NCI-N87, BCG-823 and SGC-7901, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Human gastric cancer cell lines, MKN28 and MKN45, were gifts from the laboratory of Professor Wang Linbo (Oncology Department, Sir Run Run Shaw Hospital). Unless specifically indicated, cells were cultured in RPMI-1640 medium (Invitrogen, USA) supplemented with penicillin and streptomycin and 10% fetal bovine serum, and the cells were grown in a 37˚C incubator with 5% CO_2 and 95% humidity.

Plasmid construction, transfections and stable clone establishment. The full-length human REG Iα cDNA was inserted into the pIRES2-EGFP vector to generate the pIRES2-REGIα expression vector, and the pIRES2-EGFP vector was used as the negative control. REG Iα-directed siRNA duplex oligoribonucleotides and control siRNAs were both designed and purchased from Genscript (Nanjing, China). The REG Iα target site was CTCAAGCACAGGATTCCAGAAA. The REG Iα siRNA was inserted into the pRNAT-CMV3.1 expression vector. To establish stable clones, MKN28 and MKN45 gastric cancer cells were cultured for 24 h in 12-well plates ($1.0x10^5$ cells/well). The pIRES2-REG Iα or REG Iα iRNA vector (1.6 μg plasmid for each well) was transfected with Lipofectamine 2000 (4.0 μl/well) (Invitrogen), and the pIRES2-EGFP or control siRNA was transfected as negative controls, respectively. After 18 h, the transfectants were replated and selected by growth in medium containing G418 (500 μg/ml) (Merck, Germany) for 8 weeks to obtain positive monoclonal cells.

Reverse transcription PCR and quantitative real-time PCR. REG Iα mRNA expression in gastric cancer cell lines was detected by reverse transcription polymerase chain reaction (RT-PCR) and real-time quantitative reverse transcription PCR (Q-PCR), and REG Iα mRNA from the gastric specimens was detected by Q-PCR. Total RNA (1 μg) was reverse transcribed into cDNA using Oligo (dT) 15 primers and Takara reverse transcriptase (Takara, China). RT-PCR was performed under conditions of 25 cycles of denaturation (30 sec at 95˚C), annealing (30 sec at 54˚C) and extension (30 sec at 72˚C). Q-PCR was performed using the SYBR Green Master Mix kit (Takara) in the ABI 7500 machine (ABI, USA). Human GAPDH was used as an endogenous control, and the expression levels of REG Iα mRNA were determined using the $2^{-\Delta\Delta Ct}$ method. All primers are listed in Table I.

Cell proliferation assay. Cells growth was determined in proliferation assays based on the reagent 3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). Briefly, stable transfected cells were grown into 96-well plates, and 20 μl of MTT (5 mg/ml) was added at 0, 24, 48, 72 and 96 h. The medium was discarded after 4 h. Formazan products were solubilized with dimethyl sulphoxide (DMSO), and the optical density (OD) was measured at 570 nm.

Cell apoptosis analysis. Percentages of cell apoptosis were determined by flow cytometry using the Annexin V-FITC Apoptosis Detection kit (Invitrogen). Cells were gently harvested and plated in 6-well dishes ($5x10^5$ cells/well). The cells were then suspended in 100 μl 1X binding buffer, 5 μl Alexa Fluor 488 annexin V and 1 μl propidium iodide (PI, 100 μg/ml). After a 15-min incubation, the cells were mixed with binding buffer on ice, and the apoptotic distribution of the stained cells was analyzed by flow cytometry (BD, USA).

Gastrin 17 (G17) and H_2O_2 treatment of cells. AGS cells cultured in 96-well plates ($2x10^3$ cells/well) were starved for 24 h and grown in medium containing gastrin-17 (G17) (Sigma, USA) at concentrations of 0, 10^{-8}, 10^{-7} and 10^{-6} mol/l for 0, 24, 48, 72 and 96 h. MKN28 cells stably transfected with the pIRES2-REG Iα or the pIRES2-EGFP vector were seeded in 96-well plates ($2x10^4$ cells/well) and incubated with H_2O_2 (0, 100, 200 and 800 μmol/l) for 6 h. The H_2O_2-containing medium was replaced with fresh medium, and cell activities were evaluated at 0, 24, 48 and 72 h.

