Effects of Chinese Jianpi herbs on cell apoptosis and related gene expression in human gastric cancer grafted onto nude mice

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AIM: To explore the mechanism of the Sijunzi decoction and another Chinese herbal recipe (SRRS) based mainly on the Sijunzi decoction in treatment of gastric cancer.

RESULTS: When compared with controls, tumor growth (size and weight) was significantly inhibited by treatment with the Sijunzi decoction (P<0.05) or SRRS (P<0.01). The tumor inhibitory rate in the Sijunzi decoction group was 34.33 % and SRRS group 46.53 %. AI of human gastric cancer xenografts in nude mice was significantly increased to 16.24 ±3.21 % using TUNEL method and 11.38±5.46 % by FACScan in the Sijunzi decoction group compared with the controls (TUNEL: 2.63±1.03 %, P<0.01; FACScan: 7.15±1.32 %, P<0.05). SRRS group was also found a significantly increased AI by using TUNEL method and flow cytometry analysis compared with the controls (TUNEL: 13.18±3.05 %, P<0.05; FACScan: 11.58±5.71 % (P<0.05). Under electron microscopy, cell shrinkage, nuclear chromatin condensation, formation of membrane blebs and apoptotic bodies were frequently observed in Sijunzi decoction group and SRRS group. The average labeling index (LI) for Ki-67 in SRRS group was significantly decreased to 8.43±2.22 % compared with the control group (10.37±4.91 %) (P<0.05). The average labeling index for Ki-67 in sijunzi decoction group was 7.95±2.54 % which was lower than that of the control group, but showed no significance (P=0.07). The expression level of p53 mRNA was lower in both Sijunzi decoction group and SRRS group than that in control group (P<0.05; P<0.01). The expression of bcl-2 mRNA was also decreased in SRRS group compared with the control (P<0.01).

CONCLUSION: The inhibition of gastric cancer cell growth in vivo by Chinese Jianpi herbs and SRRS is related to induction of the cell apoptosis which may be involved in aberrant expression of p53 and bcl-2 genes.

INTRODUCTION

Apoptosis plays a crucial role in the proliferation and turnover of cells in various tumors. It has been clear that its extent is often enhanced in tumor by many anticancer drugs[6,9], such as cytotoxic drugs[6], hormone[1], or some Chinese herbal medicine[7-10]. In clinic studies, some Chinese Jianpi herbs had been proved to have effect on malignant tumors, especially on gastric and colorectal tumors[11-12]. Among these herbs we found that Codonopsis pilosula (Franch) Nannf., Atractylodes macrocephala Koidz. and the Sijunzi Decoction might suppress gastric carcinoma cell proliferation and cause tumor cell loss and the nuclear condensation in vitro[14]. The Sijunzi Decoction and another Chinese herbal recipe SRRS of deheat-toxin, softening hard lumps and dissolving phlegm enhance apoptosis of human gastric cancer xenografts in nude mice[15]. Based on previous studies, we examined the apoptotic indices of human gastric cancer grafted onto nude mice after the treatment with Sijunzi Decoction and SRRS and investigate the underlying mechanism of the tumor suppressive effect of these Chinese Jianpi herbs.

MATERIALS AND METHODS

Materials

Animal models Thirty 6-7 weeks old female BALB/C-nu/nu mice (weight 18-22 g) and a human gastric carcinoma cell line SGC-7901 were obtained from Shanghai Tumor Institute (No.01842). The animals were subcutaneously grafted with the SGC-7901 cell. The tumor transplantation procedure was described previously[15].
**Drugs** The Sijunzi Decoction is composed of Codonopsis pilosula (Franch) Nannf., Atractylodes macrocephala koidz, Poria cocos (Schw.) Wolf, Glycyrrhiza uralensis Fisch. The concentration of the Sijunzi Decoction was 160 g/L; SRRS is composed of Atractylodes macrocephala koidz., Poria cocos(Schw.) Wolf, Sargentodoxa cuneata(Oliv.) Rehd. Et Wils., Prunella vulgaris L. And etc. The concentration of the SRRS decoction was 240 g/L.

