Fas expression is downregulated in gastric cancer

XUMING WANG[1,2*], ZHENGQI FU[1,2*], YING CHEN[1,2] and LIJIANG LIU[1,2]

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

Received November 10, 2015; Accepted November 10, 2016

DOI: 10.3892/mmr.2016.6037

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)-1β-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, Jianghan 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

Key words: gastric cancer, Fas, caspase-8, caspase-3, poly (adenosine diphosphate-ribose) polymerase 1

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 Jianghan 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 Jianghan 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, Jianghan 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-μm 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% CO_2.

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 ($6x10^6$ cells) in 100 mm dishes in fresh RPMI-1640 medium containing 10% FBS. The cells were transfected with 4.6 μg Fas-GFP expression vector or pEGFP-N1 using 4.8 μl 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 μm-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 ≥10% tumor cells; 2+/3+, moderate/strong staining, respectively, observed in ≥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 μl 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 μl including 100 ng cDNA and 0.4 pmol/μl 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'-GGACCCTCCTACCTCTGGTT-3', reverse 5'-ACCTGGAGGACAGGGCTTAT-3'; caspase-8, forward 5'-CCAGAGACTCCAGGAAAAGAGA-3', reverse 5'-GATAGAGCATGACCCTGTAGGC-3'; caspase-3, forward 5'-TGGCATTGAGACAGACA-3', reverse 5'-GGCACAAAGCGACTG-3'; PARP1, forward, 5'-TGATGGGTAGTACCTGTACTA-3',reverse 5'-CAGTTTTATCTACCTGGC-3'; and β-actin, forward 5'-CAACGGCTCCGGCATGTGC-3' and reverse 5'-CTCTTGCTCTGGGCCTCG-3'. The relative expression of target genes was calculated by the $2^{-\Delta\Delta Cq}$ 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 $1x10^6$ 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 ± 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

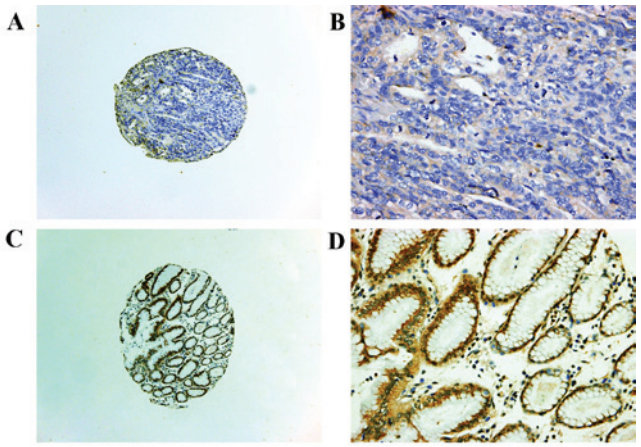

Figure 1. Immunohistochemical staining of gastric cancer tissue and corresponding healthy tissue microarrays. (A and B) Representative negative staining of Fas in gastric cancer tissue microarray (magnification x40 and x400, respectively). (C and D) Representative positive staining of Fas in corresponding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

