Comparative Proteomics of Apoptosis Initiation Induced by 5-Fluorouracil in Human Gastric Cancer

Chun-Yan Chen[1, 2], Ji-Hui Jia[1], Mao-Xiu Zhang[1], Ya-Bin Zhou[1], Rong-Mei Zhang[2], and Xiu-Ping Yu[1]

[1]*Department of Microbiology*
School of Medicine, Shandong University
and
[2]*Department of Hematology and Oncology*
the Second Hospital of Shandong University
Jinan 250012, Shandong, P.R. China

Abstract

5-Fluorouracil is the first choice chemotherapeutic drug for patients with gastric cancer, but the mechanism that 5-fluorouracil plays the anti-tumor role remains unclear. The aim of this study was to clarify correlation proteins induced by 5-fliorouracil in the apoptosis-initiation of human gastric cancer (MGC-803) cells. The time point of apoptosis-initiation induced by 5-fliorouracil in MGC-803 cells was determinated using 5-fliorouracil-withdrawal. Two-dimensional electrophoreses (2-DE) were employed to compare the differentials of protein expressions of the MGC-803 cells at the apoptosis-initiation phase and those of the MGC-803 cells untreated with 5-fluorouracil. The differential proteins included 14 upregulated proteins and 8 downregulated proteins. They indicated a more-than-doubled alteration. These proteins were digested in gels by trypsin and the mass of generated peptides were measured by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The data obtained from peptide mass fingerprinting (PMF) were searched out using the internet available database mascot (http://www.matrixscience.com). The results showed that proteomics analyses have evidenced that many kinds of proteins are involved in the apoptosis initiation of human gastric cancer MGC-803 cells. These proteins are related to metabolism, oxidation, cytoskeleton and signal transduction and other aspects of cells. In conclusion, the experiment model of apoptosis-initiation of human gastric cancer MGC-803 cells induced by 5-fluorouracil based on proteomic analysis has been established, giving an impetus to researches of the mechanism of apoptosis in human gastric cancer, and laying a foundation for the selection of potential drug precursors specific for inducing apoptosis-initiation in human gastric cancer.

Key Words: human gastric cancer cell lines, apoptosis initiation, proteomics, 5-fliorouracil

Introduction

The efficacy of conventional chemotherapeutic drugs is partially due to their ability to induce apoptosis, although this is not still under controversy (15). So it is important to enhance the researches on mechanism of apoptosis, which contributes to analyse potential anticancer drug targets by inducing tumor cell apoptosis, and to develop a new specific therapeutic strategy. Apoptosis may be divided into three different stages: initiation phase, effectuation phase and execution phase. Apoptosis initiation, just like the "check-point" in the cell cycle, determines the irreversible destiny of the cells towards apoptosis, and makes an ideal potential target of anticancer chemotherapy (26). Consequently, apoptosis initia-

Corresponding author: Dr. Ji-Hui Jia, Department of Microbiology, School of Medicine, Shandong University, 44 Wenhua Si Road, Jinan 250012, Shandong Province, China. Tel: +86-531-88382098, E-mail:jiajihui@sdu.edu.cn
Received: April 14, 2005; Revised: May 29, 2005; Accepted: June 8, 2005.

tion may play a critical role in cell apoptosis. The study on the mechanism of apoptosis initiation proves to be an important part of the entire study on the mechanism of apoptosis. Previous studies on apoptosis-related moleculars were mostly based on genome levels. However, the genome is only the carrier of genetic information, whereas the protein is the executor of living activities. Moreover, mRNA level changes do not always correlate with protein level alterations, and genomics does not analyse the regulation on protein-protein interactions, and post-translational modifications that contribute to the protein changes (8).

Proteomics, however, is probably the most appropriate approach to such a problem. Application of proteomics conveniently simplifies the identification of modified proteins by comparing the protein expression levels in different states, which makes people comprehensively understand the mechanism of life from the indirect gene to the functional executioner protein (25).

5-Fluorouracil is the first choice chemotherapeutic drug for the clinical treatment of patients with the alimentary tract cancer including gastric cancer, but the mechanism of apoptosis, especially mechanism of apoptosis-initiation, induced by 5-fluorouracil in gastric cancer remains unclear (24). This study has, on the basis of the determination of apoptosis-initiation phase of the gastric cancer cells induced by 5-fluorouracil, established the experiment model for comparative proteomic analysis so as to understand the mechanism of apoptosis induced by 5-fluorouracil in human gastric cancer. These results therefore, laid a foundation for further studies of the cellular and molecular basis of apoptotic cell death based on the proteomic profiling of human gastric cancer.