**Experimental schedule** After grafting the mice were randomly divided into 3 groups, one control and the two experimental groups assigned to receive the Sijunzi Decoction or SRRS. Each animal in the two experimental groups was given 0.5 mL of the Sijunzi Decoction or SRRS by gastric perfusion every day over a 40-day period beginning at 1st day after grafting. The control animals received normal saline according to the same schedule. Animals were killed 41 days after being grafted.

**Methods**

**Tumor growth** The effect of therapy was assessed by two ways: (1) tumor size was measured twice a week by multiplying two perpendicular diameters. (2) tumor weight was determined immediately by electron balance after the animals were killed.

**Apoptosis** For detection of apoptotic cells, apoptotic indices were examined by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate fluorescence nick end labeling (TUNEL) method and flow cytometry analysis. Morphological alterations were observed with electron microscope. (1) TUNEL: *In situ cell death detection Kit POD* (ISCDD, BOEHRINGER MANNHEIM) was used to detect the apoptotic cell. The procedures was according to protocol of the kit and the other references. The positive cells were counted, counted and analyzed under the light microscope. Non-necrotic zone was selected in the tissue section and images were sent to computer by AEC camera (Grundig Electronic Co. Ltd., Germany). 10 image at least 1000 cells were selected and the amount of RNA was determined by electrophotometry 4:260:280. The extracted RNA was converted to first strand cDNA with AMV reverse transcriptase (Promega). p53 and β-actin were amplified by polymerase chain reaction consisting of stage one: 2 minutes of denaturation at 94 °C before additon of Taq DNA polymerase; stage two: 1 minute of denaturation at 94 °C, 1 minute of primer annealing at 56 °C, and 1 minute of extension at 72 °C (Taq DNA polymerase, Shanghai Sangon Biology Engineering Technique Service Co. Ltd.). Gene expression of bcl-2 was also analyzed by RT-PCR, in the same manner except for annealing at 55 °C. The cyclic of PCR gene amplification is from Omn-E (Hybaid). Each reaction tube contained: 1.5 μl 25mM MgCl2+2.5 μl 10XPCR buffer +0.5 μl 10mM dNTP +1 μl 20 pmol/μl p53 or bcl-2 primers or β-actin primers (primer concentration: 0.8 μM) +2 μl cDNA +0.5 μl Taq (2.5-3U/μl) +16 μl ddH2O. Quantitative analysis: After the amplification step was completed, equal amounts (5 μl) of PCR products were loaded onto each lane of 1.7 % agarose gels and electrophoresed. FR-200UV/WHITE ANALYSIS (Shanghai Fu Ri Science & Technology Co. Ltd., China) took the image of electrophoresis with β-actin as internal standard, and FR-980 biological electrophoresis analysis system (Shanghai Fu Ri Science & Technology Co. Ltd., China) was used to analyze the quantity of nuclear acid.

**Statistical analysis**

The results were expressed as *X*±s and significant difference was assessed by Student’s *t* test.

**Table 1** Sequences of primers for amplified cDNA of the p53, bcl-2 and β-actin

| Primers | Sequences | Combining sites | Amplifiers |
|---------|-----------|----------------|------------|
| p53     | Sense     | 5'-GGAGGTGTGAGGGCGCTGC-3 | 645bp-663bp | 311bp |
|         | Antisense | 5'-CAGCCACCTCAAGCTGTTC-3 | 936bp-955bp |
| bcl-2   | Sense     | 5'-CAGCTGCGACGTGACGCCC-3 | 1810bp-1829bp |
|         | Antisense | 5'-CACGCACCTCAAAGCTGTTC-3 | 936bp-955bp |
| β-actin | Sense     | 5'-AGCGGGAAATCGTGCGTGAC-3 | 1103bp-1125bp |
|         | Antisense | 5'-ACTCCTGCTTGCTGATCCACATC-3 | 1103bp-1125bp |

