

Effect of apoptosis on gastric adenocarcinoma cell line SGC-7901 induced by cis-9, trans-11-conjugated linoleic acid

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Results: The growth of SGC-7901 cells was inhibited by c9,t11-CLA. Eight days after treatment with various concentrations of c9,t11-CLA, a monoisomer of c-9, t11-octadecadienoic acid (c9, t11-CLA) which are thought to be high in proportion and inducible by c9,t11-CLA, a monoisomer of c-9, t11-octadecadienoic acid (c9, t11-CLA) and its possible mechanism in the inhibition of cancer cells growth.

Methods: Using cell culture, flow cytometry and immunocytochemical techniques, we examined the cell growth, frequency of apoptosis and distribution of cell cycle, induction of apoptosis by cis-9, trans-11-conjugated linoleic acid (LA), is a minor fatty acid found especially in red meat and in dairy products. The biosynthesis of CLA in ruminants is the result of a rumen bacterium, which is known to convert linoleic acid to stearic acid via CLA. In recent years, CLA has received considerable attention as a chemopreventive agent. This is because CLA was shown to inhibit in vitro the proliferation of human gastric cancer cells(SGC-7901)[1,2], mammary cancer cells(MCF-7)[3-7], of human malignant melanoma cells, colorectal cancer cells[8], and rat hepatoma cells[9] and in animal studies to prevent the development of mouse epidermal carcinogenesis, mouse forestomach cancer[10,11] and of rat mammary tumorigenesis[12,13]. Although the exact mechanisms are not clear, the inhibitory effect of CLA on the proliferation of rapidly dividing cells has been attributed to the induction of cell cycle arrest[13] and the induction of apoptosis[12,14]. In many instances, growth inhibition following terminal differentiation or anticancer drug treatment results in apoptosis. Apoptosis, namely, programmed cell death, is an active and physiological process characterized by a series of morphological and biological alterations in which the cells become smaller, shrinking, the nuclei round up, the chromatin becomes agglutinated and marginated, the nuclear membrane breaks down, and followed by the degenerative changes of cell structure. The exact mechanisms of apoptosis are still unclear, but our earlier studies indicated that CLA can induce apoptosis in human gastric cancer SGC-7901 cells[2]

Gastric cancer is both common in China and the other parts of the world[15-18], and chemoprevention is always used as the main treatment for advanced cancer so far, which has become a focus topic in this area[19-24]. In this study, we investigate the pathways of apoptosis induced by cis-9, trans-11-CLA (c9, t11-CLA) which are thought to be high in proportion and activation as potential antioxidant and anticarcinogenic agents in CLA’s isomers and probe into the possible mechanism of apoptosis on human gastric adenocarcinoma cells SGC-7901.

INTRODUCTION

Conjugated linoleic acid (CLA), a derivative of a fatty acid (LA), is a minor fatty acid found especially in red meat and in dairy products. The biosynthesis of CLA in ruminants is the result of a rumen bacterium, which is known to convert linoleic acid to stearic acid via CLA. In recent years, CLA has received considerable attention as a chemopreventive agent. This is because CLA was shown to inhibit in vitro the proliferation of human gastric cancer cells (SGC-7901)[1,2], mammary cancer cells (MCF-7)[3-7], of human malignant melanoma cells, colorectal cancer cells[8], and rat hepatoma cells[9] and in animal studies to prevent the development of mouse epidermal carcinogenesis, mouse forestomach cancer[10,11] and of rat mammary tumorigenesis[12,13].