Immunoblotting. Cells were harvested, and whole cell lysates were prepared by extraction with radio immunoprecipitation assay (RIPA) lysis buffer containing protease inhibitors. Cytoplasmic and nuclear protein fractions were extracted using a nuclear and cytoplasmic protein extraction kit (Beyotime, China). Total protein (40 μg) was loaded in each well, and the proteins were separated by 12% SDS-PAGE and blotted onto polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with mouse monoclonal anti-caspase-3/Bcl-xL antibody (1:500) (Sigma), mouse monoclonal anti-Bad antibody (1:1,000) (Abnova, Taiwan), or rabbit polyclonal anti-human β-catenin (1:1,000) (Cell Signal, USA) overnight at 4˚C. Horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (1:2,500) (MultiSciences Biotech, China) was used for enhanced chemiluminescence detection with an LAS-4000 image system. β-actin (1:10,000) (Sigma) and lamin B (1:500) (Abcam) antibodies were used to normalize the protein levels.

Statistical analysis. All data were analyzed using SPSS 13.0 statistical software. Continuous variables are expressed as the mean ± SEM. Multi-group data were assessed by the analysis of variance (ANOVA). When comparing the means between two groups, we used LSD for homogeneity of variance and Dunnett's C test for heterogeneity of variance. T-tests were used to compare two groups of continuous variables. Fisher's exact test was used for categorical variables. A P-value <0.05 was considered to indicate statistical significance.

Results

REG Iα overexpression is associated with gastric carcinogenesis and poor differentiation in gastric cancer. We examined REG Iα mRNA expression levels in 102 gastric biopsy specimens and 30 surgically resected gastric cancer tissue samples by Q-PCR. Gastric biopsy specimens were categorized into the following groups: normal gastric tissue without *H. pylori* infection (n=25), active gastritis with *H. pylori* infection (AG, n=20), intestinal metaplasia (IM, n=40) and dysplasia (DYS, n=17). REG Iα expression was significantly upregulated in GC tissues and precancerous lesions (DYS and IM) relative to the normal *H. pylori*-negative gastric tissues (P<0.05). However, there was no statistical significant difference between the mRNA expression of REG Iα between the AG, IM and DYS samples. We determined that REG Iα was highly expressed under these clinical conditions (Fig. 1A).

We further evaluated the relationship between the pathological status of GC and REG Iα mRNA expression levels when matched with general patient characteristics, including age and gender (P>0.05) (Table II). The results showed that REG Iα mRNA was highly expressed in late-stage GC specimens (stage III, stage IV) relative to early-stage specimens (stage I/II) (P<0.01) (Fig. 1B). We also examined REG Iα mRNA expression levels in human differentiated gastric cancer cell lines (AGS, MKN28, MKN45, NCI-N87, BCG-823 and SGC-7901) using RT-PCR and Q-PCR. We found that REG Iα mRNA expression was higher in the less-differentiated AGS and MKN45 gastric cancer cell lines (Fig. 1C). Collectively, the results of these experiments showed that REG Iα overexpression was associated with gastric precancerous lesions and gastric cancer, and may predict poorly differentiated gastric cancer.

REG Iα overexpression enhances, whereas REG Iα down-regulation inhibits, gastric cancer cell growth. To determine whether REG Iα regulates cell proliferation, we stably transfected MKN28 cells, which express low levels of endogenous REG Iα, with the pIRES2-REG Iα plasmid to overexpress REG Iα. We also stably transfected MKN45 cells, which express high levels of endogenous REG Iα, with the pCMV3.1-REGIα siRNA vector to inhibit REG Iα expression. REG Iα expression levels were determined in individual clones by RT-PCR analysis (Fig. 2A). The results of MTT assays showed that cell proliferation was significantly higher in the MKN28 cells overexpressing REG Iα relative to the negative controls at 24 and 48 h (P<0.01) (Fig. 2B). In contrast, cell proliferation was significantly lower in the MKN45 cells with decreased REG Iα expression at 24, 48 and 72 h relative to the negative controls (P<0.01) (Fig. 2C). Taken together, the results of these experiments suggest that REG Iα positively regulates gastric cancer cell growth and proliferation.