**Table 2** Chinese Jianpi herbs-induced effects on gastric cancer cell SGC-7901 (*n*=< 9)

| Treatment | Tumor weight/g (n) | Tumor size/mm (n) | Percentage of control/ % | Apoptotic index(AI)/ % | ki-67/ % |
|-----------|--------------------|-------------------|--------------------------|------------------------|----------|
|           | TUNEL (n)          | FACScan (n)       |                          |                        |          |
| Sijunzi Decoction | 0.66±0.16(9)       | 371.81±52.51(9)   | 34.33                    | 16.24±5.21(9)          | 7.95±2.54(9) |
| SRRS      | 0.54±0.23(9)       | 322.66±26.14(9)   | 46.53                    | 13.18±3.05(9)          | 8.43±2.22(9) |
| Control   | 1.01±0.32(10)      | 603.61±263.39(10) |                          | 2.63±0.03(10)          | 7.51±3.32(10) |

* <0.05, ** <0.01, *** =0.070 vs control t test.
RESULTS

Chinese Jianpi herbs-induced effects on tumor growth

Each one case that the xenograft emerged later was eliminated in Sijunzi decoction group and SRRS group. Compared with the control group, tumor growth (size and weight) was significantly inhibited by treatment with the Sijunzi decoction (P<0.05) or SRRS (P<0.01). The tumor inhibitory rate of the sijunzi decoction group was 34.33 % and that in the SRRS group was 46.53 % (Table 2).

Chinese Jianpi herbs-induced effects on tumor cell apoptosis

Apoptotic index (AI) of xenografts in nude mice was significantly increased to 16.24±3.21 % using TUNEL method and 11.38±6.46 % FACSscan in the Sijunzi decoction treatment group, compared with the controls (TUNEL: 2.63±1.03 %, P<0.01; FACSscan: 7.15±1.32 %, P<0.05). SRRS group was also found a significantly increased AI by using TUNEL method and flow cytometry analysis compared with the controls (TUNEL: 13.18±3.05 %, P<0.05; FACSscan: 11.58±5.71 %; P<0.05). But there was no significant difference between Sijunzi decoction group and SRRS group by using either TUNEL method or flow cytometry analysis. Under electron microscope cell shrinkage, nuclear chromatin condensation, formation of membrane blebs and apoptotic bodies were frequently observed in Sijunzi decoction group and SRRS group (Table 2).

Chinese Jianpi herbs-induced effects on tumor cell proliferation

The average labeling index for Ki-67 (LI) in SRRS treatment group (8.43±2.22 %) was significantly lower than that in the control group (10.37±4.91 %) (P<0.01). The average labeling index for Ki-67 in Sijunzi decoction group was 7.95±2.54 % which was lower than that of the control group, but showed no significance as the P value was 0.07 (Table 2).

Chinese Jianpi herbs-induced genetic effects

Expression of p53 (exons 5-8) and bcl-2 was semiquantitatively detected with β-actin used as an internal standard. The expression level of p53 mRNA was 0.36±0.27, significantly lower in Sijunzi decoction group than that in control group (0.69±0.20) (P<0.05), in SRRS group (0.19±0.18) also significantly lower than that in control group (P<0.01). The expression of bcl-2 mRNA in SRRS group (0.33±0.23) was decreased, compared with the control (0.81±0.40) (P<0.01). In Sijunzi decoction group the expression of bcl-2 (0.41±0.15) slightly decreased, compared with the control, but no statistics difference existed (P=0.071) (Table 3, Figure 1).

| Table 3 Chinese Jianpi herbs-induced genetic effects (x±s) |
|---------------------------------|
| Treatment                  | p53(exons 5-8) | bcl-2 |
| Sijunzi Decoction           | 0.36±0.27(8)  | 0.41±0.15(8) |
| SRRS                       | 0.19±0.18(9)  | 0.33±0.23(9)  |
| Control                    | 0.69±0.20(9)  | 0.81±0.40(9)  |

*P<0.05, *P<0.01, *P<0.01 vs control t test.