Although the exact mechanisms are not clear, the inhibitory effect of CLA on the proliferation of rapidly dividing cells has been attributed to the induction of cell cycle arrest[13] and the induction of apoptosis[12,14]. In many instances, growth inhibition following terminal differentiation or anticancer drug treatment results in apoptosis. Apoptosis, namely, programmed cell death, is an active and physiological process characterized by a series of morphological and biological alterations in which the cells become smaller, shrinking, the nuclei round up, the chromatin becomes agglutinated and marginated, the nuclear membrane breaks down, and followed by the degenerative changes of cell structure. The exact mechanisms of apoptosis are still unclear, but our earlier studies indicated that CLA can induce apoptosis in human gastric cancer SGC-7901 cells[2]

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MATERIALS AND METHODS

Materials

c9, t11-CLA, a monoisomer of c-9, t11-octadecadienoic acid with 98 % purity, was obtained from Dr. Ruilai Liu (Food Science and Toxicology, Department of Food Science, Cornell
University, Ithaca, NY, USA). The c9, t11-CLA was dissolved in 96 ml·L⁻¹ ethanol, and diluted to the following concentrations: 25, 50, 100 and 200 μmol·L⁻¹.

**Methods**

**Cell culture** Human gastric adenocarcinoma cells(SGC-7901), purchased from Cancer Research Institute of Beijing (China), were cultured in RPMI 1640 (Gibco) medium, supplemented with calf serum 100 ml·L⁻¹, penicillin (100×10⁶U·L⁻¹) and streptomycin (100 mg·L⁻¹). The pH was maintained at 7.2-7.4, by equilibration with 5 % CO₂. The temperature was kept at 37°C. The cells were sub-cultured with a mixture of Ethyleneedinitrile tetraacetic acid (EDTA) and trypsin.

**Cell growth curve** The SGC-7901 cells were seeded in six 24-well plates (Nuc, Co.), each well containing 2×10⁴ cells. After 24 h, the medium of different plates was replaced with media supplemented with c9, t11-CLA at different concentrations. On the next day, the numbers of cells of 3 wells from each plate was determined by using the trypan blue staining. The means were obtained on each of eight days and were used to draw a cellular growth curve. The inhibitory rates (IR) on the 8th day was calculated as follows:

\[
\text{IR(\%)} = \frac{\text{Number of cells in negative control (8d)- Number of cells in test groups(8d)}}{\text{Total number of cells in negative control(8d)}} \times 100%
\]

**Apoptosis detection and cell cycle analysis**

SGC-7901 cells (5×10⁴ cells in 25 ml bottles) were seeded in appropriate medium for 24 h prior to the beginning of the experiment. The medium was then replaced with different concentrations of c9, t11-CLA. After 24 h and 48 h, the cells were harvested using a mixture of trypsin/EDTA, washed twice with cold PBS, fixed in 70 % ethanol on ice for 30 minutes, and washed once again. Cells were then stained by adding 1 ml of PI mixture (containing 50 μg propidium iodide, 0.2 mg RNase, 5 μL Triton X-100, and 1 mg citromalic acid) in the dark (4°C, 30 minutes). Cell apoptosis and cell cycle analysis were subsequently performed by flow cytometry using a FACSCalibur Analyzer (BD Biosciences) with a 15-milliwatt air-cooled argon laser (excitation=488 nm). Sub G₀ peak was observed and DNA content in phases of cell cycle was analyzed using software of Modifux LT.

**Cell samples**

SGC-7901 cells were treated for 24 h and 48 h with various concentrations of c9, t11-CLA and collected by centrifugation. Specimens were fixed immediately in 40 g·L⁻¹ formaldehyde polymersiam and embedded in paraffin. Gastric cancer tissue from a patient served as a reference.

**Primary antibody**

To examine the expression of Ki67 cell proliferation and the expression of bcl-2, c-myc and Fas in SGC-7901 cells, we used four primary antibodies: corresponding rabbit polyclonal antibodies for bcl-2 and Fas and corresponding mouse monoclonal antibodies for ki67 and c-myc. Antibodies of bcl-2 and Fas were purchased from the Calbiochem Co. USA; and others from Zhongshan Co. China.

