ANTICANCER RESEARCH *25*: 971-980 (2005)

Ellagic Acid Induced p53/p21 Expression, G1 Arrest and Apoptosis in Human Bladder Cancer T24 Cells

TE-MAO LI[1], GUANG-WEI CHEN[1], CHIN-CHENG SU[2], JAUNG-GUNG LIN[1], CHIN-CHUNG YEH[3], KWORK-CHU CHENG[4] and JING-GUNG CHUNG[5]

[1]Schools of Chinese Medicine, [5]Department of Microbiology, China Medical University, Taichung City 400, Taiwan; Departments of [2]Surgery and [3]Urology, China Medical University Hospital, Taichung City 400, Taiwan; [4]Department of Surgery, Jen-Ai Hospital, No 483, Tong-Rong Road, Tali, Taichung, Taiwan, R.O.C.

Abstract. *It is well known that dietary phenolic compounds can elicit vital cellular responses such as cytotoxicity, cell cycle arrest and apoptosis by activating a cascade of molecular events. Ellagic acid is one of these phenolic compounds, but the exact mechanism of its action is still unclear. The objective of this study was to investigate ellagic acid-induced cell cycle arrest and apoptosis in T24 human bladder cancer cells in vitro. Assays were performed to determine cell viability, cell cycle arrest, apoptosis, caspases-3 activity and gene expression, measured by flow cytometric assay, polymerase chain reaction (PCR) and determination of caspase-3 activity. Ellagic acid significantly reduced the viable cells, induced G0/G1-phase arrest of the cell cycle and apoptosis. Ellagic acid also increased p53 and p21 and decreased CDK2 gene expression, that may lead to the G0/G1 arrest of T24 cells. Ellagic acid also promoted caspase-3 activity after exposure for 1, 3, 6, 12 and 24 h, which led to induction of apoptosis. Furthermore, the ellagic acid-induced apoptosis on T24 cells was blocked by the broad-spectrum caspase inhibitor (z-VAD-fmk).*

The "People's Health Bureau of Taiwan" reported that about 3.47/100,000 people die annually of bladder cancer in Taiwan. The most frequently used clinical therapeutic treatments are radiotherapy, chemotherapy and surgery. However, these strategies for treatment of human bladder cancer are not totally satisfactory. Many compounds purified from plants have shown anticancer activity, such as taxol (1) and camptothecin (2), both of which can induce cancer cells to differentiate and undergo apoptosis. Furthermore, some anticancer drugs are known to induce apoptosis *via* the inhibition of topoisomerase II and activation of caspase-3 activity (2-5).

Ellagic acid is a phenolic compound present in fruits and nuts. It has been demonstrated that ellagic acid can inhibit tumor growth caused by chemical carcinogens in an animal model (6-10). It had also been reported that ellagic acid inhibits TPA-induced ornithine decarboxylase activity, N-acetyltransferase activity, hydroperoxide production and DNA synthesis (11-16). Further, it was reported that ellagic acid induced G0/G1 arrest and apoptosis in human cervical carcinoma Ca Ski cells (17). Recently, it was demonstrated that ellagic acid induced cell cycle arrest in human colon cancer cells resulting in the down-regulation of IGF-II (18). However, there are no reports that address whether ellagic acid affects cell cycle arrest and apoptosis of human bladder cancer T24 cells. This study showed that ellagic acid induced G0/G1 arrest through increased p53 level and induced apoptosis through activation of caspase-3 activity in human bladder cancer T24 cells.

Correspondence to: Dr. J.-G. Chung, Department of Microbiology, China Medical University, No 91, Hsueh-Shih Road, Taichung City 404, Taiwan, R.O.C. Tel: 886-4-2205-3366-8501, Fax: 886-4- 2205-3764, e-mail: jgchung@mail.cmu.edu.tw

Key Words: Ellagic acid, cell cycle arrest, apoptosis, p53, p21, caspase-3.

Materials and Methods

Chemicals and reagents. Ellagic acid, Tris-HCl, triton X-100, ribonuclease-A, trypan blue and propidium iodide (PI) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO), potassium phosphates and TE buffer were purchased from Merck Co. (Darmstadt, Germany). McCoy's 5a medium, fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA and glutamine were obtained from Gibco BRL (Grand Island, NY, USA). The caspase-3 activity assay kit was from Boehringer Mannheim (Mannhein, Germany).

Human bladder cancer cell line. The human bladder cancer cell line (T24: human; female; 81 years old) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were placed into 75-cm^3 tissue culture flasks and grown at 37˚C in a humidified 5% CO_2 and 95% air atmosphere in McCoy's

5a medium supplemented with 10% FBS, 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin) and 1% glutamine.

Cell viability was determined by using flow cytometry. T24 cells were plated in 12-well plates at a density of $5x10^5$ cells/well and grown for 24 h. Then, various concentrations of ellagic acid (0, 1, 5, 10, 25, and 50 μM) were added and the cells were grown for different periods of time. DMSO (solvent) was used for the control regimen. For determining cell viability, the flow cytometric protocol was used, as previously described (19).

Flow cytometry analysis of DNA content for cell cycle and apoptosis analysis in T24 cells co-treated with different concentrations of ellagic acid. About $5x10^5$ T24 cells/well in 12-well plate were incubated with ellagic acid (0, 1, 5, 10, 25 and 50 μM) for different time periods before the cells were harvested by centrifugation. The cells were fixed gently (drop by drop) in 70% ethanol (in PBS) in ice overnight and were then resuspended in PBS containing 40 μg/mL PI, 0.1 mg/mL RNase (Sigma) and 0.1% triton x-100. After 30 min at 37˚C in the dark, the cells were analyzed with flow cytometry (Becton-Dickinson, San Jose, CA, USA) equipped with an argon laser at 488 nm. Then, cell cycle and apoptosis were determined and analyzed (20).