DISCUSSION

Despite its declining incidence, the gastric carcinoma remains one of the most common cause of cancer-related death in the world[25-27]. At present gastric carcinoma is still detected later in most patients throughout the world, and even with curative resection, they remain at a high risk of relapse. Thus, there is a great need for effective adjuvant therapy for patients with gastric carcinoma[28-30]. Our previous clinic paired comparative studies suggested that Chinese herbal recipe SRRS have therapeutic effects on advanced gastric cancer, with increasing the surviving period of the patients, improving the life quality, and decreasing the metastasis and recurrence rates after operation[12,14]. Because of its lower toxic side-effect compared with chemical therapy, it is worth to make a further research on its anti-cancer mechanism.

As the other malignant tumor, gastric carcinoma is not only a disease with abnormal cell proliferation and differentiation, but also a disease with abnormal apoptosis[5,31-35]. The enhanced induction of apoptosis in human gastric carcinoma cells can be observed after treatment with 5-Fluorouracil[36], Cisplatin[37], arsenic oxide[38], etc. These data suggest that inducing cancer cell apoptosis may be a therapeutic method for gastric carcinoma. The present study indicated that tumor growth was significantly inhibited by treatment with the Sijunzi decoction or SRRS. TUNEL method and cytometry analysis clarified the apoptotic effects of Chinese Jianpi herbs described here is related to inducing apoptosis. Immunohistochemical staining for Ki-67 showed that SRRS inhibited cell proliferation. So the inhibition of gastric cancer by SRRS is also related to suppressing the proliferation.

Apoptosis is a complex, tightly regulated, and active cellular process whereby individual cells are triggered to undergo self-destruction in a manner that will neither injure neighboring cells nor elicit any inflammatory reaction[1,39-42]. Various triggering factor initiate corresponding proteolytic cascade reaction depending on mitochondrial or APO-1/FAS/CD95 receptor mediated apoptotic pathways[40,41]. There are many oncopgenes and tumor suppressor gene products in the regulation and execution of apoptosis. Among them are p53, Rb, myc, ras, raf, etc[25,40,41,44,45]. p53, because of its role in apoptosis, has earned the name “guardian of the genome”. It monitors the state of DNA, and in case of DNA damage, stalls the cell cycle. This takes place through the induction of CIP/WAF1/p21. In the absence of phosphorylated, active cyclin-dependent kinases, also another regulator of the cell cycle, Rb, remains inactive(unphosphorylated), and hence, the cell cycle halts. This then leads to activation of DNA repair machinery. If the DNA repair fails, p53 takes over again and triggers apoptosis in a process that involves upregulation of the apoptotic-inducing bax and down-regulation of the apoptotic bcl-2[23]. p53 also upregulates KILLER/DR5, a 45-kd apoptosis-inducing member of the tumor necrosis factor receptor family. Analogous to the APO-1/FAS/CD95 receptor