**Immunocytochemistry**

Immunocytochemical staining was performed on serial sections at room temperature using the horseradish peroxidase method. The sections were deparaffinized in xylene and rehydrated through graded alcohol. The sections were incubated for 10 min at 95°C in 10 mmol·L⁻¹ sodium citrate(pH 6.0) buffer for ki67 staining. Endogenous peroxidases were inactivated by immersing the sections in hydrogen peroxide for 10 min, and then were incubated for 10 min with 100 ml·L⁻¹ normal goat serum in PBS to block the non-specific binding. The sections were subsequently incubated overnight at 4°C with relevant antibodies(1:50 dilution) respectively. The next day, the sections were incubated with biotinylated anti-mouse or anti-rabbit IgG (Zhongshan Co., China) for 30 min, followed by peroxidase-conjugated streptavidin (Zhongshan Co., China) for 30 min. The chromogenic reaction was developed with DAB (diaminobenzidine) for 10 min, and all sections were counterstained with hematoxylin. Controls consisted of omission of the primary antibody. The positive rate (PR) was calculated as follows:

\[
\text{PR(\%)} = \frac{\text{Number of positive cells}}{\text{Total number (2×10⁴)}} \times 100%
\]

**Statistical analysis**

Analysis of data was performed using the Student’s t test or χ² test. A value of P<0.05 is considered to be statistically significant.

**RESULTS**

**Effect of c9, t11-CLA on SGC-7901 cell growth**

As shown in Figure 1, growth of the cells in various concentrations (except for 25 μmol·L⁻¹ and 50 μmol·L⁻¹) of c9, t11-CLA did differ from the negative control within 8d. SGC-7901 cells incubated in 25 μmol·L⁻¹ of c9, t11-CLA grew at a higher rate than that of the negative control, while in 100 and 200 μmol·L⁻¹ concentrations of c9, t11-CLA, proliferation of SGC-7901 cell was significantly inhibited. The inhibitory rate of various c9, t11-CLA concentrations were 5.9 %, 20.2 %, 75.6 % and 82.4 %, respectively.

**Analysis of flow cytometry**

To investigate the influence of c9, t11-CLA on apoptosis and cell cycle progression of SGC-7901 cells, we determined apoptosis and cell cycle distribution by flow cytometry. The results are shown in Table 1. We observed that apoptotic peaks and intention of the cells accumulating in the G₀/G₁ phases and of cells decreased in the S phase of the cell cycle in SGC-7901 cells with different c9, t11-CLA concentrations at various time periods, whereas G₂/M did not. The results suggested that c9, t11-CLA may induce apoptosis and arrest the progression of cell cycle of SGC-7901 cells.

**Cell proliferation**

Ki67 is a marker for the proliferation of cells. To determine the
effect of c9, t11-CLA on the proliferation of SGC-7901 cells, we investigated the expression of Ki67 using immunocytochemistry. The results are shown in Figure 2. Expression rates of Ki67 on SGC-7901 cells gradually decreased after SGC-7901 cells were incubated with different concentrations of c9, t11-CLA at various time periods. Moreover, SGC-7901 cells expressed significantly less Ki67 than did the negative control \((P < 0.01)\). The expression rate of Ki67 on SGC-7901 cells displayed a dose-response relationship as the concentrations of CLA increased.