Caspase activity determination. T24 cells were plated in 12 well plates at a density of $5x10^5$ cells/well with various concentrations of ellagic acid using only DMSO as a solvent for the control regimen. The cells were grown at 37˚C in a humidified 5% CO_2 chamber for 12 h. About $5x10^6$ cells (T24) were lysed in lysis buffer (1% Triton X-100, 0.32 M sucrose, 5mM EDTA, 10 mM Tris-HCl, pH 8.0, 2mM dithiothreitol, 1 mM PMSF, 1 μg/ml aprotinin, 1 mg/ml leupeptin) for 30 min at 4˚C followed by centrifugation at 10,000 xg for 30 min. For caspase-3 activity determination, 50 μL reaction mixtures with fluorogenic report substrate peptides were used. The substrate peptide (200 μM) was incubated at 37˚C with cytosolic extracts (15 μg of total protein) in reaction buffer (100 mM HEPES, 10% sucrose, 10 mM DTT, 0.1% 3-[3-chlo-amidopropyl] dimethylammonio 1-propanesulfonate). Fluorescence was determined after 2 h (excitation wavelength, 400 nm; emission wavelength, 505 nm) with a fluorescence plate reader (Fluoroskan Ascent; Labsystems).

Inhibition of ellagic acid-induced apoptosis by the caspase inhibitor z-VAD-fmk in T24 cells. In order to examine whether or not caspase-3 activation was involved in apoptosis triggered by ellagic acid, T24 cells were pretreated with the cell permeable broad-spectrum caspase inhibitor z-VAD-fmk 3 h prior to treatment with 25 μM ellagic acid. Apoptosis and caspase activity were then determined as described above.

Reverse transcriptase polymerase chain reaction (RT-PCR) for examining the gene expression of p53, p21, CDK2 and β-actin in T24 cells after exposure to ellagic acid. The total RNA was extracted from T24 cells using Qiagen RNeasy Mini Kit after 24 h cotreatment with different concentrations of ellagic acid, as described previously (14). Total RNA (1.5 μg), 0.5 μg of oligo-dT primer and DEPC (diethyl pyrocarbonate)-treated water were combined in a micro-centrifuge tube to a final volume of 12.5 μl. The entire mixture was heated at 70˚C for 10 min and chilled on ice for at least 1 min. The subsequent procedures for conducting reverse transcription were as described in the instruction manual (First-strand cDNA synthesis kit, Novagen). The reverse transcription products from total RNA served as a template for PCR. When amplifying target cDNA, the components in 50 μl of solution were as follows: 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 20 pmoles of each primer, cDNA template corresponding to the amount synthesized from 50 ng of total RNA and 2 units of DyNAzyme DNA polymerase. The sequence of primers was as follows:

p53: CAGCCAAGTCTGTGACTTGCACGTAC and
CTATGTCGAAAAGTGTTTCTGTCATC
p21: AGTGGACAGCGAGCAGCTGA and
RTAGAAATCTGTCATGCTGGTCTG
CDK2: GCATTGTTTTGGAGGCTGGTT and
AGAACTGGCCACGCAGT
β-actin: GCTCGTCGTCGACAACGGCTC and
CAAACATGATCTGGGTCATCTTCTC (21).

Statistical analysis. The Student's *t*-test was used to analyze the statistical significance between the ellagic acid-treated and control groups.

Results

Effects of various concentrations of ellagic acid on cell morphology and viability of human bladder cancer T24 cells. In the presence of ellagic acid (1-50 μM), the cells were collected and stained by propidium iodine and analyzed by flow cytometry. The results indicated that cells were increasingly stained as the time and concentration increased, suggesting that ellagic acid induced cell death on T24 cells. Further increasing the concentration of ellagic acid resulted in increased morphological changes (cell death) and a greater decrease of viable T24 cells (Figures. 1, 2 and 3).

Ellagic acid induced cell cycle arrest and apoptosis in human bladder cancer T24 cells. The flow cytometry results for cell cycle analysis indicated that, during the 48 h time period, ellagic acid increased the percentage of cells in G0/G1 (enhanced G0/G1 peak), and decreased the percentage of cells in G2/M. The control cells showed a typical pattern of DNA content that reflected G0/G1-, S- and G2/M- phases of the cell cycle (Figures. 4A and B). The ellagic acid-treated cells showed a typical pattern of DNA content that reflected G0/G1-, S- and G2/M- phases of the cell cycle together with a sub-G0/G1- phase (corresponding to apoptotic cells), as shown in Fig. 4A. A pre-G0/G1 apoptotic peak was very clear after cells were treated for 48 h. The percentage of apoptotic cells in various concentrations of ellagic acid are shown in Fig. 4C. An increase of ellagic acid concentration led to an increase of apoptosis in T24 cells.

Effects of ellagic acid on p53, p21 and CDK2 gene expression in intact T24 cells. Changes in p53, p21 and CDK2 mRNA levels in response to varying concentrations of ellagic acid