![Figure 1](image-url)
system, its activation also lead to a caspase activation[37]. Thus p53 is known as one of the essential genes for cells to undergo apoptosis. In gastric cancer, mutant p53 expression decreased cancer cell apoptosis, p53 mutant provided selective growth superiority to the tumor cell. In our investigation we adopt the human gastric cancer cell line SGC-7901 which has point mutation in exon 6 codon 204 GAG→GCC coding Glu→Ala, which expresses mutant p53[46, 47]. RT-PCR detected p53 mRNA level was significantly lower in Sijunzi decoction group than that in control group. The expression level of p53 mRNA in SRRS group was also significantly lower than that in control group. The data suggest that these two recipes composed of Jianpi herbs induce SGC-7901 cancer cell apoptosis by down-regulation of mutant p53 mRNA expression. We also detected apoptosis-inhibiting member of the bcl-2 family[41, 42]; bcl-2 mRNA. The expression of bcl-2 mRNA was decreased in SRRS group, compared with the control. In Sijunzi decoction group the expression of bcl-2 was slightly decreased compared with the control, but there was no statistics difference. It suggested that SRRS could suppress bcl-2 expression, but it was not stronger for than that of Sijunzi decoction. bcl-2 is the epitome of an antiapoptotic or survival gene. Attesting to its role in an apoptosis checkpoint, it counteracts apoptosis initiated by quite disparate signals, such as chemotherapeutic drugs, oxidative stress, viral infections, and p53. In gastric carcinoma, bcl-2 over-expressed at both protein and mRNA level in many cases[42]. In our study SRRS and Sijunzi decoction down-regulated the expression of p53 mRNA and SRRS also decrease the expression of bcl-2 mRNA in SGC-7901 cancer cell undergoing apoptosis. We inferred that p53 and bcl-2 may be involved in the regulation of these Chinese Jianpi herbs inducing gastric cancer cell SGC-7901 apoptosis. The interaction contact of p53 and bcl-2 in SRRS or Sijunzi decoction inducing apoptosis needs further investigation.