REG Iα mediates an anti-apoptotic effect in gastric cancer cells via the Bad/Bcl-xL/caspase-3 pathway. We also carried out experiments to determine whether REG Iα has an anti-apoptotic effect in gastric cancer cells. We found a significantly higher number of early apoptotic cells in REG Iα siRNA-transfected MKN45 cells relative to the control cells (12.96±0.5 vs. 3.99±0.3%, respectively; P<0.001) (Fig. 3A). To induce cell apoptosis, MKN28 cells overexpressing REG Iα were treated with increasing concentrations of H_2O_2 for 6 h. Flow cytometry showed that MKN28 cells overexpressing REG Iα exhibited lower apoptosis relative to the negative controls when exposed to H_2O_2 (200 μmol/l) for 6 h (21.32±1.25 vs. 27.14±0.82%, respectively; P<0.05) (Fig. 3B). The results of the MTT assays showed that cell growth was higher in MKN28 cells overexpressing REG Iα relative to the negative controls following treatment with H_2O_2 (200 μmol/l) for 6 h (OD value: 1.73±0.067 vs 1.44±0.131, P<0.01) (Fig. 3C). We also carried out experiments to determine whether REG Iα

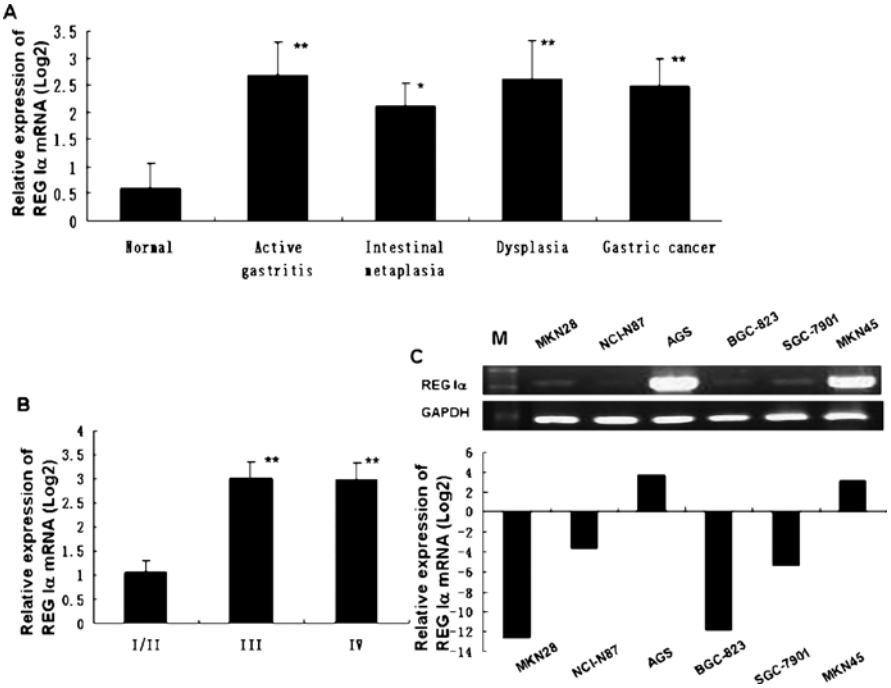

Figure 1. REG Iα mRNA expression levels in gastric tissues and cell lines. (A) Q-PCR analysis of REG Iα mRNA expression levels in gastric cancer tissue, precancerous lesions (dysplasia and intestinal metaplasia) and *H. pylori*-positive active gastritis relative to normal *H. pylori*-negative gastric tissue (Normal). (B) Relative levels of REG Iα mRNA in gastric cancer of stage I/II or III/IV according to the TNM classification system. (C) The expression levels of REG Iα mRNA in various gastric cancer cell lines, including MKN28, NCI-N87, AGS, BGC-823 SGC-7901 and MKN45, as determined by RT-PCR (upper panel) and Q-PCR (lower panel). Bars, ±SE; *P<0.05, **P<0.01.

