

Fas expression is downregulated in gastric cancer

XUMING WANG^{1,2*}, ZHENGQI FU^{1,2*}, YING CHEN^{1,2} and LIJIANG LIU^{1,2}

¹Department of Pathology and Pathophysiology, School of Medicine; ²Department of Pathology, Jiangda Pathology Institute, Jiangnan University, Wuhan, Hubei 430056, P.R. China

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Abstract. The aim of the present study was to investigate Fas expression in tumor samples from patients with gastric cancer, in order to determine the involvement of the Fas signaling pathway. The protein expression levels of Fas, caspase-8, caspase-3 and poly (adenosine diphosphate-ribose) polymerase 1 (PARP1) were examined in gastric cancer specimens and their associations with clinical pathological parameters were analyzed with immunohistochemical staining and western blot analysis. The mRNA expression was quantified with quantitative PCR and apoptosis was examined with a FACScan flow cytometer. The results demonstrated that the downregulation of Fas expression was correlated with less histological differentiation, gender (male), and increased lymph node and distant metastases ($P < 0.05$). In the AGS established gastric cancer cell line, upregulation of the Fas signaling pathway promoted the apoptosis of gastric cancer cells by upregulating the expression of caspase-8 and caspase-3, and downregulating the expression of PARP1. The present study demonstrated that Fas was associated with gastric cancer and promoted the apoptosis of gastric cancer cells via caspase-8, caspase-3 and PARP1. These results suggested that caspase-8, caspase-3 and PARP1 may be triggers of gastric cancer, and upregulation of caspase-8 and caspase-3 expression, or inhibition of PARP1 expression may improve the therapeutic outcome in patients with gastric cancer.

Introduction

Gastric cancer is a frequently occurring cancer, which is responsible for ~989,600 novel diagnoses and ~738,000 cases

of mortality annually worldwide (1-2). The goal of this study was to show relationship between Fas (including its downstream signaling molecules) and gastric cancer. Fas, which is also known as apoptosis antigen-1 or cluster of differentiation 95, is a member of the death receptor family, a subfamily of the tumor necrosis factor receptor superfamily (3). Interactions between Fas and its natural ligand (FasL) or agonistic antibodies induce apoptosis in responsive cells (3). The death-inducing signaling complex (DISC), which contains oligomerized Fas, the adaptor protein Fas-associated death domain (FADD), two isoforms of procaspase-8, procaspase-10 and cellular FADD-like interleukin (IL)-1 β -converting enzyme-inhibitory protein, is formed following FasL stimulation (4), resulting in the induction of programmed cell death or apoptosis (5). Downregulated expression of Fas has been observed in numerous types of tumor, including head and neck, esophageal, pancreatic, non-small cell lung and bladder cancer (4-8).

Gastric cancer with reduced frequency of apoptosis usually increase during tumor progression (9-12). The present study investigated Fas expression in specimens from patients with gastric cancer, in order to determine the involvement of the Fas signaling pathway in gastric cancer.

Materials and methods

Reagents. The rabbit polyclonal anti-Fas (N-18; cat. no. sc-714), rabbit polyclonal anti-caspase-3 (H-277; catalog no. sc-7148), mouse monoclonal anti-caspase-8 (cat. no. sc-81656), mouse monoclonal anti-poly (adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP1) (B-10; cat. no. sc-74470) and mouse monoclonal anti- β -actin (C4; cat. no. sc-47778) antibodies were all purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Polyvinylidene difluoride (PVDF) membranes were purchased from EMD Millipore (Billerica, MA, USA). The radioimmunoprecipitation buffer and enhanced bicinchoninic acid assay kit were purchased from Beyotime Institute of Biotechnology (Haimen, China). The LipoFiter™ Liposomal Transfection reagent was from Hanbio Biotechnology Co., Ltd. (Shanghai, China). The Biotin-Streptavidin HRP Detection System (SP test kit; SP-9000) and diaminobenzidine (DAB) colorization test kit were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). The Annexin V-phycoerythrin (PE)/7-aminoactinomycin D (AAD) apoptosis detection kit was obtained from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China). Reverse

Correspondence to: Dr Lijiang Liu, Department of Pathology and Pathophysiology, School of Medicine, Jiangnan University, Building J12, 8 Sanjiaohu Road, Wuhan Economy and Technology Development Zone, Wuhan, Hubei 430056, P.R. China
E-mail: liulijiang@163.com

*Contributed equally

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Transcription-Polymerase Chain Reaction (PCR) kit was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). THUNDERBIRD® SYBR® quantification PCR Mix was purchased from Toyobo Co., Ltd. Primer sequences were synthesized by Sangon Biotech Co., Ltd.