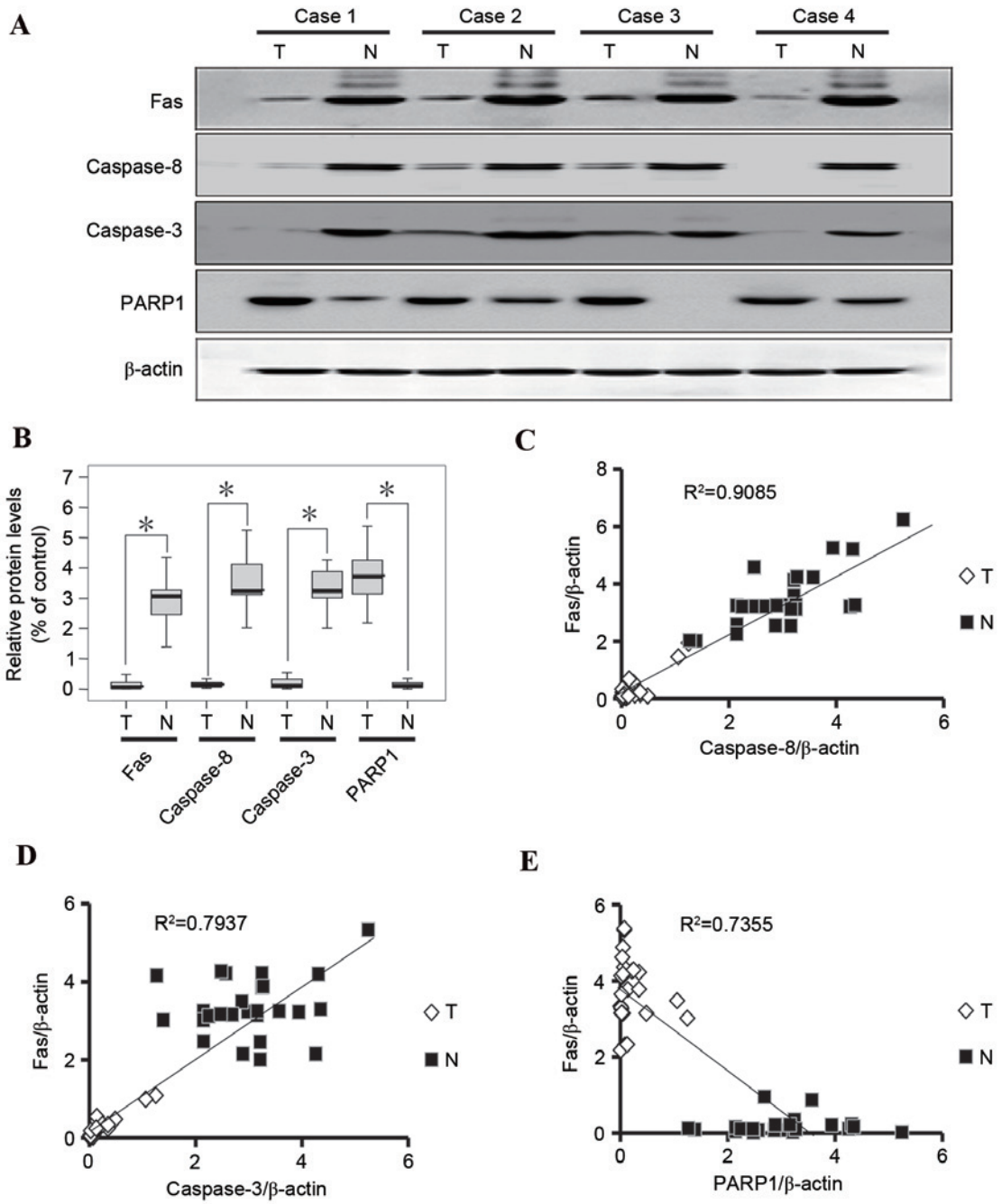

Figure 2. Expression of Fas is accompanied by expression of caspase-8, caspase-3 and PARP1. (A) Representative western blot of four samples. (B) Analysis of the protein expression of Fas, caspase-8, caspase-3 and PARP1 in T specimens and corresponding N tissue (*P<0.05). The correlation between Fas and (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-1β-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