### Table 1: Cell cycle analysis of SGC-7901 cells induced by c9, t11-CLA at 24 h and 48 h \((x \pm s, n = 4)\)

| c9,t11-CLA (µmol) | Apoptosis | G0/G1 | G2/M | S |
|-------------------|-----------|-------|------|---|
| 24 h | 48 h | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h |
| 0 | 0.55±0.09 | 0.69±0.21 | 53.13±3.44 | 58.57±0.90 | 11.13±2.75 | 12.64±1.18 | 35.74±2.04 | 28.80±0.89 |
| 25 | 1.27±0.73 | 3.89±2.12 | 53.00±8.35 | 56.81±0.09 | 15.55±1.11 | 15.95±0.36 | 31.40±9.23 | 26.52±1.46 |
| 50 | 4.12±0.55 | 8.18±1.55 | 56.58±0.87 | 56.32±0.78 | 14.55±3.45 | 15.17±0.61 | 28.87±3.32 | 28.51±1.32 |
| 100 | 7.95±0.35 | 12.33±5.53 | 58.35±2.44 | 61.18±4.94 | 13.12±1.00 | 14.99±3.10 | 28.52±0.96 | 25.08±2.85 |
| 200 | 12.79±1.12 | 14.75±5.97 | 60.67±4.28 | 63.82±7.84 | 12.95±4.48 | 13.35±3.91 | 26.38±0.92 | 22.82±4.63 |

![Figure 2](image)

**Figure 2** Expression of Ki67 on SGC-7901 cells treated with c9, t11-CLA

**Expressions of bcl-2, Fas and c-myc**

We detected the expression of bcl-2, Fas, and c-myc on SGC-7901 cells treated by various concentrations of c9, t11-CLA with immunocytochemical technique. The expression rates of bcl-2 and c-myc (Figure 3B, 3D) on SGC-7901 cells was decreased (Table 2) after SGC-7901 cells were incubated with different concentrations of c9, t11-CLA for 24 h and 48 h while expression of Fas increased (Table 2; Figure 3C). In the meantime, there was no expression of Fas at doses of c9,t11-CLA (25 and 50 µmol·L⁻¹ at 24 h).

### Table 2: Positive rates of bcl-2, Fas and c-myc on SGC-7901 cells treated with c9, t11-CLA (%)

| C9,t11-CLA (µmol/L) | 24 h bcl-2 | 48 h bcl-2 | 24 h Fas | 48 h Fas | 24 h c-myc | 48 h c-myc |
|---------------------|-------------|-------------|---------|---------|-----------|-----------|
| 0 | 6.80 | 6.00 | 5.50 | 8.00 | 0.45 | 5.30 |
| 25 | 4.30 | 4.00 | 4.85 | 8.05 | 0.85 | 4.75 |
| 50 | 2.50 | 2.40 | 4.20 | 8.05 | 0.85 | 4.75 |
| 100 | 1.45 | 1.95 | 3.80 | 3.80 | 0.30 | 4.10 |
| 200 | 0.15 | 2.75 | 2.20 | 0 | 5.95 | 0.30 |

\(P < 0.05, ^aP < 0.01\) vs negative control.

![Figure 3](image)

**Figure 3** A: The expression of Ki67 on SGC-7901 cells of the negative control. Immunocytochemistry staining SP method, original magnification, ×400; B: The expression of bcl-2 on
SGC-7901 cells of the negative control. Immunocytochemistry staining SP method, original magnification, ×400. C: The expression of Fas on SGC-7901 cells of c9, t11-CLA group (200 µmol·L⁻¹/48 h). Immunocytochemistry staining SP method, original magnification, ×400; D: The expression of c-myc on SGC-7901 cells of the negative control. Immunocytochemistry staining SP method, original magnification, ×400

**DISCUSSION**

CLA is a natural fatty acid in animal’s food. CLA has a mixture of positional (9/11 or 10/12 double bonds) and geometric (various cis/trans combinations) isomers of linoleic acid (LA) formed by rumen and colonic bacteria. There are eight potential isomers of CLA, but the cis 9, trans 11 and trans 9, cis 11 isomers are thought to be active potential antioxidant and anticarcinogenic agents. Therefore, it is of interest to investigate extensively the mechanism of anticancer activities of CLA.