Human gastric cancer tissue and tissue microarray. A total of 26 frozen tumor samples and their corresponding healthy gastric tissues were obtained from 26 patients with gastric cancer diagnosed between 2006 and 2013 at Jiangnan University Medical School (Wuhan, China) including 20 males and 6 females with an average age of 53.5 years. Formalin-fixed, paraffin-embedded tissue samples were obtained from 113 patients with gastric cancer diagnosed between 2006 and 2013 at Jiangnan University Medical School (Wuhan, China), including 81 males and 32 females with an average age 51.3 years old. All patients had not treatment before the samples were obtained. The 113 gastric cancer tissues were fixed in 10% neutral buffered formalin for >24 h within half an hour following surgical removal and paraffin-embedded with standard procedures. Furthermore, there were 41 cases of normal gastric tissues that were paired to 41 cases of gastric cancer tissues from the 113 gastric cancer tissues were also collected. The 41 cases of normal gastric tissues were collected from the incisal margin where were away from the tumor at least 5 cm. They were all confirmed by two pathologists. The normal gastric tissues were also fixed in 10% neutral buffered formalin and paraffin-embedded, written informed consent was obtained from all patients. These samples were used following approval by the Institutional Review Board. The present study was approved by the ethics committee of the School of Medicine, Jiangnan University (Wuhan, China). A single tissue core, 1.0 mm in diameter and 3-4 mm in depth, was removed from each block using a manual microarray device (Beecher Instruments Inc., Sun Prairie, WI, USA) with a total of 113 gastric cancer tissue cores and 41 paired normal gastric tissues inserted into the recipient paraffin-block. The tissue microarray was sectioned at 4- μ m thickness. Subsequently, 41 gastric cancer tissues from the 113 gastric cancer tissue and 41 corresponding normal gastric tissues were selected from the paraffin-embedded samples for immunohistochemistry (IHC) to analyze Fas expression.

Cell lines. The AGS, BGC823 and SGC7901 gastric cancer cell lines, and GES-1 healthy gastric cell line were obtained from the Cell Center of Basic Medicine, Chinese Academy of Medical Sciences (Beijing, China).

Cell culture. All cells were maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C in an atmosphere containing 5% CO₂.

Fas-green fluorescent protein (GFP) expression plasmid and DNA transfection. The Fas-GFP expression plasmid and the empty expression control vector (pEGFP-N1) were obtained from Dr. Junfeng Zhang (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences,

Beijing, China). All transfections were performed using LipoFiter™ Liposomal Transfection reagent according to the manufacturer's protocol. The AGS cells were plated (6x10⁶ cells) in 100 mm dishes in fresh RPMI-1640 medium containing 10% FBS. The cells were transfected with 4.6 μ g Fas-GFP expression vector or pEGFP-N1 using 4.8 μ l LipoFiter™ reagent. The media was replaced 6 h following transfection and the transfected cells were incubated at 37°C for 48 h. These transfected cells were used for subsequent experiments.

IHC. Immunohistochemical staining was performed on 4 μ m-thick tumor sections of the tissue microarray using a 'two-step' method. The tissue slides were deparaffinized with 100% xylene and rehydrated gradually in an alcohol series. Antigen retrieval was performed with immersing the slides in 0.5 mol/l ethylenediamine tetraacetic acid buffer (pH 8.0), followed by boiling in a water bath. The endogenous peroxidase activity was attenuated by incubation in a 3% hydrogen peroxide/methanol buffer for 10 mins at room temperature. The slides were washed in phosphate-buffered saline (PBS) and blocked according to the protocol from the Biotin-Streptavidin HRP Detection System (SP test kit; SP-9000, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) and subsequently incubated with 1:100 primary antibodies in PBS overnight at 4°C in a humidified chamber. Following incubation, the slides were washed with PBS containing 0.05% Tween-20. The slides were then incubated with the Biotin-Streptavidin HRP Detection System (SP test kit) (SP-9000, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) according to the protocol. The secondary antibody was the Biotin labeled Goat anti-rabbit/mouse IgG from the SP test kit. The slides were then washed, incubated with DAB chromogen and washed with tap water, prior to counterstaining with Mayer's hematoxylin (Sangon Biotech Co., Ltd).

A total of 100 gastric tumor cells per high magnification field with five high magnification fields in total were randomly examined with an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan) and cells that exhibited Fas-positive staining were scored by two pathologists. The normal gastric cells and apoptotic cells were examined as negative controls or positive controls in the same field. The experiments were performed at least three times to ensure reproducibility of results. Fas staining was scored as follows: 0, no staining or staining observed in <10% tumor cells; 1+, faint/barely perceptible staining detected in \geq 10% tumor cells; 2+/3+, moderate/strong staining, respectively, observed in \geq 10% tumor cells. A score of 0/1+ was considered negative and a score of 2+/3+ was considered positive. The immunostained slides were evaluated independently by two pathologists in a blinded-manner. In the majority of cases, the evaluation of the two pathologists was identical and discrepancies were resolved by re-examination and consensus.