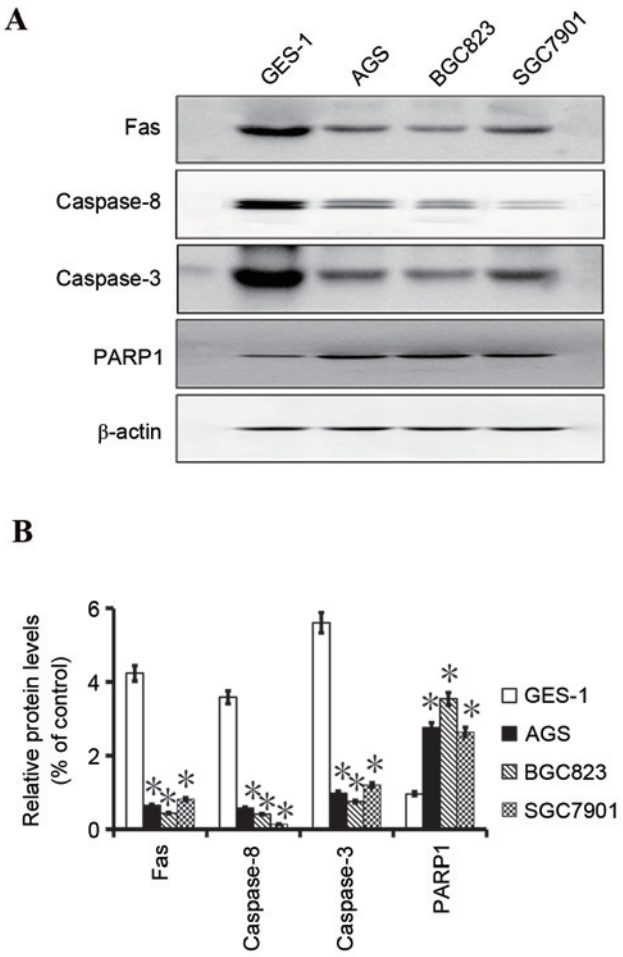

Figure 3. Expression of Fas is accompanied by expression of caspase-8, caspase-3 and PARP1 in gastric cancer cell lines. (A) Western blot and (B) analysis of Fas, caspase-8, caspase-3 and PARP1 expression in AGS, BGC823 and SGC7901 gastric cancer cell lines compared with GES-1 healthy gastric cell line.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

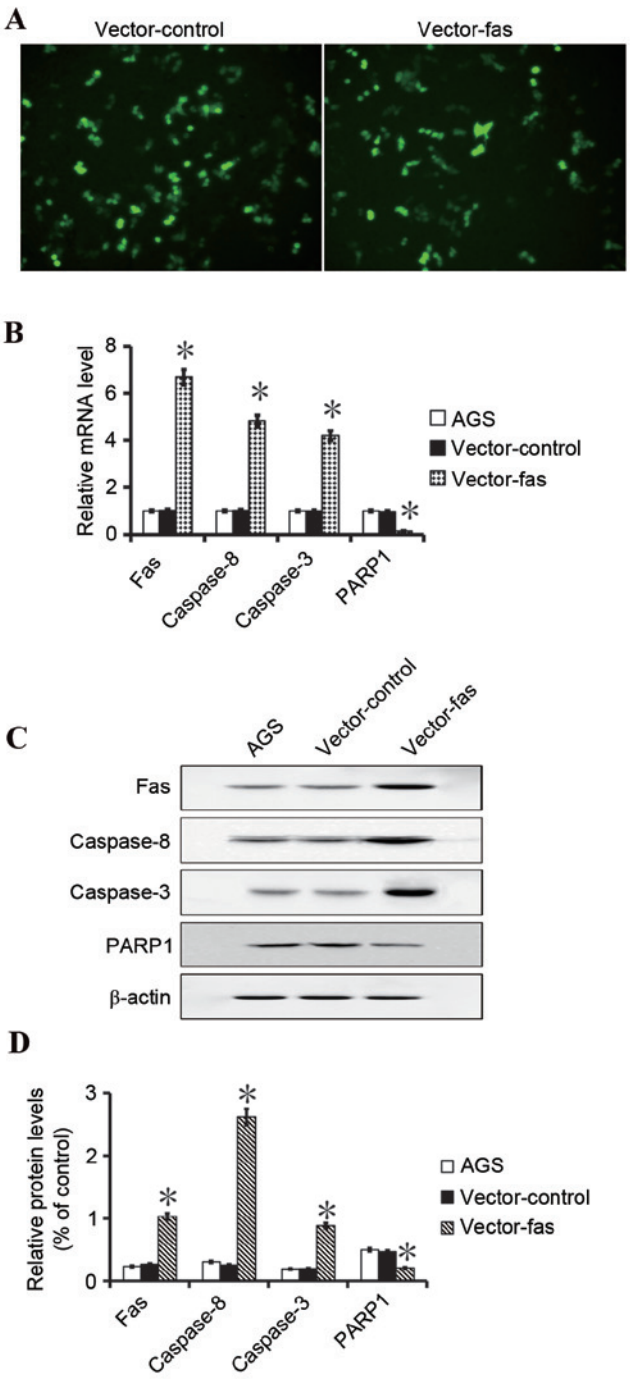

Figure 4. Fas, caspase-8, caspase-3 and PARP1 expression in Fas recombinant AGS gastric cancer cells. (A) Green fluorescence indicates cells successfully transfected with control and Fas-green fluorescent protein expression vectors. (B) mRNA expression levels of Fas, caspase-8, caspase-3 and PARP1 in 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 ± standard deviation from three independent experiments (*P<0.05 vs. AGS control cells). 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.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant nos. 81470110, 81272754 and 30870981) and the Science Foundation of Health Office of Hubei Province (grant no. WJ2015Z059).