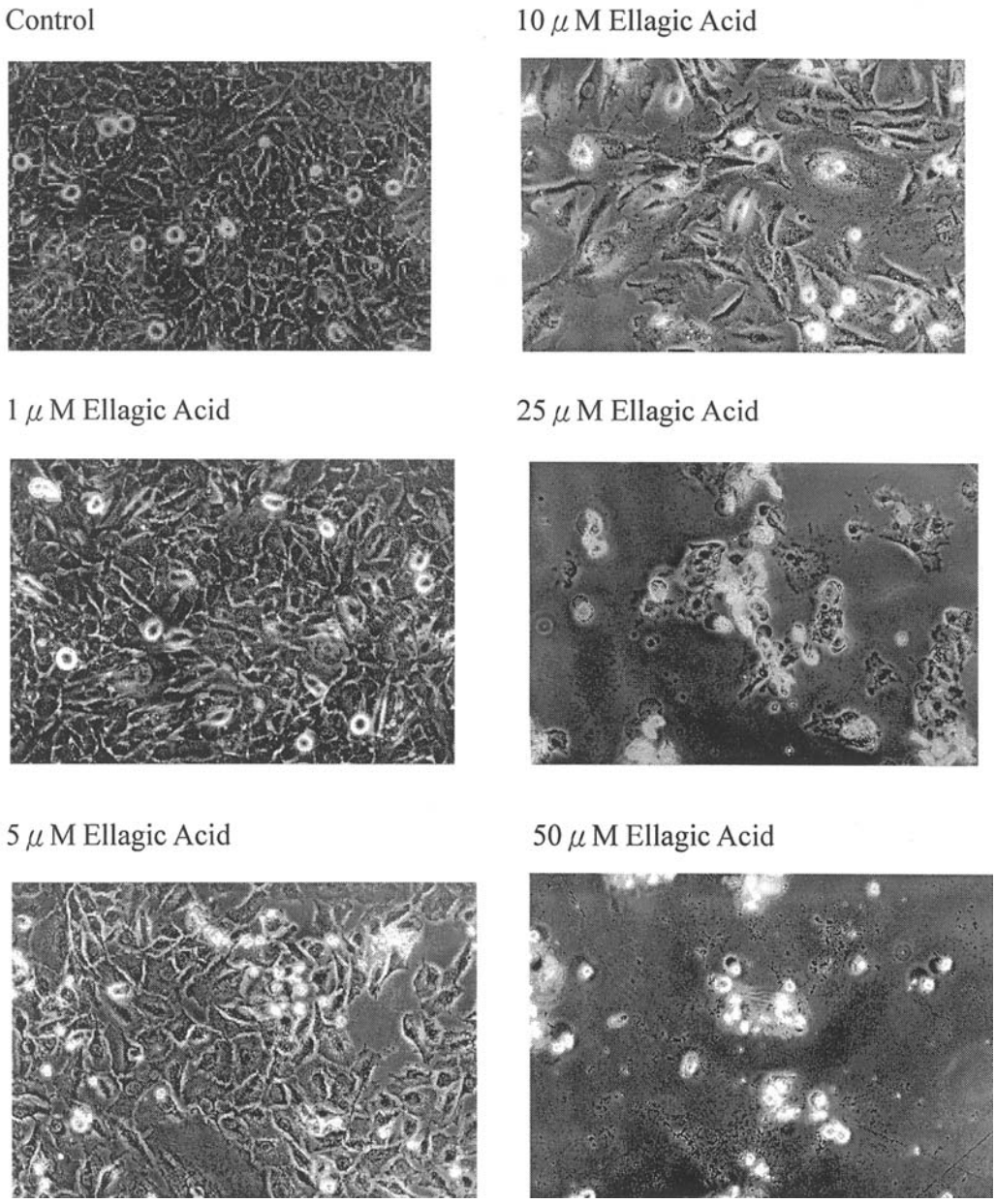

Figure 1. *Morphological changes of human bladder cancer T24 cells in response to ellagic acid. T24 cells were treated with varying concentrations of ellagic acid for 24 hours. Cells were examined under contrast-phase microscope and photographed.*

were studied by PCR. The results from PCR are presented in Figures. 5A, B and C. The data demonstrated that 1-50 μM ellagic acid increased p53 and p21 levels and decreased CDK2 levels. These effects of ellagic acid on p53, p21 and CDK2 were also dose–dependent.

Inhibition of ellagic acid-induced caspase activity and apoptosis by the caspase inhibitor z-VAD-fmk in human bladder tumor T24 cells. These experiments detected ellagic acid induced caspase-3 activity and also examined whether caspase-3 activation was involved in apoptosis triggered by ellagic acid. The data is presented in Figures. 6A and B and indicates that ellagic acid increased caspase-3 activity. This effect was dose-and time-dependent. The caspase inhibitor z-VAD-fmk decreased caspase-3 activity. T24 cells were pretreated with the cell permeable broad-spectrum caspase inhibitor z-VAD-fmk 3 h prior to treatment with ellagic acid. When cotreated with ellagic acid and z-VAD-fmk,

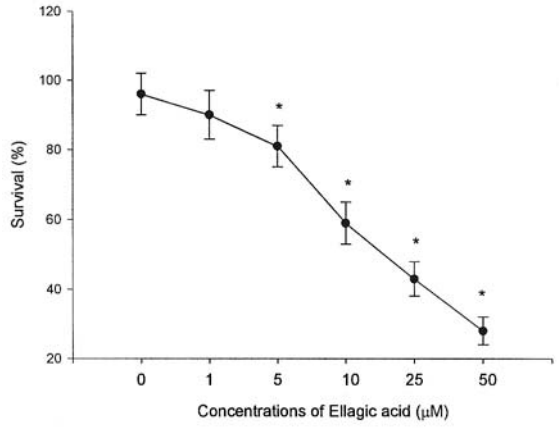

Figure 2. *Percentage of the viable T24 cells after ellagic acid treatment for 24 h. T24 cells (5x10^5 cells/well; 12-well plates) were cultured in McCoy's 5a medium + 10% FBS with ellagic acid for 24 h. Then cells were collected by centrifugation and the viable cells were determined by flow cytometry as described in Materials and Methods. Data represents mean ±S.D. of three experiments. *p<0.05*