RNA extraction and reverse transcription-PCR. Total RNA was extracted from the gastric cancer cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was then reverse transcribed into cDNA using the Reverse Transcription-PCR

kit (M-MuLV First Strand cDNA Synthesis kit; cat. no. B532435; Sangon Biotech Co. Ltd., Shanghai, China) according to the manufacturer's protocol. The reaction volume was 20 μ l per sample. Subsequently, a PCR reaction was carried out using the StepOnePlus Real-Time PCR system (Thermo Fisher Scientific, Inc.) with 35 cycles with a SYBR-Green-based approach and SYBR[®] qPCR mix (LOT:246900, THUNERBIRD; Toyobo Co., Ltd., Osaka, Japan) in a final volume of 20 μ l including 100 ng cDNA and 0.4 pmol/ μ l of each primer. Thermocycling conditions were as follows: An initial denaturation for 1 min at 95°C and 35 cycles consisting of denaturation at 95°C for 15 sec, an annealing step at 56°C for 30 sec and an extension step at 72°C for 30 sec. Primers for Fas, caspase-8, caspase-3, PARP1 and β -actin were designed using Primer 5.0 (Premier Biosoft International, Palo Alto, CA, USA) and were used simultaneously in the same reaction. The following primers were used: Fas, Forward 5'-GGACCCTCTACCTCTGGTT-3', reverse 5'-ACCTGGAGGACAGGGCTTAT-3'; caspase-8, forward 5'-CCAGAGACTCCAGGAAAAGAGA-3', reverse 5'-GATAGAGCATGACCCTGTAGGC-3'; caspase-3, forward 5'-TGGCATTGAGACAGACA-3', reverse 5'-GGC ACAAAGCGACTG-3'; PARP1, forward, 5'-TGATGG GTAGTACCTGTACTA-3', reverse 5'-CAGTTTTATCTACCT GGC-3'; and β -actin, forward 5'-CAACGGCTCCGGCAT GTGC-3' and reverse 5'-CTCTTGCTCTGGGCCTCG-3'. The relative expression of target genes was calculated by the $2^{-\Delta\Delta C_q}$ method (13). The data were presented as the relative quantity of target mRNA normalized to the expression of β -actin mRNA and relative to a calibrator sample. Each experiment was performed at least three times.

Western blot analysis. For western blot analysis, gastric cancer and normal gastric cells were washed with cold PBS and lysed in lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.25 mM EDTA (pH 8.0), 0.1% SDS, 1% Triton X-100 and 50 mM NaF, supplemented with MS-SAFE[™] Protease and Phosphatase Inhibitor Cocktail and phosphatase inhibitors, all obtained from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany). The protein concentrations were determined using an Enhanced Bicinchoninic Acid Protein Assay kit (Beyotime Institute of Biotechnology). The cell lysates were subsequently mixed with loading buffer (Beyotime Institute of Biotechnology), separated by 12% SDS-PAGE and transferred onto PVDF membranes. The PVDF membranes were incubated for 45 min in 2% (wt/vol) bovine serum albumin (BSA, Boster Biological Technology Co, Wuhan, China). The membranes were subsequently probed overnight at 4°C with 1:1,000 (vol/vol) different primary antibodies (the rabbit polyclonal anti-Fas; N-18; cat. no. sc-714; rabbit polyclonal anti-caspase-3; H-277; cat. no. sc-7148; mouse monoclonal anti-caspase-8; cat. no. sc-81656; mouse monoclonal anti-PARP1; B-10; cat. no. sc-74470; and mouse monoclonal anti- β -actin; C4; cat. no. sc-47778; antibodies). Then the membranes were washed with TBS-T (20 mM Tris-HCl; pH 7.6; containing 150 mM NaCl, 0.1% Tween-20) for 5 mins/3 times at room temperature. The membranes were subsequently probed with 1:2,000 (vol/vol) appropriate secondary antibodies (goat anti-mouse IgG-HRP; cat. no.

sc-2005; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and goat anti-rabbit IgG-HRP (cat. no. sc-2004; Santa Cruz Biotechnology, Inc.) for 1 h at 37°C. The membranes were then washed with TBS-T for 5 mins/3 times at room temperature and visualized using enhanced chemiluminescence detection reagents (DNR Bio-Imaging Systems, Ltd., Jerusalem, Israel). The density of the protein bands was assessed using TotalLab analysis software, version 2.01 (Nonlinear USA, Inc., Durham, NC, USA). The above antibody were diluted with the 2% (wt/vol) BSA (Boster Biological Technology Co, Wuhan China).