Table II. Clinical characteristics of the enrolled patients.

					Gastric cancer				
	Normal	Active gastritis	Intestinal metaplasia	Dysplasia	Total	I/II	III	IV	P-value
Number	25	20	40	17	30	9	7	14	
Male/Female	13/12	11/9	18/22	11/6	17/13	5/4	4/3	8/6	P>0.05
Age (years)	52.52±9.25	52.15±7.94	53.75±8.96	57.00±9.96	54.53±10.80	54.44±13.09	52.00±9.42	57.79±9.63	P>0.05
H. pylori (-/+)	25/0	0/20	24/16	13/4	18/12	5/4	5/2	8/6	P<0.05
Tumor size (cm)					5.59±2.63	4.44±3.60	5.91±1.68	6.28±1.82	P<0.05
Differentiation									
High					4	2	0	2	P>0.05
Moderate					6	2	2	2	
Low					5	0	2	3	
Undifferentiated					15	5	3	7	

affects the expression of apoptotic signaling proteins during H_2O_2-induced apoptosis in MKN28 cells. Bad and caspase-3 expression was significantly decreased in MKN28 cells overexpressing REG Iα relative to the negative control cells, whereas Bcl-xL expression was significantly increased, after induction of apoptosis with 200 μmol/l H_2O_2 for 6 h (Fig. 3D). The results of these experiments suggest that REG Iα exhibits a critical anti-apoptotic activity in gastric cancer cells via the Bad/Bcl-xL/caspase-3 pathway.

Gastrin stimulates REG Iα expression and induces β-catenin nuclear translocation in AGS cells. To determine whether gastrin stimulated REG Iα expression, AGS cells were treated with increasing concentrations of gastrin-17 (G17), and cell proliferation was measured. The results of these experiments showed that G17 significantly promoted AGS cell proliferation in a dose-dependent manner (Fig. 4A). Q-PCR experiments showed that REG Iα mRNA levels were elevated 4.18-fold following stimulation with G17 (10^{-6} mol/l) for 96 h (Fig. 4B). We also examined the total, cytoplasmic and nuclear β-catenin levels in AGS cells stimulated with G17 (10^{-7} or 10^{-6} mol/l) for 24 and 72 h. Immunoblot analysis showed that the nuclear β-catenin levels were elevated, whereas cytoplasmic β-catenin levels decreased significantly in the G17-treated AGS cells (10^{-6} mol/l) at 72 h. However, cytoplasmic and nuclear β-catenin levels were unchanged in the AGS cells treated with