Over the past ten years, a number of animal experiments have supported the observation that CLA is an effective chemopreventive agent for cancer, and that it can inhibit carcinogenesis of different tissues at various stages of induction by chemical agents[14,15]. Several investigators in our group have reported that c9, t11-CLA is an effective agent to prevent carcinogenesis[10,11] and cancer[13,17]. Zhu’s study[10] demonstrated that c9, t11-CLA could significantly inhibit the mice forestomach neoplasia induced by B(a)P (50 mg·kg⁻¹) in post-initiation in short term (23 weeks). The incidences of tumors in mice of B(a)P group, B(a)P with high dose CLA (5 µL·g⁻¹) group and B(a)P with low dose CLA (2.5 µL·g⁻¹) group were 100 %, 60 % and 69 %, respectively (P<0.05). Xue’s study[10] also indicated that the incidence of neoplasm in mouse forestomach in the B(a)P group, 75 % pure c9, t11-CLA group, 98 % pure c9, t11-CLA group and 98 % pure t10, c12-CLA group were 100.0 %, 75.0 %, 69.2 % and 53.8 %, respectively. This may be due to an inhibiting mitogen of activated protein kinase (MAPK)- an approach to reduce carcinogenesis.

The data in this series suggested that c9,t11-CLA could inhibit the proliferation of cancer cells, i.e. SGC-7901 cells[14] and MCF-7 cells[15], and induced cancer cell (SGC-7901) apoptosis[21]. Liu’s study[14] indicated that cell growth and proliferation and DNA synthesis of SGC-7901 cells were inhibited and SGC-7901 cells preincubated in media supplemented with different c9,t11-CLA concentrations at various times significantly decreased the expressions of PCNA (the expression rates were 7.2-3.0 %, at 24 h and 9.1-0.9 % at 48 h, respectively), Cyclin A (11.0-2.3 %, at 24 h and 8.5-0.5 %, at 48 h), B (4.8-1.8 % at 24 h and 5.5-0.6 % at 48 h) and D1, (3.6-1.4 % at 24 h and 3.7-0.0 % at 48 h) as compared with those in the negative controls (the expressions of PCNA, cyclin A, B, and D1 were 6.5 % at 24 h and 9.0 % at 48 h, 4.2 % at 24 h and 5.1 % at 48 h, 9.5 % at 24 h and 6.0 % at 48 h, respectively) (P<0.01), whereas the expressions of p16[14] and p21[16], cyclin-dependent kinases inhibitors (CDKI) were increased. Our results showed that the proliferation marker Ki67 was inhibited and the cells were accumulating in the G0/G1 phase and cells decreasing in the S phases of the cell cycle on SGC-7901 cells with different c9, t11-CLA concentrations at various time periods, whereas G2/M did not have. All these results suggested that c9, t11-CLA may arrest the progression of cell cycle of SGC-7901 cells. Our previous works[21] indicated that at the early stage morphological changes of cell apoptosis were observed using fluorescent dye (Hoechst 33342) under electronic microscope and SGC-7901 cells preincubated in media supplemented with different c9, t11-CLA concentrations at various times significantly decreased the expressions of mutant p53 as compared with those in the negative control. The inhibitory rates of mutant p53 on SGC-7901 cells induced by various c9,11-CLA concentrations (25-200 µmol·L⁻¹) were -19.2 %, 13.7 %, 53.4 %, and 89.0 % at 24 h and 1.8 %, 29.1 %, 87.3 %, and 86.8 % at 48 h, respectively. p53 is one of the major factors controlling cell proliferation, suppressing both growth and transformation of cells. A common idea[15] is that p53 acts as “guardian of the genome” by preventing damaging of DNA in cell proliferation or DNA damage leads to an increase in the level of p53, resulting in p21[17,18,19], -mediated cell cycle arrest in the G, phase, which persists until DNA repair is completed or by arresting the cell division cycle induces damaged-cell apoptosis. In the absence of functional wild-type p53, the “guardian” function is lost; cells accumulate genetic damage and show marked genetic instability, often to the extent of gross aneuploidy. However, wild-type p53 protein has a very short half-time, many point mutants have a greatly enhanced stability, allowing for the immunohistochemical detection of mutant p53 in clinical material. Mutant p53 protein loses its biochemical functions which may facilitate DNA repair as well as apoptosis, and displayed over-proliferation of cancer cells. In addition, Natalie et al[20] found that a fraction of p53 protein localizes to mitochondria at the onset of p53-dependent apoptosis. The accumulation of p53 to mitochondria is rapid (within 1 h after p53 activation) and precedes changes in mitochondrial membrane potential, cytochrome c release, and procaspase-3 activation. Overexpression of anti-apoptotic bcl-2 inhibits signal-mediated mitochondrial p53 accumulation and apoptosis but not cell cycle arrest. Our results showed that c9, t11-CLA may reduce the expression of mutant p53 protein and may recover the bio-function of wild p53 protein that blocks the cell cycle of SGC-7901 cells by p21[17,18,19] and processes to another apoptotic pathway of mitochondria by bcl-2.