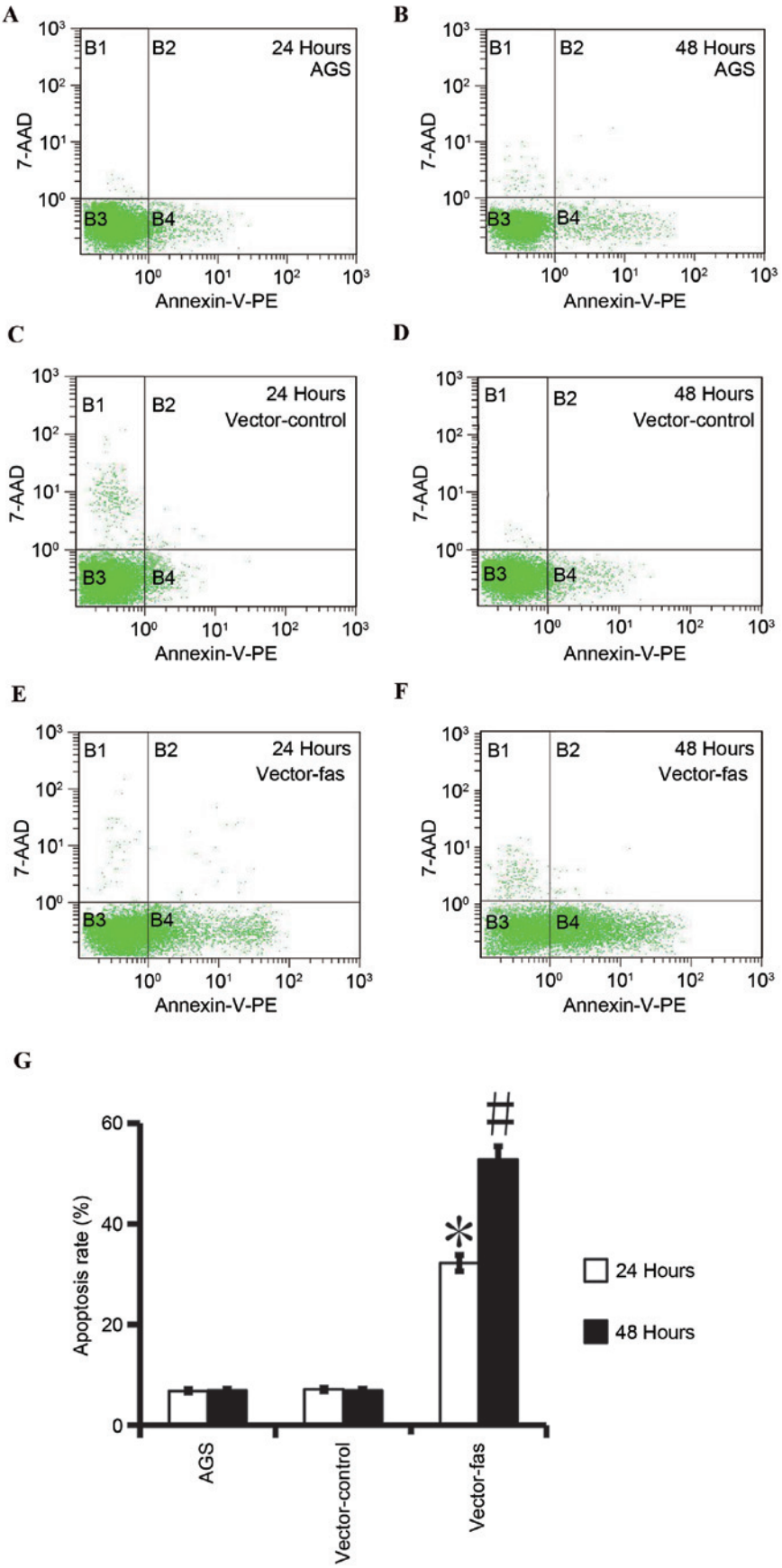

Figure 5. Flow cytometric analysis of Fas-expressing AGS cells. B1 quadrant indicates cell death from mechanical damage, B2 quadrant indicates late apoptotic or necrotic cells, B3 quadrant indicates healthy cells and B4 quadrant indicates early apoptotic cells. Cell apoptosis rate (B2 quadrant and B4 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 ± 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.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
2. Aguiar PN Jr, Tadokoro H, Forones NM and de Mello RA: Treating operable patients with gastric cancer: Macdonald's protocol versus adjuvant chemotherapy. Future Oncol 11: 2247-2249, 2015.
3. Neumann L, Pforr C, Beaudouin J, Pappa A, Fricker N, Krammer PH, Lavrik IN and Eils R: Dynamics within the CD95 death-inducing signaling complex decide life and death of cells. Mol Syst Biol 6: 352, 2010.
4. Laytragoon-Lewin N, Jönson F, Lundgren J, Lundgren J, Rutqvist LE, Wikby A, Löfgren S and Lewin F: Perforin, CD28 and CD95 expression in circulating CD4 and CD8 cells as predictors of head and neck (H&N) cancer patient survival. Med Oncol 31: 290, 2014.
5. He C, Jiang H, Geng S, Sheng H, Shen X, Zhang X, Zhu S, Chen X, Yang C and Gao H: Expression and prognostic value of c-Myc and Fas (CD95/APO1) in patients with pancreatic cancer. Int J Clin Exp Pathol 7: 742-750, 2014.
6. Wang L, Pan XD, Xie Y, Zhang GB, Jiang M, Zheng L, Wang JH, Shi JF and Zhang XG: Altered CD28 and CD95 mRNA expression in peripheral blood mononuclear cells from elderly patients with primary non-small cell lung cancer. Chin Med J (Engl) 123: 51-56, 2010.
7. Gratas C, Tohma Y, Barnas C, Taniere P, Hainaut P and Ohgaki H: Up-regulation of Fas (APO-1/CD95) ligand and down-regulation of Fas expression in human esophageal cancer. Cancer Res 58: 2057-2062, 1998.
8. Watson CJ, O'Kane H, Maxwell P, Sharaf O, Petak I, Hyland PL, O'Rouke D, McKnight J, Canning P and Williamson K: Identification of a methylation hotspot in the death receptor Fas/CD95 in bladder cancer. Int J Oncol 40: 645-654, 2012.
9. Heidari S, Akrami H, Gharaei R, Jalili A, Mahdiuni H and Golezar E: Anti-tumor activity of ferulago angulata boiss. Extract in gastric cancer cell line via induction of apoptosis. Iran J Pharm Res 13: 1335-1345, 2014.
10. Zhao ZG and Shen WL: Heat shock protein 70 antisense oligonucleotide inhibits cell growth and induces apoptosis in human gastric cancer cell line SGC-7901. World J Gastroenterol 11: 73-78, 2005.
11. Liu W, Guo QL, You QD, Zhao L, Gu HY and Yuan ST: Anticancer effect and apoptosis induction of gambogic acid in human gastric cancer line BGC-823. World J Gastroenterol 11: 3655-3659, 2005.