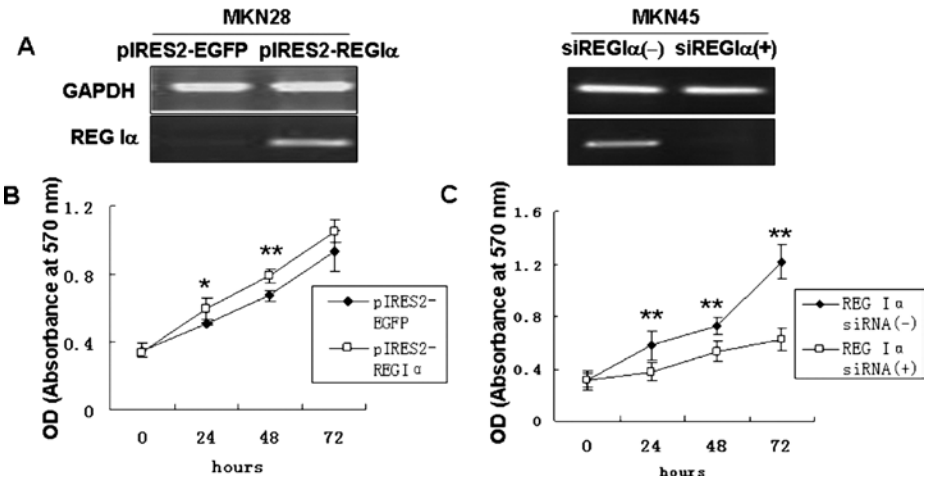

Figure 2. Analysis of REG Iα function in gastric cancer cell proliferation. (A) Results of RT-PCR experiments for REG Iα expression in MKN28 cells stably transfected with the pIRES2-REG Iα expression vector or the pIRES2-EGFP negative control vector. (B) Results of MTT assays to analyze the cell growth of MKN28 cells stably transfected with the pIRES2-REG Iα expression vector or the pIRES2-EGFP negative control vector at selected time points. (C) Results of MTT assays to analyze the growth of MKN45 cells stably transfected with the pCMV3.1-REG Iα siRNA vector or a control siRNA vector at selected time points. OD (optical density, absorbance at 570 nm) values represent the proliferative value, Bars, ±SE; *P<0.05, **P<0.01.

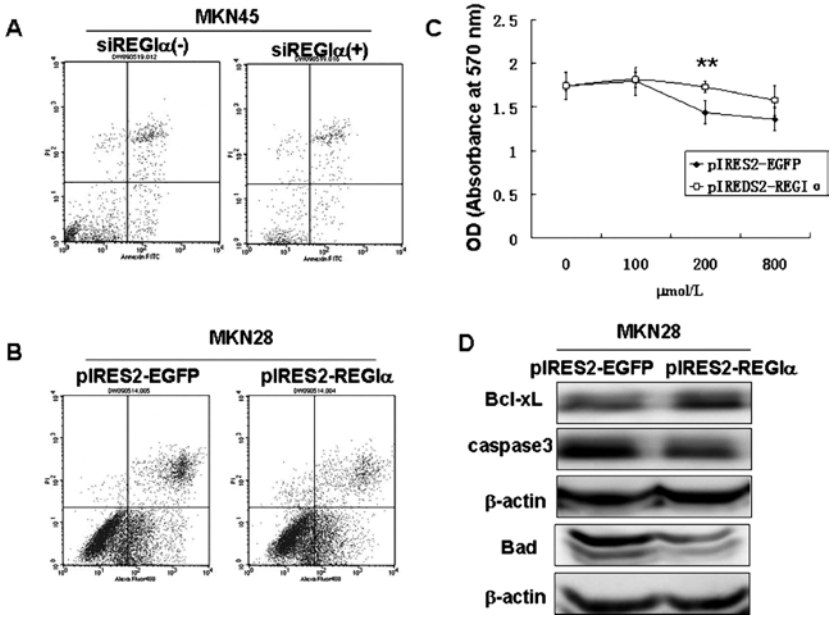

Figure 3. Anti-apoptotic activity of REG Iα in gastric cancer cells associated with the Bad/Bcl-xL/caspase-3 pathway. (A) Annexin V-FITC flow cytometry to determine the percentage of apoptotic cells when MKN45 cells were stably transfected with either the pCMV3.1-REG Iα siRNA vector (12.96±0.5%) or the control siRNA vector (3.99±0.3%). (B) Annexin V-FITC flow cytometry to determine the percentage of apoptotic cells (21.32±1.25 and 27.14±0.82% for MKN28 cells transfected with either pIRES2-REG Iα or pIRES2-EGFP). (C) MTT assays to analyze cell proliferation of MKN28 cells transfected with pIRES2-REGIα or pIRES2-EGFP. REG Iα-overexpressed MKN28 cell showed stronger cell activities than the empty vector transfected cells (1.73±0.067 vs. 1.44±0.131, P<0.01). (D) Immunoblot analysis to determine Bcl-xL, Bad and caspase-3 expression of MKN28 cells stably transfected with either the pIRES2-REG Iα vector or the control vector after treatment with H_2O_2 (200 μmol/l) for 6 h. Bars, ±SE; *P<0.05, **P<0.01.

10^{-7} mol/l G17 (Fig. 4C). The results of these experiments suggest that gastrin may stimulate REG Iα expression and induce β-catenin nuclear translocation in AGS cells.

Discussion

Recent studies have shown that inflammation plays a critical role in the development of cancer, including gastric cancer (4). REG Iα protein is expressed at low levels in non-inflammatory gastric mucosa; in contrast, REG Iα is markedly upregulated in inflammatory conditions and contributes to the maintenance of gastric tissue regeneration (8,12). In the present study, REG Iα expression was found to increase in patients with active *H. pylori* infection gastritis and in a rat model of active gastritis (data not shown).