At the same time, we investigated further the expressions of bcl-2 and Fas from pathways of cell apoptosis such as mitochondria and Fas-associated death domain (FADD) as well as expression of c-myc on SGC-7901 cells treated with various concentrations of c9,t11-CLA. Bcl-2 is an inhibitor of apoptosis and shown to exert anti-apoptotic activity by one of the following mechanisms[21]: 1) sequestration of the proforms of two major initiator caspases, pro-caspase-9 (through binding to Apaf-1) and pro-caspase-8 (through unidentified molecules); 2) inhibition of apoptotic mitochondrial changes, including cytochrome c release and the mitochondrial membrane potential (ΔΨ) loss resulting in AIF (apoptosis-inducing factor) release, as demonstrated using isolated mitochondria bearing endogenous bcl-2 and recombinant forms of these proteins; and 3) Inhibition of accumulation of p53 in mitochondrial membrane[31]. Fas is a potent inducer of apoptosis in tumor cells but not in normal cells. Fas/CD95 requires ligand receptor (FasL/CD95L) which binds to FasL and via downstream signaling molecules FADD activates caspase-8. Fas binding to FasL can activate two routes downstream of caspase-8 activation[31]: type I apoptosis signaling: direct activation of effector’s caspases by caspase-8; type II apoptosis (inhibition of bcl-2 overexpression): cleavage of proapoptotic members of bcl-2 family, ΔΨ, release of cytochrome c and activation of caspase-9. The present study indicated that expression of bcl-2 protein decreased and expression of Fas increased on SGC-7901 cells induced by c9, t11-CLA as compared with that in negative control. c9, t11-CLA may induce apoptosis via pathways of bcl-2-associated mitochondria, p53-associated cell cycle and FADD on SGC-7901 cells. The relationship among p53, bcl-2 and Fas is still
unclear in the effect of apoptosis on SGC-7901 cells induced by c9, t11-CLA. The c-myc oncogene product (c-myc) is a transcription factor that dimerizes with Max and recognized E-box sequence, and it plays key functions in cell proliferation, differentiation and apoptosis[90]. C-myc expression can not only promote proliferation but also induce or sensitize cells to apoptosis. Overexpression of c-myc under the circumstances that this gene is usually down regulated such as serum deprivation, results in apoptotic cells in nonhepatic cells and in a hepatoma cell line[80]. But our result showed that expression of c-myc with 10 % calf serum was lowered with c9, t11-CLA in SGC-7901 cells. We have not known the reason why expression of c-myc on SGC-7901 cells is lower than that in negative control, and this needs further studies.