12. Song MQ, Zhu JS, Chen JL, Wang L, Da W, Zhu L and Zhang WP: Synergistic effect of oxymatrine and angiogenesis inhibitor NM-3 on modulating apoptosis in human gastric cancer cells. World J Gastroenterol 13: 1788-1793, 2007.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
14. Peter ME, Hadji A, Murmann AE, Brockway S, Putzbach W, Pattanayak A and Ceppi P: The role of CD95 and CD95 ligand in cancer. Cell Death Differ 22: 885-886, 2015.
15. Zhang Y, Tong S, Guan L, Na F, Zhao W and Wei L: CD95 rs1800682 polymorphism and cervical cancer risk: Evidence from a meta-analysis. Tumour Biol 35: 1785-1790, 2014.
16. De la Rosa AJ, Gomez MA, Morales S, Padillo FJ and Muntane J: CD95 signaling in cancer treatment. Curr Pharm Des 20: 2809-2818, 2014.
17. Li F, Liu Y, Fu T, Tong W and Zhang A: Associations of three common polymorphisms in CD95 and CD95L promoter regions with gastric cancer risk. Tumour Biol 34: 2293-2298, 2013.
18. Martin-Villalba A, Llorens-Bobadilla E and Wollny D: CD95 in cancer: Tool or target? Trends Mol Med 19: 329-335, 2013.
19. Wu J, Nihal M, Siddiqui J, Vonderheid EC and Wood GS: Low FAS/CD95 expression by CTCL correlates with reduced sensitivity to apoptosis that can be restored by FAS upregulation. J Invest Dermatol 129: 1165-1173, 2009.
20. Lavrik IN, Golks A, Riess D, Bentele M, Eils R and Krammer PH: Analysis of CD95 threshold signaling: Triggering of CD95 (FAS/APO-1) at low concentrations primarily results in survival signaling. J Biol Chem 282: 13664-13671, 2007.
21. Yap R, Veliceasa D, Emmenegger U, Kerbel RS, McKay LM, Henkin J and Volpert OV: Metronomic low-dose chemotherapy boosts CD95-dependent antiangiogenic effect of the thrombospondin peptide ABT-510: A complementation antiangiogenic strategy. Clin Cancer Res 11: 6678-6685, 2005.
22. McCabe MJ Jr, Eckles KG, Langdon M, Clarkson TW, Whitekus MJ and Rosenspire AJ: Attenuation of CD95-induced apoptosis by inorganic mercury: Caspase-3 is not a direct target of low levels of Hg2+. Toxicol Lett 155: 161-170, 2005.
23. Strand S, Strand D, Seufert R, Mann A, Lotz J, Blessing M, Lahn M, Wunsch A, Broering DC, Hahn U, *et al*: Placenta-derived CD95 ligand causes liver damage in hemolysis, elevated liver enzymes, and low platelet count syndrome. Gastroenterology 126: 849-858, 2004.
24. Takarada S, Imanishi T, Hano T and Nishio I: Oxidized low-density lipoprotein sensitizes human vascular smooth muscle cells to FAS (CD95)-mediated apoptosis. Clin Exp Pharmacol Physiol 30: 289-294, 2003.