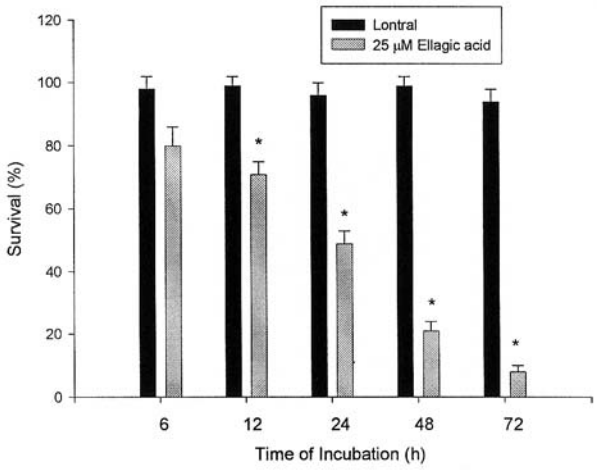

Figure 3. *Percentage of the viable T24 cells after 25 μM ellagic acid co-treatment. T24 cells (5x10^5 cells/well; 12-well plates) were cultured in McCoy's 5a medium + 10% FBS with varying concentrations of ellagic acid for 6, 12, 24, 48 and 72 h. Then cells were collected by centrifugation and the viable cells were determined by flow cytometry as described in Materials and Methods. Data represents mean ± S.D. of three experiments. *p<0.05*

inhibition of ellagic acid-mediated caspase-3 activation was accompanied by the marked attenuation of ellagic acid-induced apoptotic cell death (Figures. 7A and B). The data also indicated that activation of caspase-3 contributed to ellagic acid-induced apoptosis in T24 cells.

Discussion

In the present work, we investigated the possibility that ellagic acid-induced apoptosis can occur dependently of cell cycle arrest. Our results indicate that ellagic acid was able to induce, in a dose-dependent manner, G1 arrest and apoptotic cell death in T24 cells, as determined by flow cytometric analysis of hypodiploid nuclei. Ellagic acid-induced G1 arrest and apoptosis appeared to be statistically significant at 10, 25 and 50 μM. The mechanisms underlying the ellagic acid-induced G1 arrest and apoptosis of T24 cells were also investigated. Specifically, the critical elements of drug-induced cell cycle arrest and apoptosis, including the checkpoint elements of the cell cycle, activation of caspases and mitochondrial function with concomitant release of apoptogenic factors, were analyzed.

In general, two major pathways are involved in apoptosis: the mitochondrion-initiated pathway and the cell surface death receptor pathway. Both pathways are associated with caspase-3. Apoptosis was induced by activating caspase-3, amongst many other caspases. Caspase-3, one of the downstream effector caspases, works as a key trigger of apoptosis, which eventually results in cell death (22). Evidence indicates that subapoptotic concentrations of cytotoxic drugs can induce growth arrest with senescence features and that p53 and p21$^{WAF-1/CIP-1}$ are major players in this process (23, 24). We have demonstrated that ellagic acid induced death of the tumor cells mainly by caspase-dependent mechanisms. Inhibition of caspase activity by z-VAD-fmk significantly reduced apoptosis induction by ellagic acid.

Furthermore, in addition to its role as a cell cycle regulator, p21$^{WAF-1/CIP-1}$ functions to prevent apoptosis, mediating the survival function of phosphatidylinositol through phosphorylation of AKT (24, 25). It is possible that p21$^{WAF-1/CIP-1}$ functions in protecting DNA-damaged cells from becoming apoptotic, while p53 mediates DNA repair in the process.

In cancer treatment, cell death is an important factor influencing cell number and susceptibility to neoplastic transformation, as well as sensitivity to chemotherapeutic agents. It is well documented that cell death can be distinguished biochemically and morphologically into two distinct types, called necrosis and apoptosis (26). Morphological changes occur in cells undergoing apoptosis, including shrinkage of the cell and DNA fragmentation into membrane-bound apoptotic bodies in which most of the organelles remain intact before being rapidly phagocytosed by neighbouring cells (27). However, the results from agarose gel electrophoresis indicated the present of a ladder pattern of DNA fragments from T24 cells after exposure to ellagic acid (data not shown). Therefore, ellagic acid-triggered apoptosis might be induced in some fractions of the cells existing in the G0/G1-phase, which are committed