Notably, studies have shown that REG Iα has the ability to direct gastric cell differentiation towards parietal and chief cells in the neck zone, which is believed to house progenitor

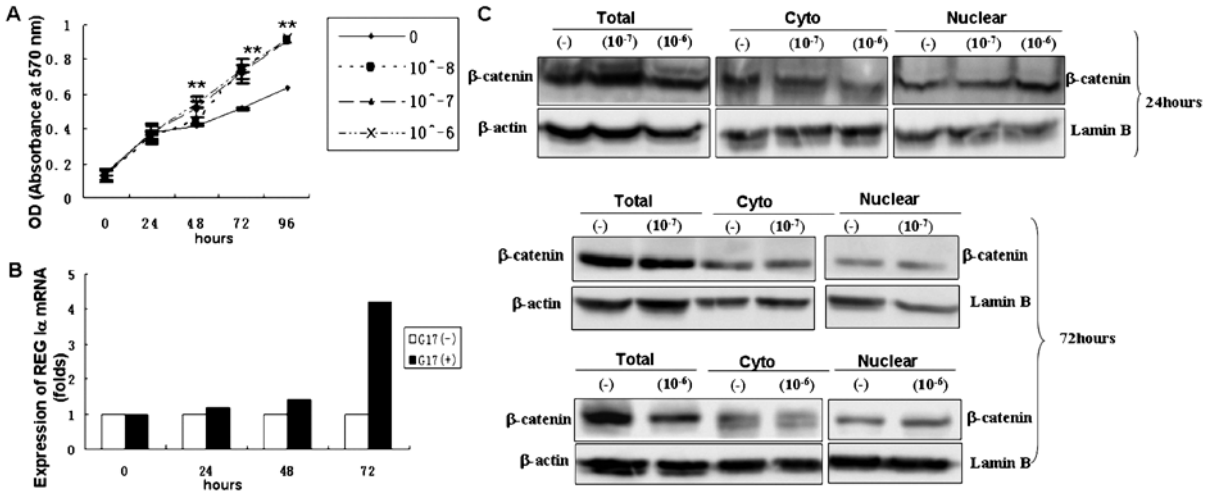

Figure 4. REG Iα and β-catenin expression in AGS cells upon gastrin stimulation. (A) MTT assays to analyze the proliferation of AGS cells treated with different concentrations of gastrin (G17) (0, 10^{-8}, 10^{-7} and 10^{-6} mol/l). (B) Q-PCR analysis of REG Iα mRNA levels in AGS cells incubated with G17 (10^{-6} mol/l). (C) Immunoblot analysis of β-catenin expression in ABS cells treated with G17 (0, 10^{-7} and 10^{-6} mol/l) for 24 and 72 h. β-catenin expression was determined in whole-cell lysates (Total), cytoplasmic fractions (Cyto), and nuclear fractions (Nuclear) of AGS cells. *P<0.05, **P<0.01.

cells of the gastric fundic mucosa (13,14). In general, frequent stimulation of inflammation can lead to destruction and incomplete regeneration of the gastric gland, which predisposes cells to cancer. On the other hand, *in vitro* and *in vivo* studies have shown that inflammatory cytokines, such as CINC-2β and IL-6, may promote REG Iα expression (22). REG Iα may function as a growth factor in gastric gland tissue repair under inflammatory conditions. However, we cannot rule out the possibility that the growth effect of REG Iα was exerted through an indirect mechanism involving the release of other growth factors, such as gastrin. Therefore, it is important to determine whether REG Iα is involved in carcinogenesis associated with gastric inflammation, which may in turn lead to gastric premalignant lesions and eventually gastric cancer, as well as which factors may promote REG Iα expression under these conditions.