25. Amendola A, Poccia F, Martini F, Gioia C, Galati V, Pierdominici M, Marziali M, Pandolfi F, Colizzi V, Piacentini M, *et al*: Decreased CD95 expression on naive T cells from HIV-infected persons undergoing highly active anti-retroviral therapy (HAART) and the influence of IL-2 low dose administration. Irhan Study Group. Clin Exp Immunol 120: 324-332, 2000.
26. Munker R, Younes A, Cabanillas F and Andreeff M: Soluble CD95 in the serum of patients with low and intermediate grade malignant lymphomas: Absence of prognostic correlations. Leuk Lymphoma 27: 517-521, 1997.
27. Elrod HA, Fan S, Muller S, Chen GZ, Pan L, Tighiouart M, Shin DM, Khuri FR and Sun SY: Analysis of death receptor 5 and caspase-8 expression in primary and metastatic head and neck squamous cell carcinoma and their prognostic impact. PLoS One 5: e12178, 2010.
28. Shi LS, Wang H, Wang F, Feng M, Wang M and Guan WX: Effects of gastrokine-2 expression on gastric cancer cell apoptosis by activation of extrinsic apoptotic pathways. Mol Med Rep 10: 2898-2904, 2014.
29. Liu M, Li CM, Chen ZF, Ji R, Guo QH, Li Q, Zhang HL and Zhou YN: Celecoxib regulates apoptosis and autophagy via the PI3K/Akt signaling pathway in SGC-7901 gastric cancer cells. Int J Mol Med 33: 1451-1458, 2014.
30. Stupack DG: Caspase-8 as a therapeutic target in cancer. Cancer Lett 332: 133-140, 2013.
31. Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH and Peter ME: FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 16: 2794-2804, 1997.
32. Zheng HC, Sun JM, Wei ZL, Yang XF, Zhang YC and Xin Y: Expression of Fas ligand and caspase-3 contributes to formation of immune escape in gastric cancer. World J Gastroenterol 9: 1415-1420, 2003.
33. Nho RS, Peterson M, Hergert P and Henke CA: FoxO3a (Forkhead Box O3a) deficiency protects Idiopathic Pulmonary Fibrosis (IPF) fibroblasts from type I polymerized collagen matrix-induced apoptosis via caveolin-1 (cav-1) and Fas. PLoS One 8: e61017, 2013.
34. Hu Q, Peng J, Liu W, He X, Cui L, Chen X, Yang M, Liu H, Liu S and Wang H: Elevated cleaved caspase-3 is associated with shortened overall survival in several cancer types. Int J Clin Exp Pathol 7: 5057-5070, 2014.
35. Ran X, Diao JX, Sun XG, Wang M, An H, Huang GQ, Zhao XS, Ma WX, Zhou FH, Yang YG and Miao CM: Huangzhi oral liquid prevents arrhythmias by upregulating caspase-3 and apoptosis network proteins in myocardial ischemia-reperfusion injury in rats. Evid Based Complement Alternat Med 2015: 518926, 2015.
36. Hua RX, Li HP, Liang YB, Zhu JH, Zhang B, Ye S, Dai QS, Xiong SQ, Gu Y and Sun XZ: Association between the PARP1 Val762Ala polymorphism and cancer risk: Evidence from 43 studies. PLoS One 9: e87057, 2014.