Apoptotic assay. The cells were stained using an Annexin V-PE/7-AAD apoptosis detection kit according to the manufacturer's protocol, in order to detect early apoptotic cells (Annexin V⁺/7-AAD⁻) and necrotic or late apoptotic cells (Annexin V⁺/7-AAD⁺) by flow cytometry. Briefly, the cells transfected with the Fas-GFP expression vector or the control vector were collected and re-suspended at a density of 1×10^6 cells/ml. The cells were then stained with Annexin V-PE and 7-AAD in binding buffer, according to the manufacturer's protocol. Quantification of the apoptotic cells was performed using a FACScan flow cytometer (Beckman Coulter, Inc., Brea, CA, USA) and data were collected and analyzed using CXP Version 2.2 software (Beckman Coulter, Inc.).

Statistical analysis. Statistical analysis was performed using SPSS software version 12.0 (SPSS, Inc., Chicago, IL, USA). The data are expressed as the mean \pm standard deviation of three replicates and were analyzed using an unpaired Student's t-test and one-way ANOVA. The Pearson's correlation coefficient test was used to assess the strength of the relationship between two continuous variables. $P < 0.05$ was considered to indicate a statistically significant difference. All experiments were performed at least three times to ensure reproducibility of results.

Results

Fas expression and clinicopathological features in gastric cancer. Fas expression was examined in 113 samples of gastric cancer using IHC. Fas expression was primarily detected in the cytoplasm and on the plasma membrane of gastric cancer cells (Fig. 1). Positive expression of Fas was detected in 21 of the 113 samples of gastric cancer. Downregulated Fas expression was correlated with various factors including low histological differentiation ($P=0.012$), male gender ($P=0.00002$), and lymph node ($P=0.022$) and distant metastases ($P=0.026$). However, no correlation was observed with age ($P=0.617$) and T stage ($P=0.173$; Table I). A total of 41 samples of gastric cancer and paired healthy tissues were examined for Fas expression using IHC. Positive staining of Fas was detected in 7 of the 41 samples of gastric cancer and in 36 of the 41 corresponding normal tissues ($P < 0.001$; Table II; Fig. 1). These results indicated that Fas expression was downregulated in gastric cancer.

Fas expression was accompanied by caspase-8, caspase-3 and PARP1 expression. Expression of Fas, caspase-8, caspase-3

Table I. Fas expression and clinicopathological features of gastric cancer.

Factor	Fas expression		P-value
	Positive	Negative	
Gender			
Male	7	74	P=0.00002
Female	14	18	
Age			
≤50 years	5	20	P=0.617
>50 years	16	72	
Histological differentiation			
High	10	17	P=0.012
Medium	6	28	
Low	5	47	
T stage			
T1	1	18	P=0.173
T2	6	21	
T3	8	19	
T4	6	34	
N stage			
N0	13	32	P=0.022
N1-3	8	60	
M stage			
M0	15	41	P=0.026
M1	6	51	

T, tumor stage; N, node stage; M, metastasis stage.

Table II. Fas expression in T and corresponding N tissues.

Tissue	Fas expression		P-value
	Positive	Negative	
T	7	34	P<0.001
N	36	5	

T, tumor tissues; N, normal tissues.

and PARP1 was examined in tumor tissues and corresponding healthy tissues of 26 cases of gastric cancer using western blot analysis. Fas, caspase-8 and caspase-3 expression in gastric cancer tissues was significantly downregulated (Fig. 2) compared with in paired healthy tissues. However, PARP1 expression was higher in gastric cancer tissues compared with healthy gastric tissues (Fig. 2). Fas expression was positively correlated with caspase-8 and caspase-3 expression, and was inversely correlated with PARP1 expression in tumor and normal gastric specimens (Fig. 2). The expression levels of Fas, caspase-8, caspase-3 and PARP1 in AGS, BGC823 and SGC7901 gastric cancer cell lines, and the GES-1 healthy

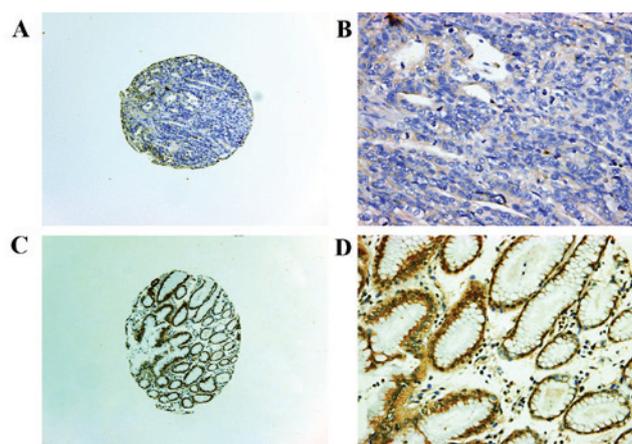


Figure 1. Immunohistochemical staining of gastric cancer tissue and corresponding healthy tissue microarrays. (A and B) Representative negative staining of Fas in gastric cancer tissue microarray (magnification x40 and x400, respectively). (C and D) Representative positive staining of Fas in corresponding healthy tissue microarray (magnification, x40 and x400, respectively).

gastric cell line were also examined. It was observed that Fas, caspase-8 and caspase-3 expression in the gastric cancer cell lines was downregulated compared with the healthy GES-1 gastric cell line. Conversely, PARP1 expression was increased in the gastric cancer cell lines compared with the normal gastric cell line (Fig. 3).