REFERENCES

1. Kerr JFR, Winterford CM, Harmon BV. Apoptosis its significance in cancer and cancer therapy. Cancer 1994; 73: 2013-2026
2. Lu XP, Li BJ, Chen SL, Lu B, Jiang NY. Effect of chemotherapy or targeting chemotherapy on apoptosis of colorectal carcinoma. Shijie Huaren Xiaohua Zazhi 1999; 7: 332-334
3. Liang WJ, Huang ZY, Ding YQ, Zhang WD. Lovo cell line apoptosis induced by cycloheximide combined with TNF-α. Shijie Huaren Xiaohua Zazhi 1999; 7:326-329
4. Kong XP, Zou QY, Li RB, Zheng PL, Yang LP, Jin SW. Apoptosis of neoplasm cell lines induced by hepatic peptide extracts from sucking porcine hepatocytes. World J Gastroenterol 1999; 5:435-439
5. Majno G, Joris I. Apoptosis, oncosis, and necrosis an overview of cell death. Am J Pathol 1995; 146: 3-15
6. Wu JY, Zhou XF, Jiang WX, Wang JL, Yang F, Cai XS, Zhang ZG. Effects of chemotherapeutic drugs on Bcl-2, p53 and Ki67 expression of gastric cancer. Shijie Huaren Xiaohua Zazhi 1999; 7:399
7. Tu SP, Jiang SH, Qiao MM, Cheng SD, Wang LF, Wu YL, Yuan YZ, Wu YX. Effect of trichosanthin on cytotoxicity and induction of apoptosis of multiple drugs resistance cells in gastric cancer. Shijie Huaren Xiaohua Zazhi 2000; 8: 150-152
8. Zhu QX, Wang GS, Zhang XJ, Zhao Q. Apoptosis of human liver cancer EBL-7404 cells induced by traditional Chinese medicine Sodium Asafetida. Shijie Huaren Xiaohua Zazhi 1999; 7:715-716
9. Fan RY, Ma L. Apoptosis of gastric cancer SGC-7901 cell induced by free radical. Shijie Huaren Xiaohua Zazhi 1999; 7:807-808
10. Xu AG, Li SG, Liu JH, Shen JG, Gan AH. A apoptosis of gastric cancer induced by Huangsheng capsule and mechanism of nitrous oxide. Shijie Huaren Xiaohua Zazhi 1999; 7:364-365
11. Shen HX, Chen L, Zhou SJ, Chen YQ, Fan Y, Lu LP. Clinical and experimental study on therapeutic effect of compound shengqitang on gastric carcinoma. Shijie Huaren Xiaohua Zazhi 1998; 6:837-840
12. Qiu JX, Jia JS, Yang JK, Zheng JH, Zheng JG, Tang LD, Wang N, Shen KP, Pang HF, Ji GR, Qin DP, Li YM, Zhou XY, Luo XX, Liu MS, Qiu ZF, Cao LH, Dong MZ. Probing into the treatment of advanced stage of stomach carcinoma mainly by spleen-strengthening method. Zhongyi Zazhi 1992; 33:23-25
13. Wang GT, Zhao JS, Xu WY, Wang Y, Zhao AG. Clinical and experimental studies on FuZheng anti-cancer granulacombined with chemotherapy in advanced gastric cancer. Shijie Huaren Xiaohua Zazhi 1996; 6:214-218
14. Qiu JX, Tang LD, Zuo JP, Gao WP, Chen FZ. Study on mechanism of herbal medicines for strengthening spleen in treatment of malignant tumor of digestive tract. Shanghai Zhongyiyao Zazhi 1987; 6:45-47
15. Zhao AG, Yang JK, Zhao HL, Liu LK. Chinese Jianpi herbs induce apoptosis of human gastric cancer grafted onto nude mice. Shijie Huaren Xiaohua Zazhi 2000; 8: 737-740
16. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992; 119:493-501
17. Zhang XL, Liu L, Jiang HQ, Salvia millitorrhiza monomer IH764-3 induces hepatic stellate cell apoptosis via caspase-3 activation. World J Gastroenterol 2002; 8: 515-519
18. Li J, Wang WL, Wang WY, Liu B, Wang BY. Apoptosis in human hepatocellular carcinoma by terminal deoxynucleotidyl transferase mediatedUTP-FITC nick end labeling. Shijie Huaren Xiaohua Zazhi 1998; 6: 491-494
19. Nicotelli I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods 1991; 139:271-279
20. Gong J, Traganos F, Darzynkiewicz Z. An effective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. Anal Biochem 1994; 218:314-319
21. Darzynkiewicz Z, Bruno S, Bino GD, Gorczyca W, Hotz MA. Features of apoptotic cells measured by flow cytometry. Cytometry 1992; 13: 795-808
22. Kondo S, Shinomura Y, Kanayama S, Higashimoto Y, Kiyohara T, Zushi S, Kitamura S, Ueyama H, Matsuzawa Y, Komai H. Recent advances in surgical treatment of colorectal carcinoma. Cancer 2002; 99:1628-1633
23. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B, Reed JC. Suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 1994; 9:1799-1805
24. Yamamoto M, Maehara Y, Sakaguchi Y, Kusumoto T, Ichiyoshi Y, Sugimachi K. Transforming growth factor-β1 induces apoptosis in gastric cancer cells through a p53-independent pathway. Cancer Supplement 1996; 77:1628-1633
25. Otsuji E, Yamaguchi T, Sawai K, Hagiwara A, Taniguchi H, Takahashi T. Recent advances in surgical treatment have improved the survival of patients with gastric carcinoma. Cancer 1998; 82:1233-1237
26. Nakajima T, Nashimoto T, Kitamura M, Kito T, Iwanaga T, Koizumi G. Adjuvant mitomycin and fluorouracil followed by oral uracil plus tegafur in serosa-negative gastric cancer: a randomised trial. Gastric Cancer Surgical Study Group. Lancet 1999; 354: 277-277
27. Cirera L, Balil A, Batiste-Alentorn E, Tusquets I, Cardona
Randomized clinical trial of adjuvant mitomycin plus tegafur in patients with resected stage III gastric cancer. J Clin Oncol 1999; 17: 3810-3815

28 Hermans J, Bonenkamp JJ, Boon MC, Bunt AMG, Ohyama S, Sasako M, Van de Velde CJH. Adjuvant therapy after curative resection for gastric cancer: Meta-analysis of randomized trials. J Clin Oncol 1999; 17: 1441-1447