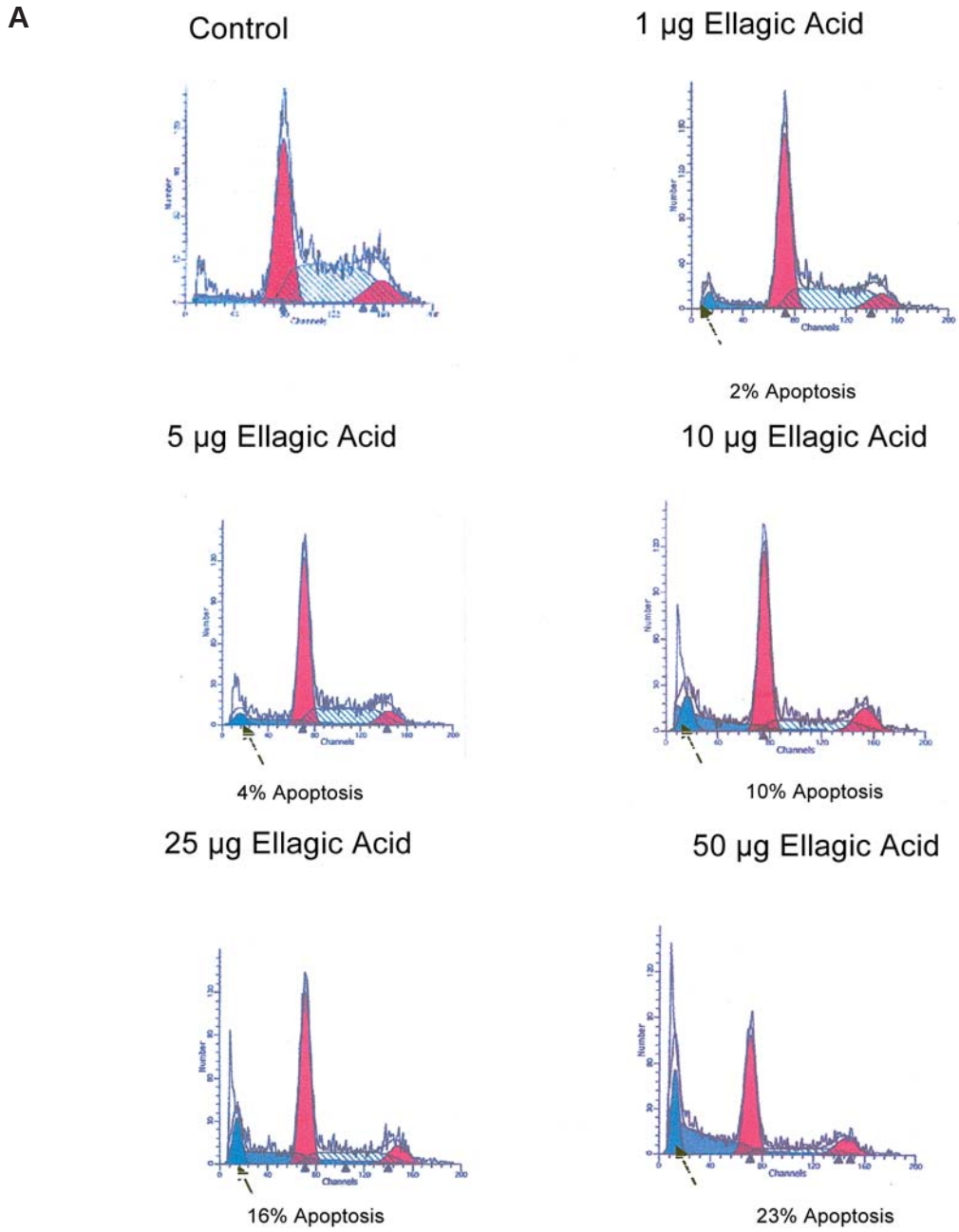

Figure 4

to G0/G1 arrest. It is conceivable that cells overexpressing wtp53 will not survive *in vivo* due to p53-induced G1 arrest and apoptosis (28, 29). It has also been reported that p53 status in tumor cells influence apoptotic decisions in response to hypoxia (30).

The PCR assay demonstrated that ellagic acid promoted p53 and p21 gene expression and inhibited CDK2 gene expression, and therefore may lead to G0/G1 arrest in the cell cycle. Ellagic acid has been demonstrated to exhibit antineoplastic activity, through induction of apoptosis and cell cycle arrest. During the normal cell cycle, the kinase activity of CDK2/cyclin E is elevated specifically at the G0/G1-phase transition and decreases at the onset of anaphase. Many studies have reported that cyclin-dependent kinase 2 (CDK2) and cyclin-E are involved in the cell cycle from G1- into S-phase, and our PCR data showed that ellagic acid inhibited

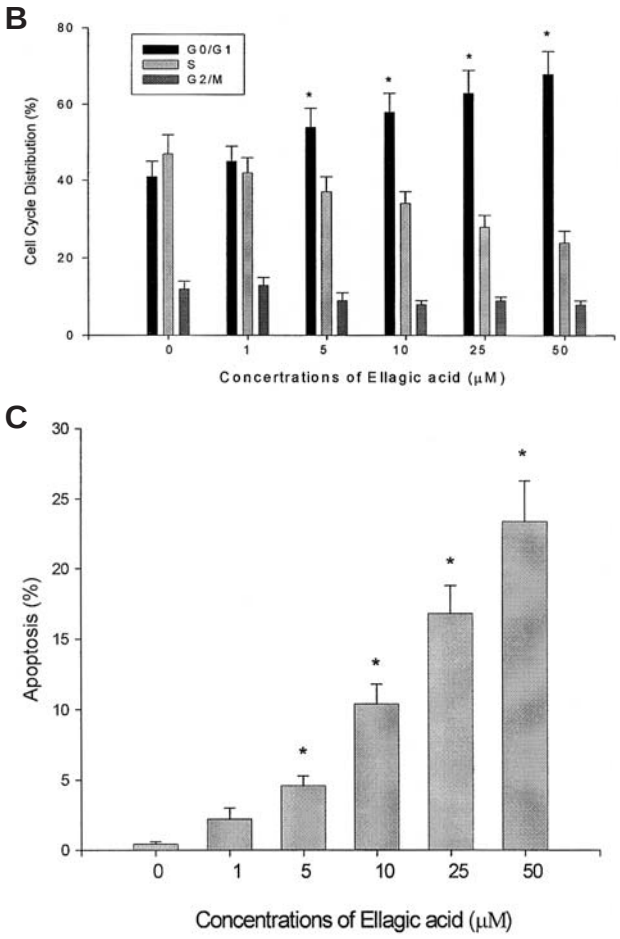

Figure 4. *Effects of ellagic acid on the T24 cell cycle and apoptosis (sub-G1 group). T24 cells were cultured with varying concentrations of ellagic acid for 48 hours, and the cells were harvested and analyzed for cell cycle and sub-G1 group (panel A: control and 1, 5, 10, 25, 50 μM ellagic acid). The percent of T24 cells in phase (panel B) and apoptosis (panel C) were evaluated by flow cytometry as described in Materials and Methods. Data represents mean ± S.D. of three experiments. *p<0.05*

CDK2, which may be the mechanism resulting in G1/M arrest in the cell cycle of T24 cells. The data from the present study showed that ellagic acid induced apoptosis, since cell death was mediated by caspase-3 activation. Investigators had demonstrated that caspase-3 plays an important role in chemotherapy of HL-60 cells (31) and growth factor withdrawal of hematopoietic cells (32). Apoptosis was completely inhibited by pretreatment with the caspase inhibitor z-VAD-fmk. This phenomenon also showed that the decrease of caspase-3 activity led to an increase in the number of viable T24 cells when treated with ellagic acid. Apparently ellagic acid-induced apoptosis is caspase-3-dependent.