Fas expression and apoptosis in AGS gastric cancer cells.

To examine if Fas expression promotes gastric cell apoptosis, Fas-GFP and the control expression vector were transfected into gastric cancer AGS cells. The transfection efficiency of the vectors was similar as revealed by the green fluorescence in transfected cells (Fig. 4). The PCR and western blotting assays indicated that Fas, caspase-8 and caspase-3 expression was increased in the Fas-transfected cells compared with cells transfected with the control vector ($P<0.05$; Fig. 4). Western blot analysis and PCR revealed that the PARP1 expression was lower in the Fas-expressing cells compared with control cells ($P<0.05$; Fig. 4). Fas, caspase-8, caspase-3 and PARP1 expression did not indicate a difference between the parental AGS cells and the control cells. The flow cytometry results revealed that in the Fas-expressing cells, apoptosis was significantly increased compared with AGS control cells (Fig. 5). These results indicated that increased Fas expression promotes apoptosis in gastric cancer AGS cells.

Discussion

Downregulation of Fas expression has been implicated in tumor progression in various types of cancer, including gastric, ovarian, lung and renal carcinoma (5,14-18), and has been hypothesized to result in reduced tumor apoptosis (19-26). The present study revealed that Fas expression was correlated with caspase-8, caspase-3 and PARP1 expression in the gastric cancer tissues and cell lines.

Caspase-8 is a primary component of the extrinsic apoptotic pathway and the first caspase activated in death receptor-initiated apoptosis (27). Decreased expression of