29 Shimada K, Ajani JA. Adjuvant therapy for gastric carcinoma patients in the past 15 years. Cancer 1999; 86: 1657-1668

30 Shimoyama S, Shimizu N, Kaminishi M. Type-oriented intraoperative and adjuvant chemotherapy and survival after curative resection of advanced gastric cancer. World J Surg 1999; 23: 284-292

31 Pan CJ, Zhong P, Huang X, Liu KY, Wang SX. Study on the correlation between proliferation and apoptosis in atrophy and intestinal metaplasia of gastric mucosa. Shijie Huaren Xiaohua Zazhi 2000; 8: 143-146

32 Wagner S, Beil W, Westermann J, Logan RPH, Bock CT, Trautwein C, Bleck JS, Manns MP. Regulation of gastric epithelial cell growth by Helicobacter pylori. J Exp Med 1998; 188: 2033-2045

33 Ikeguchi M, Cai J, Yamane N, Maeta M, Kaibara N. Clinical significance of spontaneous apoptosis in advanced gastric carcinoma. Cancer 1999; 85: 2329-2335

34 Liu HF, Liu WW, Fang DC, Men RF, Wang ZH. Apoptosis and its relationship with Fas ligand expression in gastric carcinoma and its precancerous lesion. Shijie Huaren Xiaohua Zazhi 1999; 7: 951-954

35 Sugamura K, Makino M, Shirai H, Kimura O, Maeta M, Itoh H, Kaibara N. Enhanced induction of apoptosis of human gastric carcinoma cells after preoperative treatment with 5-Fluorouracil. Cancer 1999; 79: 12-17

36 Muller M, Wilder S, Bannasch D, Israel D, Lehlbach K, Min LW, Friedman SL, Galle PR, Stresemann W, Oren M, Kramer PH. p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. J Exp Med 1998; 188: 2033-2045

37 Tu SP, Jiang SH, Tan JH, Jiang XH, Qiao MM, Zhang YP, Wu YL, Wu, YX. Proliferation inhibition and apoptosis induction by arsenic trioxide on gastric cancer SGC-7901. Shijie Huaren Xiaohua Zazhi 1999; 7: 18-21

38 Brown JM, Wouters BG. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. Cancer Res 1999; 59: 1391-1399

39 Green DR. Apoptotic pathways: the roads to ruin. Cell 1998; 94: 695-698

40 Soini Y, Paakko P, Lehto VP. Histopathological evaluation of apoptosis in cancer. Am J Pathol 1998; 153: 1041-1053

41 Pan G, Ni J, Wei YF, Yu GL, Gentz R, Dixit VM. An Antiagonist Decoy Receptor and a Death Domain-Containing Receptor for TRAIL. Science 1997; 277: 815-818

42 Ashkenazi A, Dixit VM. Death Receptors: Signaling and Modul abion. Science 1998; 281: 1305-1308

43 Reed JC. Bcl-2 and the regulation of programmed cell death. J Cell Biol 1994; 124: 1-6

44 Arends MJ, McGregor AH, Wyllie AH. Apoptosis is inversely related to necrosis and determines net growth in tumors bearing constitutively expressed myc, ras, and HPV oncogenes. Am J Pathol 1994; 144: 1045-1057

45 Zhang QY, Lu YY, Li Z, Li WM, Li Z, Li WM, Li Z, Li WM. P53 gene mutation in Chinese gastric carcinoma cell lines. Shengwu Huaxue Zazhi 1995; 11: 311-315

46 Buchman VL, Chumakov PM, Ninkina NN, Samarina OP, Georgiev GP. A variation in the structure of the protein-coding region of the human p53 gene. Gene 1988; 70: 245-252

47 Kondo S, Shinomura Y, Kanayama S, Higashimoto Y, Miyagawa JI, Minami T, Kiyohara T, Zushi S, Kitamura S, Isozaki K, Matsuzawa Y. Over-expression of bcl-x, gene in human gastric adenomas and carcinomas. Int J Cancer 1996; 68: 727-730

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