In this study, we reported that ellagic acid induced apoptosis in T24 cells as well as caspase-3 activation.

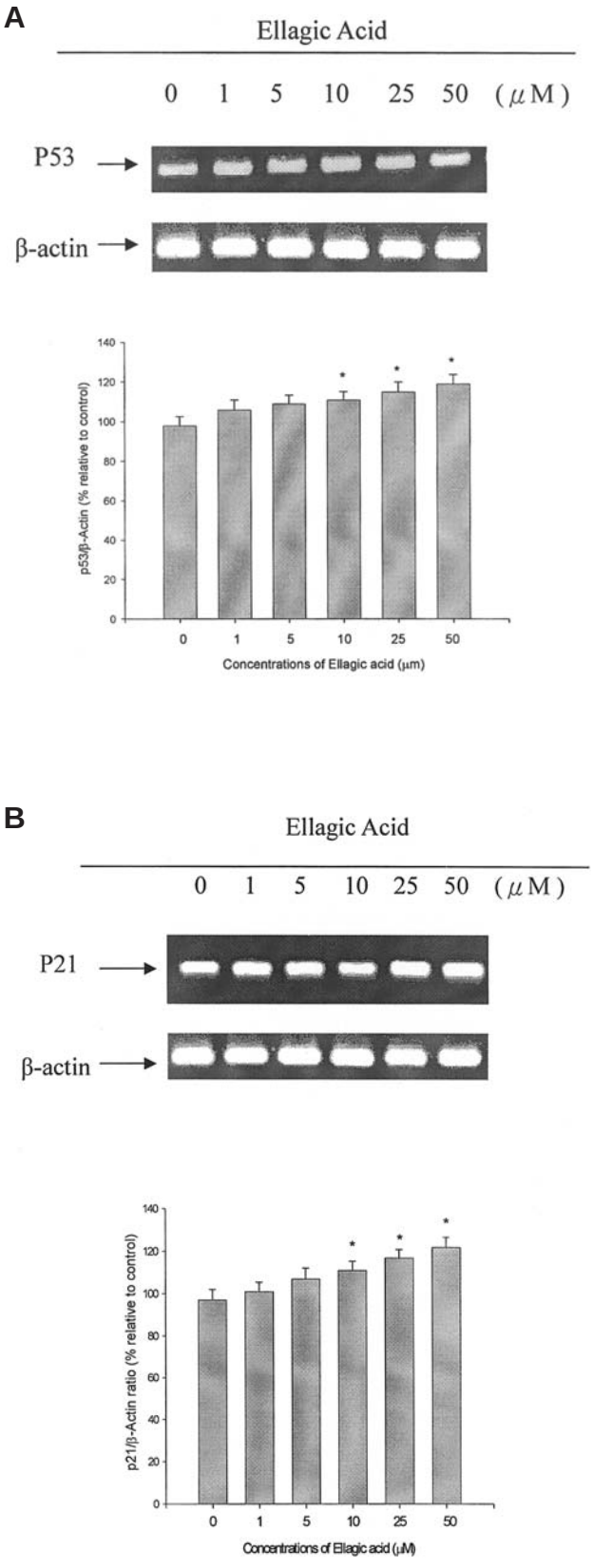

Figure 5

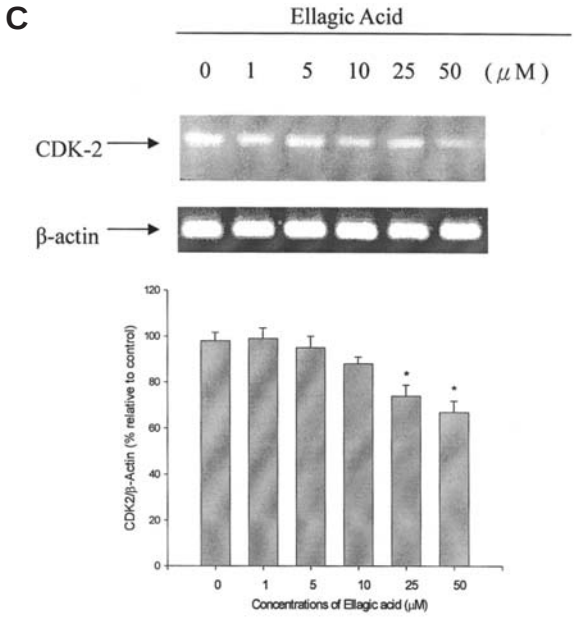

Figure 5. *Representative PCR showing changes in the levels of p21, p53 and CDK2 in human T24 cells after exposure to ellagic acid. T24 cells (5x10^6/ml) were treated with 0, 1, 5, 10, 25 and 50 μM ellagic acid for 24 hours before total RNA was prepared and PCR was performed, as described in Materials and Methods.*

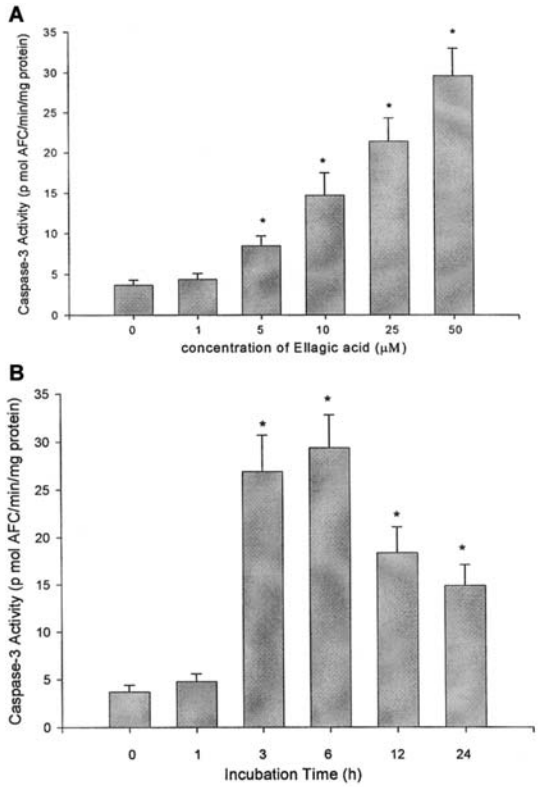

Figure 6. *Effects of ellagic acid on caspase-3 activity. T24 cells were incubated with various concentrations of ellagic acid for various time periods. Then, the cells were harvested and were analyzed for caspase-3 activity (panel A and B), as described in Materials and Methods. Data represents mean ± S.D. of three experiments. *p<0.05*