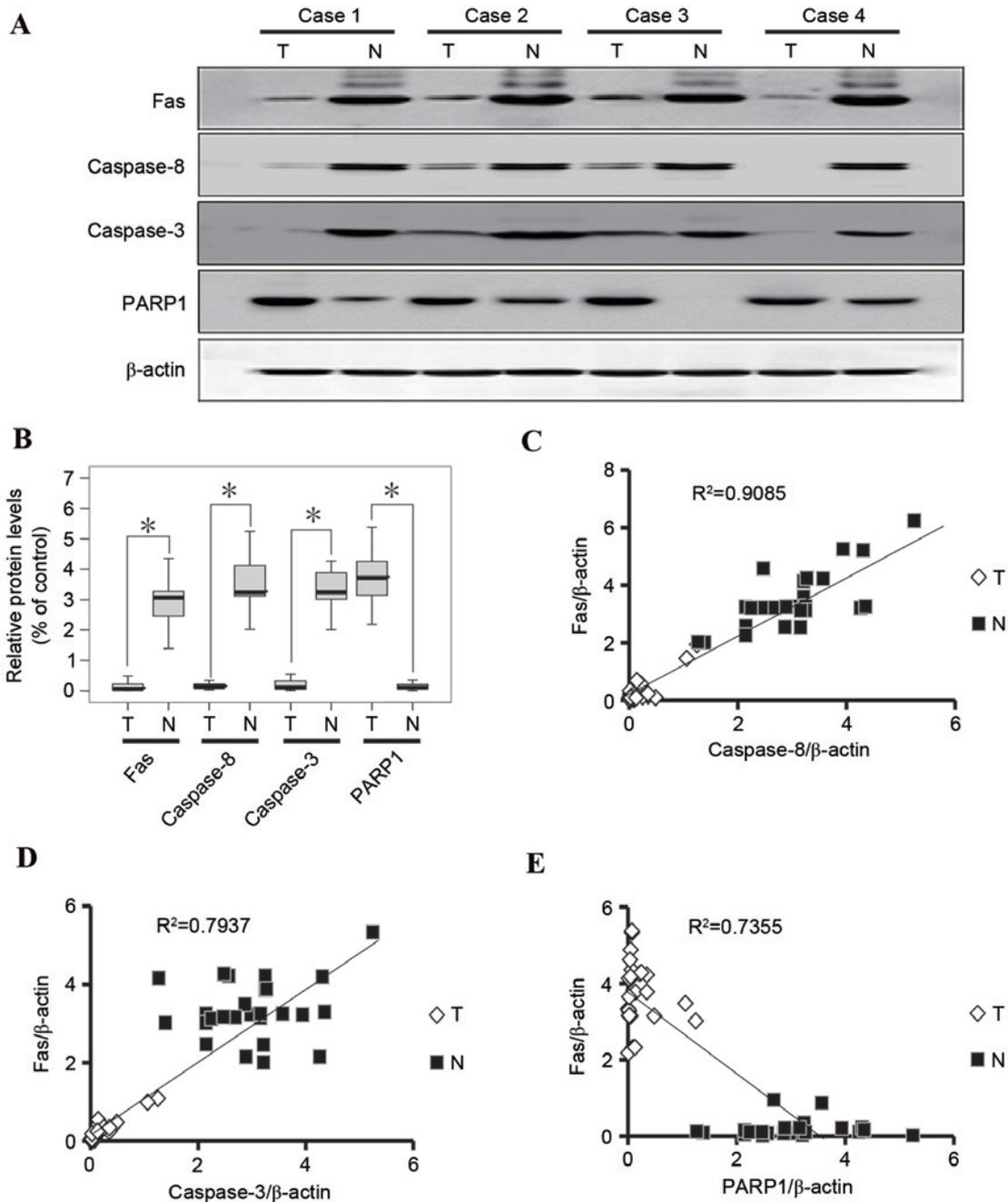


Figure 2. Expression of Fas is accompanied by expression of caspase-8, caspase-3 and PARP1. (A) Representative western blot of four samples. (B) Analysis of the protein expression of Fas, caspase-8, caspase-3 and PARP1 in T specimens and corresponding N tissue (* $P < 0.05$). The correlation between Fas and (C) caspase-8, (D) caspase-3 and (E) PARP1 expression levels in T specimens and corresponding N tissue. T, gastric tumor specimens; N, corresponding normal specimens; PARP1, poly (adenosine diphosphate-ribose) polymerase 1.

caspase-8 has been previously observed in several types of cancer including gastric, colorectal and rectal, hepatocellular, and pancreatic cancers (27). The present study demonstrated that the caspase-8 expression was lower in gastric cancer tissues compared with healthy gastric tissues and was correlated with Fas expression in gastric cancer tissues and cell lines. Previous studies revealed that functional grade purified Fas activated caspase 8 in extrinsic apoptosis, and recruited procaspase-8 to form DISC in extrinsic apoptosis in gastric cancer cells (28,29). Furthermore, a previous study reported that Fas facilitates the caspase-8 dimerization and maturation process (30). The present study demonstrated that Fas

expression was correlated with caspase-8 expression and this was consistent with previously reported data (28-30).

Caspase-3 is a member of the cysteine-aspartic acid protease (caspase)/IL-1 β -converting enzyme family (31) and is activated directly by caspase-8, -9 and -10 via extrinsic and intrinsic pathways to initiate apoptosis. The present study indicated that expression of caspase-3 is lower in gastric cancer tissues compared with healthy gastric tissues. Previous studies revealed that caspase-3 expression was positively associated with Fas expression and FasL expression in human cells (32,33). Furthermore, a previous study indicated that patients with low caspase-3 expression

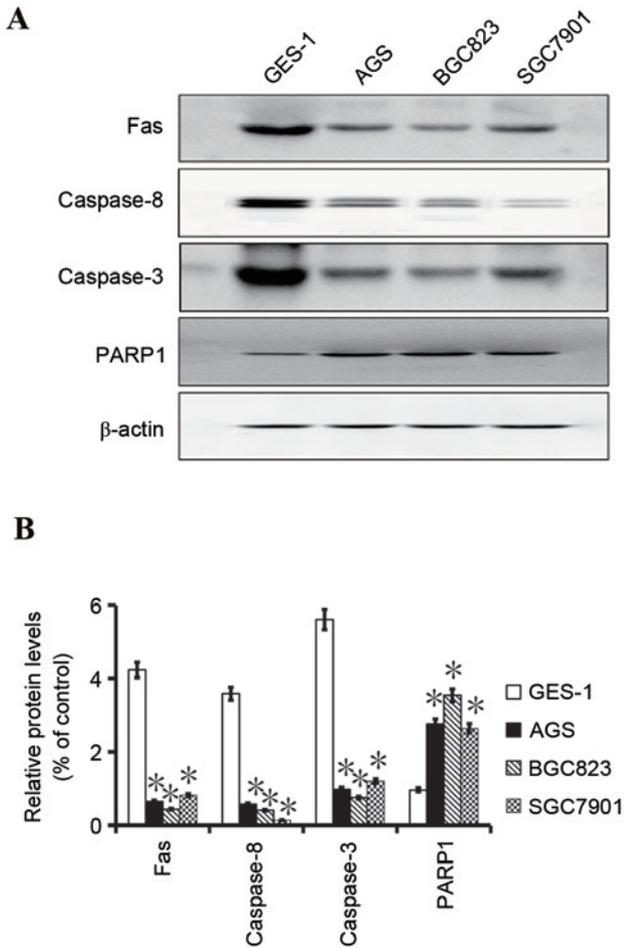


Figure 3. Expression of Fas is accompanied by expression of caspase-8, caspase-3 and PARP1 in gastric cancer cell lines. (A) Western blot and (B) analysis of Fas, caspase-8, caspase-3 and PARP1 expression in AGS, BGC823 and SGC7901 gastric cancer cell lines compared with GES-1 healthy gastric cell line. (* $P < 0.05$ vs. GES-1 control). PARP1, poly (adenosine diphosphate-ribose) polymerase 1.

signified an ominous prognosis in gastric cancer (34). The present study demonstrated that caspase-3 expression was correlated with Fas expression, and this was supported by former reports (32,33,35).