In conclusion, c9, t11-CLA may inhibit proliferation and induce apoptosis by decreasing in the expression of ki67, bcl-2 and increasing that of Fas in SGC-7901 cells. This result suggested that the inhibition effect of c9, t11-CLA on SGC-7901 cell proliferation is related to the pathways of bcl-2-associated mitochondria, p53-associated cell cycle and FADD on SGC-7901 cells. In the meantime, we found that expression of c-myc in SGC-7901 cell line is related to the pathways of bcl-2-associated mitochondria, p53-associated cell cycle and FADD. The proliferative effect of conjugated linoleic acid (CLA) inhibits growth of human mammary cancer cells (MCF-7), Chang[21]: 87-94; and Colonic mucosa in 1,2-dimethyl-4-phenylimidazo[4,5-b]pyridine (PhIP). Cell Death Differ 2001; 8: 129-134. Tovey FI, Hobsley M. Post-gastrectomy patients need to be followed up for 20-30 years. World J Gastroenterol 2000; 6: 45-48. Pan QS, Fang ZP, Zhao YX. Immunocytochemical identification and localization of APUD cells in the gut of seven stomachless teleost fishes. World J Gastroenterol 2000; 6: 96-101. Chen GY, Wang DR. The expression and clinical significance of CD44v in human gastric cancers. World J Gastroenterol 2000; 6: 125-127. Xu CT, Huan LT, Pan BR. Current gene therapy for stomach carcinoma. World J Gastroenterol 2001; 7: 752-759. Fang DC, Yang SM, Zhou XD, Wang DX, Luo YH. Telomere erosion is independent of microsatellite instability but related to loss of heterozygosity in gastric cancer. World J Gastroenterol 2001; 7: 522-526. Xu AG, Li SG, Liu JH, Gan AH. Function of apoptosis and expression of the proteins Bcl-2, p53 and c-myc in the development of gastric cancer. World J Gastroenterol 2001; 7: 403-406. Mornegner M, Mihelske S, Sottke M, Nubauer A, Alpen B, Thiede C, Klann H, Hiermeier FX, Eil C, Ehninger G, Bayerdorfer E. Development of early gastric cancer 4 and years after complete remission of Helicobacter pylori associated gastric low grade marginal zone B cell lymphoma of MALT type. World J Gastroenterol 2001; 7: 248-253. Niu WX, Qin XY, Liu H, Wang CP. Clinicopathological analysis of patients with gastric cancer in 1200 cases. World J Gastroenterol 2001; 7: 281-284. Xin Y, Li XL, Wang YP, Zhang SM, Zheng HC, Wu DY, Zhang YC. Relationship between phenotypes of cell2function differentiation and pathobiological behavior of gastric carcinomas. Wrold J Gastroenterol 2001; 7: 53-59. Yao YL, Xu B, Song YG, Zhang WD. Overexpression of cyclin E in Mongolian gerbil with Helicobacter pylori-induced gastric precancerosis. World J Gastroenterol 2002; 8: 60-63. Gao HJ, Yu LZ, Bai JF, Peng YS, Sun G, Zhao HL, Miu K, LüZX, Zhang XY, Zhao ZQ. Multiple genetic alterations and behavior of cellular biology in gastric cancer and other gastric mucosal lesions. H: pylori infection, histological types and staging. World J Gastroenterol 2000; 6: 486-854. Deng DJ. Progress of gastric cancer etiology: N-nitosamides 1999s. World J Gastroenterol 2000; 6: 613-618. Liu ZM, Shou NH, Jiang XH. Expression of lung resistance protein in patients with gastric carcinoma and its clinical significance. World J Gastroenterol 2000; 6: 433-434. Zou SC, Qiu HS, Zhang CW, Tao HQ. A clinical and long-term follow-up study of peri-operative sequential triple therapy for gastric cancer. World J Gastroenterol 2000; 6: 284-286. Wang X, Liu FK, Li X, Li JS, Xu GX. Inhibitory effect of endostatin expressed by human liver carcinoma SMMC-7721 on endothelial cell proliferation in vitro. World J Gastroenterol 2002; 8: 253-257. Cao W, Ou JM, Fei XF, Zhu ZG, Yin HY, Yan M, Lin YZ. Methionine-dependence and combination chemotherapy on human gastric cancer cells in vitro. World J Gastroenterol 2002; 8: 230-232. Li Y, Lu YY. Applying a highly specific and reproducible cDNA reference.

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