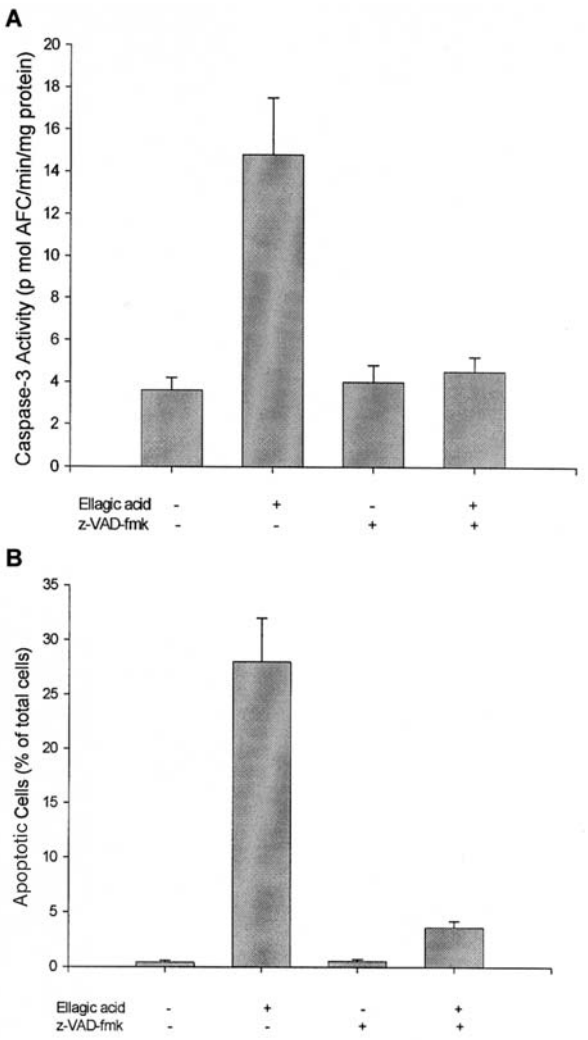

Figure 7. *Effects of ellagic acid on caspase-3 activity and apoptosis. T24 cells were cultured with various concentrations of ellagic acid with or without z-VAD-fmk co-treatment for 48 h. Then, the cells were harvested and were analyzed for caspase-3 activity (panel A) and apoptosis (panel B), as described in Materials and Methods. Data represents mean ± S.D. of three experiments. *p<0.05*

However, ellagic acid did not affect the activity of caspases-1 and -8 (data not shown). It is well known that the ratio between pro- and anti-apoptotic proteins determines, in part, the susceptibility of cells to a death signal which leads to apoptosis (33-35). Our results indicated that ellagic acid-induced apoptosis in T24 cells was prevented by the presence of catalase (data not shown). Catalase induced photolytic activation of procaspase-3, which led to inhibition of caspase-3 activity, and finally to decrease of the frequency of apoptosis. Interestingly, susceptibility to the apoptosis-inducing effects of chemotherapeutic drugs may depend on the intrinsic ability of tumor cells to respond by apoptosis

and, thus, defects in the process of apoptosis may be closely associated with carcinogenesis (36). Therefore, multiple mechanisms may be involved in the growth-inhibitory effects of ellagic acid. In the future, studies focusing on cell signaling and the biological significance of ellagic acid-induced apoptosis and cell cycle arrest would elucidate the mechanisms of the chemotherapeutic potency of ellagic acid in human cancer.

Acknowledgements

This study was supported by grant CMU93-M-03 and CMC91-CM-04 from the Research Section of China Medical University, Taichung city, Taiwan, Republic of China.

References

1 Bhalla K, Ibrado AM, Tourkina E, Tang C, Mahoney ME and Huang Y: Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. Leukemia *7*: 563-568, 1993.

2 Kaufman SH: Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. Cancer Res *49*: 5870-5878, 1989.

3 Walker PR, Smith C, Youdale T, Leblanc J, Whitfield JF and Sikorska M: Topoisomerase II-reactive chemotherapeutic drugs induce apoptosis in thymocytes. Cancer Res *51*: 1078-1085, 1991.

4 Ling YH, Priebe W and Perze SR: Apoptosis induced by anthracycline antibiotics in P388 parent and multidrug-resistant cells. Cancer Res *53*: 1845-1852, 1993.

5 Stevnsner T and Bohr VA: Studies on the role of topoisomerase in general, gene and strand-specific DNA repair. Carcinogenesis *14*: 1841-1850, 1993.

6 Bate-Smith EC: Detection and determination of ellagitannins. Phytochemistry *11*: 1153-1156, 1972.

7 Perchellet JP, Gali EM, Perchelle DS and Klish AD: Armbrust, antitumor promoting activities of tannin acid, ellagic acid, and several gallic acid derivatives in mouse skin, Basic Life Sci *59*: 783-801, 1992.

8 Stoner GD and Morse AM: Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. Cancer Lett *114*: 113-119, 1997.

9 Dow LR, Chou TT, Bechle MB, Goddard C and Larson RE: Identification of tricyclic analogs related to ellagic acid as potent/selective tyrosine protein kinase inhibitors. J Med Chem *37*: 224-231, 1994.