PARP1 is a DNA-binding enzyme, which is important in the base excision repair pathway via detection of DNA strand breaks and poly ADP-ribosylation of nuclear acceptor proteins responsible for DNA repair programs and/or apoptotic decision (36). The results of the present study indicated that PARP1 expression was increased in the gastric cancer tissues compared with healthy gastric tissues. The results indicated that greater PARP1 expression may be a trigger or accompaniment in gastric cancer. In addition, PARP1 expression was decreased as Fas expression increased in the AGS cells, suggesting that inhibition of PARP1 expression may be of therapeutic benefit in patients with gastric cancer.

In summary, the results of the present study demonstrated that increased expression of Fas and its downstream signaling molecules caspase-8 or caspase-3 or inhibition of the expression of PARP1 might improve outcomes in patients with gastric cancer.

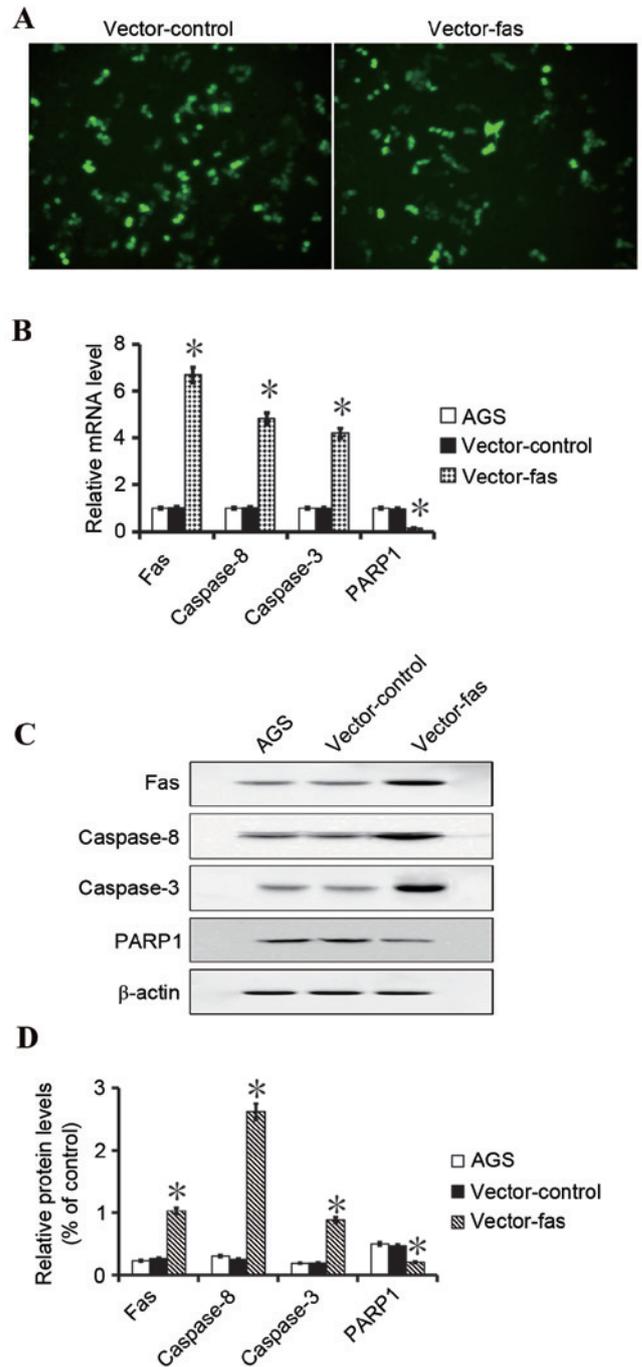


Figure 4. Fas, caspase-8, caspase-3 and PARP1 expression in Fas recombinant AGS gastric cancer cells. (A) Green fluorescence indicates cells successfully transfected with control and Fas-green fluorescent protein expression vectors. (B) mRNA expression levels of Fas, caspase-8, caspase-3 and PARP1 in recombinant AGS gastric cancer cells detected by polymerase chain reaction. (C and D) Protein expression levels of Fas, caspase-8, caspase-3 and PARP1 in recombinant AGS gastric cancer cells detected by western blotting. Data are expressed as the mean \pm standard deviation from three independent experiments (* $P < 0.05$ vs. AGS control cells). PARP1, poly (adenosine diphosphate-ribose) polymerase 1.