A noteworthy finding of this study is that REG Iα was frequently overexpressed not only in gastric cancer tissues, but also in intestinal metaplastic and atypical dysplasia, which are considered precancerous lesions (3,5). Similarly, Harada *et al* (17) reported that REG Iα protein was expressed in hyperplasias and low- or high-grade dysplasia in hepatolithiasis, suggesting that REG Iα may play a role in the early stages of biliary carcinogenesis. A relatively small number of studies have suggested that REG Iα is involved in pancreatic cancer and endocrine pancreas regeneration. In transgenic ElasCCK2 mice that express the gastrin receptor CCK2R, REG Iα protein levels increased during the early steps of pancreatic carcinogenesis (23). Another REG family member, REG IV, was found to be upregulated in gastric IM, but was downregulated during malignant transformation of gastric epithelial cells (24). Unexpectedly, REG Iα expression was unusually high in late TMN stage gastric cancer patients and poorly differentiated gastric cancer cells in the present study. We presumed that gastric cancer cells acquire the ability to produce the REG Iα protein during the multistep carcinogenesis progression, resulting in a considerable growth advantage and the development of malignant phenotypes. The role of REG Iα during the development from gastric premalignancy to gastric cancer should be addressed and clarified in future studies.

We found that REG Iα overexpression enhanced, whereas REG Iα downregulation inhibited, gastric cancer cell growth in *in vitro* experiments. Previous reports found that REG Iα protein stimulated gastric epithelial cell proliferation in a dose-dependent manner (19,25). In the present study, we also showed that REG Iα mediates an anti-apoptotic effect on gastric cancer cells via the Bad/Bcl-xL/caspase-3 pathway. REG Iα protein has been shown to rapidly induce tyrosine phosphorylation of cellular proteins and activate the extracellular-signal regulated kinase (ERK) 1/2 pathway, suggesting that REG Iα may stimulate gastric cancer cell proliferation via the ERK1/2 pathway (26). Sekikawa *et al* (27) reported that REG Iα protein promoted cell proliferation by enhancing Akt phosphorylation and Bcl-xL expression. Furthermore, a recent study suggested that REG Iα protein mediates the anti-apoptotic effect of STAT3 signaling via activation of the Akt/Bad/Bcl-xL pathway by IL-6 stimulation (28). Taken together, it is clear that REG Iα exhibits not only a growth-promoting effect, but also an anti-apoptotic effect on gastric cancer cells. Therefore, silencing the expression of REG Iα through small RNA interfering techniques may provide a useful anticancer target therapy for gastric cancer and should be addressed in future studies.

In the present study, we confirmed that gastrin is an important regulator of REG Iα in gastric cancer cell lines. However, the pathway for gastrin-mediated REG Iα regulation has not yet been elucidated. We found that β-catenin accumulated and translocated to the nucleus when AGS cells were cultured with increasing concentration of gastrin. Therefore, we suggest that gastrin induces REG Iα expression in gastric cancer cell via the β-catenin pathway. A recent study of liver cancer reported that REG Iα and REG IIIα were possible downstream targets of the Wnt/β-catenin

pathway during liver tumorigenesis; β-catenin mutations induced REG Iα and REG IIIα expression in liver cancer cells (21). β-catenin is a transcription cofactor with T-cell factor/lymphoid enhancer factor (TCF/LEF) in the Wnt pathway. Wnt stimulation leads to the inactivation of APC mutations or the activation of β-catenin mutations, resulting in nuclear accumulation of β-catenin, which subsequently complexes with TCF/LEF transcription factors to activate gene transcription (29). Activating mutations of the Wnt/β-catenin pathway have been reported in gastric cancer and adenocarcinoma (30-32). Several individual TCF/LEF target genes have been identified, including c-MYC and cyclin D1, most of which have significant effects on cell proliferation, migration and metastasis. However, many questions remain regarding the possible pathways involved in REG Iα regulation in response to gastrin stimulation.

In summary, increased REG Iα expression was associated with late stages of gastric cancer and precancerous lesions. REG Iα also promoted cell growth and exerted an anti-apoptotic effect on gastric cancer cells. Finally, gastrin stimulated REG Iα expression via a potentially novel mechanism that may involve β-catenin phosphorylation and nuclear translocation. Taken together, these findings suggest a potential role for REG Iα as a novel candidate biomarker for early diagnosis and prognosis of gastric cancer and as a possible therapeutic target.

Acknowledgements

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