10 Barch DH, Rundhaugen LM, Stoner GD, Pillay NS and Rosche WA: Structure-function relationships of the dietary anticarcinogen ellagic acid. Carcinogenesis *17*: 265-269, 1996.

11 Das M, Bickers DR and Mukhtar H: Effect of ellagic acid on hepatic and pulmonary xenobiotic metabolism in mice: studies on the mechanism of its anticarcinogenic action. Carcinogenesis *6*: 1409-1413, 1985.

12 Ahn D, Putt D, Kresty L, Stoner GD, Fromm D and Hollenberg PF: The effects of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes p450 and phase II enzymes. Carcinogenesis *17*: 821-828, 1996.

13 Chung JG: Inhibitory actions of ellagic acid on growth and arylamine N-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. Microbios *93*: 115-127, 1998.

14 Lin SS, Hung CF, Tyan YS, Yang CC, Hsia TC, Yang MD and Chung JD: Ellagic acid inhibits arylamine N-acetyltransferase activity and DNA adduct formation in human bladder tumor cell lines (T24 and TSGH 8301). Urol Res *29*: 371-376, 2001.

15 Teel RW: Ellagic acid binding to DNA as a possible mechanism for its antimutagenic and anticarcinogenic action. Cancer Lett *30*: 329-336, 1986.

16 Thresiamma KC and Kuttan R: Inhibition of liver fibrosis by ellagic acid. Indian J Physiol Pharmacol *40*: 363-366, 1996.

17 Narayanan BA, Geoffroy O, Willingham MC, Re GG and Nixon DW: P53/p21(WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Lett *136*: 215-221, 1999.

18 Narayanan BA and Re GG: IGF-II down-regulation associated cell cycle arrest in colon cancer cells exposed to phenolic antioxidant ellagic acid. Anticancer Res *21*: 359-364, 2001.

19 Li YC, Tyan YS, Kuo HM, Chang WC, Hsia TC and Chung JG: Baicalein induced *in vitro* apoptosis undergo caspases activity in human promyelocytic leukemia HL-60 cells. Food Chem Toxicol *42*: 37-43, 2004.

20 Ormerod MG: *In:* Flow Cytometry: A Practical Approach. Oxford University Press, New York, pp. 69-81, 1990.

21 Chung JG, Yeh KT, Wu SL, Chen GW, Hsu NY and Ho HC: Novel transmembrane GTPase of non-small cell lung cancer identified by mRNA differential display. Cancer Res *61*:8873-8879, 2001.

22 Drexler HG, Dirks W, MacLeod RAF, Quentmeier H, Steube K and Uphoff CC (eds): DSMZ Catalogue of Human and Animal Cell Lines, Seventh edition, Braunschweig, Germany, 1999.

23 Chang BD, Swift ME, Shen M, Fang J, Broude EV and Roninson IB: Molecular determinants of terminal growth arrest induced in tumor cells by chemotherapeutic agent. Proc Natl Acad Sci USA *99*: 389-394, 2002.

24 Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, Hendrickson EA, Balan KV, Pantazis P and Wyche JH: Role of p21 in apoptosis and senescence of human colon cancer cells treated with camptothecin. J Biol Chem *277*: 17154-17160, 2002.

25 Zhou BP, Liao Y, Xia W, Sophn B, Lee MH and Hung MC: Cytoplasmic localization of p21CIP1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat Cell Biol *3*: 245-252, 2001.

26 Wyllie AH, Kerr RG and Currie AR: Cell death: the significance of apoptosis. Int Rev Cytol *68*: 251-306, 1980.

27 Kerr JFR, Wyllie AH and Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer *26*: 239-257, 1972.

28 Li H, Alonso-Vanegas M, Colicos MA, Jung SS, Lochmuller H, Sadikot AF, Snipes GJ, Seth P, Karpati G and Nalbantoglu J: Intracerebral adenovirus-mdiated p53 tumor suppressor gene therapy for experimental human glioma. Clin Cancer Res *5*: 637-642, 1999.

29 Hsiao M, Tse V, Carmel J, Tsai Y, Felgner PL, Haas M and Silverberg GD: Intracavitary liposome-mediated p53 gene transfer into glioblastoma with endogenous wild-type p53 *in vivo* in tumor suppression and long-term survival. Biochem Biophys Res Commun *233*: 359-364, 1997.

30 Yu JL, Coomber BL and Kerbel RS: A paradigm for therapy-induced microenvironmental changes in solid tumors leading to drug resistance. Differentiation *70*: 599-609, 2002.
31 Ibrado AM, Huang Y, Fang G, Liu L and Bhalla K: Overexpression of Bcl-2 or Bcl-xL inhibits Ara-C-induced CPP32/Yama protease activity and apoptosis of human acute myelogenous leukemia HL-60 cells. Cancer Res *56*: 4743-4748, 1996.
32 Ohta T, Kinoshita T, Naito M, Nozaki T, Masutani M, Tsuro T and Miyajima A: Requirement of the caspase-3/CP32 protease cascade for apoptotic death following cytokine deprivation in hematopoietic cells. J Biol Chem *272*: 23111-23116, 1997.
33 Gross A, McDonnell JM and Korsmeyer SJ: BCL-2 family members and the mitochondria in apoptosis. Genes Develop *13*: 1899-911, 1999.
34 Vander Heiden MG and Thompson CB: Bcl-2 proteins: regulators of apoptosis or of mitochondrial homeostasis. Nature Cell Biol *1*: E209-16, 1999.
35 Zhang H, Huang Q, Ke N, Matsuyama S, Hammock B, Godzik A and Reed JC: Drosophila pro-apoptotic Bcl-2/Bax homologue reveals evolutionary conservation of cell death mechanism. J Biol Chem *275*: 27303-6, 2000.
36 Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momosaki S and Kojiro M: The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res 54, 448-454, 1994.

Received June 30, 2004
Revised December 20, 2004
Accepted February 2, 2005