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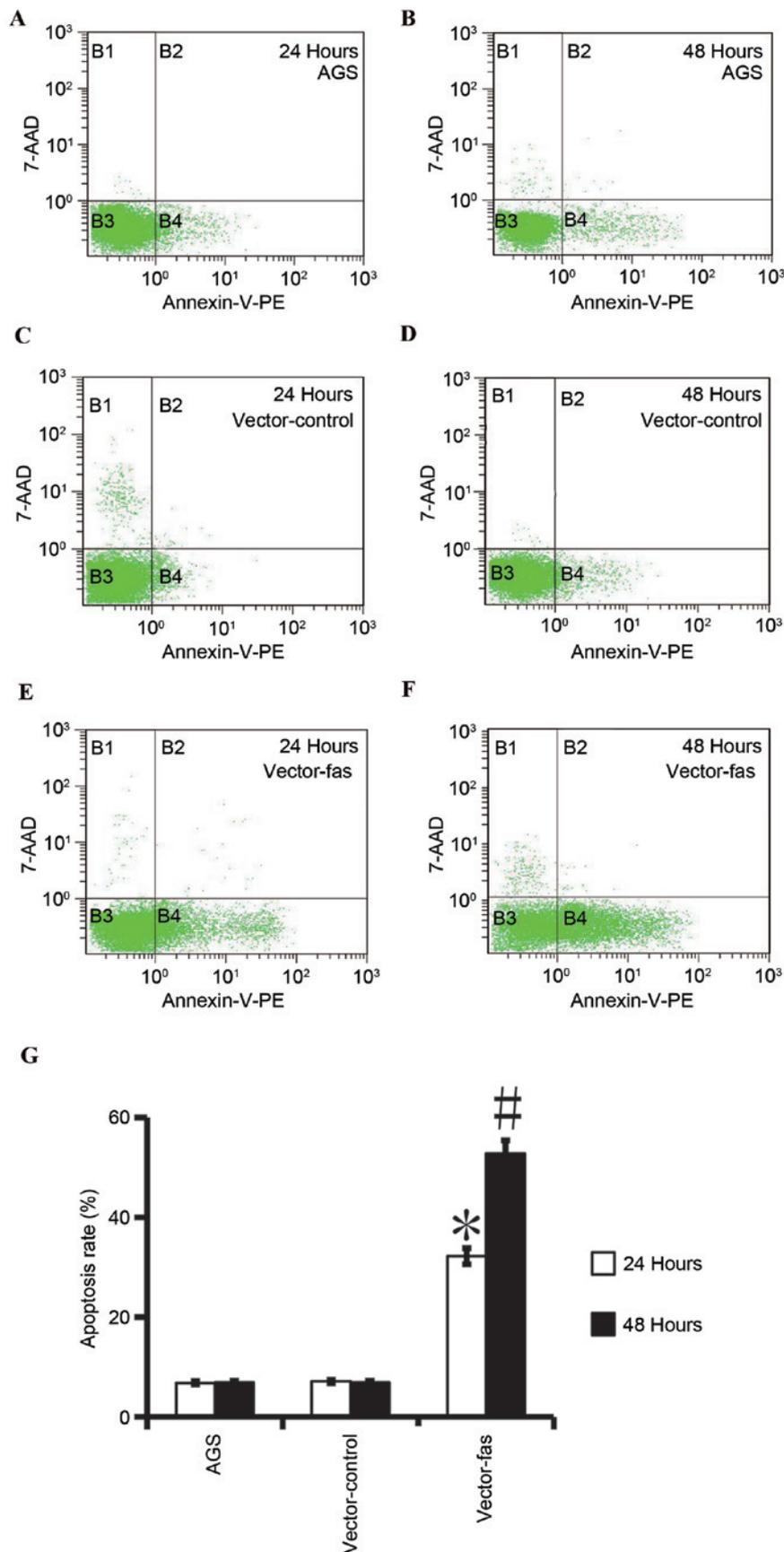


Figure 5. Flow cytometric analysis of Fas-expressing AGS cells. B1 quadrant indicates cell death from mechanical damage, B2 quadrant indicates late apoptotic or necrotic cells, B3 quadrant indicates healthy cells and B4 quadrant indicates early apoptotic cells. Cell apoptosis rate (B2 quadrant and B4 quadrant) at (A) 24 and (B) 48 h. Apoptosis rate of the cells transfected with the control vector at (C) 24 and (D) 48 h. Apoptosis rate of cells transfected with the Fas-green fluorescent protein expression vector at (E) 24 and (F) 48 h. (G) Statistical analysis of apoptosis rate in AGS control and Fas expressing cells. Data are expressed as the mean \pm standard deviation from three independent experiments ($P < 0.05$ vs. AGS control cells at 24h; $\#P < 0.05$ vs. AGS control cells at 48 h). PE, phycoerythrin; 7-AAD, 7-aminoactinomycin D.

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