Materials and Methods

Cells and Chemical Agents

Human poorly-differentiated gastric adenocarcinoma cells (MGC-803) were purchased from the Cell Bank of Chinese Academy of Medical Sciences; N, N-methylenebisacrylamide (Bis), N, N, N', N'-tetramethylethylenediamine, urea, dithiothreitol, iodoacetamide, α-cyano-4-hydroxycinnamic acid and (3-[(3-cholamidopropyl) dimethylamino]-1-propanesulfonate (CHAPS), immobilize pH gradient drystrip (IPG, pH 4~7, L = 18), IPG buffer, IPG covering fluid, pharmalyte (pH 4~7) from Amersham-Pharmacia Biotech (APB, Uppsala, Sweden); PMSF, pepstatin and leupeptin from Sigma (St. Louis, MO, USA); trifluoroacetic acid (TFA) and acetonitrile (ACN) from Acros (Springfield, NJ, USA); and sequencing grade trypsin from Boehringer Mannheim (Mannheim, Germany).

Establishment of Apoptosis Initiation Model of MGC-803 Cells Inducd by 5-Fluorouracil

Human gastric cancer MGC-803 cells were incubated at 37°C in 5% CO_2-95% humidified air and passaged in RPMI 1640 containing 10% fetal bovine serum to logarithm growth phase. According to Wan's methods (26), the experimental cells were divided into three groups: In the 5-Fluorouracil-treated group, 10 mg/l 5-fluorouracil was added into MGC-803 cells suspension. 2×10^6 5-fluorouracil-treated MGC-803 cells were taken out at 3, 6, 12, 24, 48 and 72 hours; in the 5-fluorouracil-withdrawl group, after being treated with 5-fluorouracil, the MGC-803 cells were rinsed three times in RPMI 1640 to remove 5-fluorouracil and then cultured in fresh RPMI 1640 medium for 72 hours and harvested; and the MGC-803 cells without 5-fluorouracil serve as the control group. The effects of the 5-fluorouracil on MGC-803 cells were estimated by a growth curve. The final cell structures were observed under transmission electron microscope and a scanning electron microscope. The DNA contents were detected by means of flow cytometry analysis of propidium iodide (PI)-stained nuclei.

Protein Extraction and Two-Dimensional Electrophoreses

According to the methods (3), proteins were extracted from 5-fluorouracil -treated MGC-803 cells at the apoptosis-initiation phase or MGC-803 cells without 5-fluorouracil respectively. The protein concentration level was determined by means of a Bradford assay, using bovine serum albumin as a standard, the protein levels ranging between 5 and 10 g/l. Then, the two-dimensional electrophoreses were performed by means of 18-cm IPG strips (pH 4~7) and an IPGphor instrument (APB, Uppsala, Sweden). After this, protein spots were stained for 2 hours with Coomassie Brilliant Blue G-250, and then destained and left alone overnight until the background was clear. The gels were scanned with an ImageScanner at the 256 grayscale and 300 dpi degree level. Quantification of spot abundance was carried out using ImageMaster 2D Elite 3.01 (APB, Uppsala, Sweden). One of the MGC-803 cells gels was selected as the reference gel. Other gels including the gels treated with 5-fluorouracil at the apoptosis initiation phase were matched with the reference gel. After the removal of noise and background, PI and Mr calibration and volume normalization, the spots abundances were expressed as percent volume where V% = (spot volume / Σvolumes of all spots resolved in the gel).

Mass Spectrometry Analysis

Spots of protein associated with apoptosis initia-

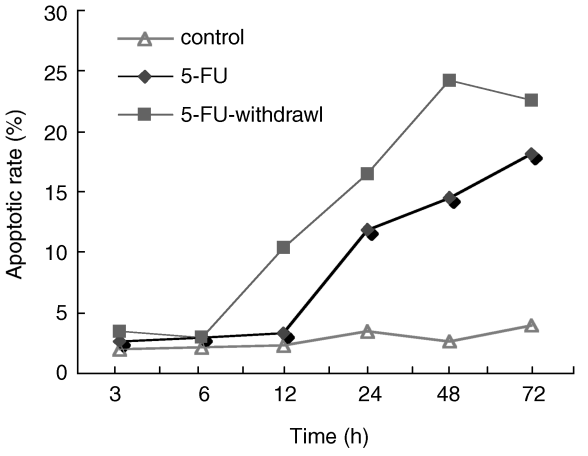

Fig. 1. The apoptotic rate of 5-fluorouracil-treated cells and 5-fluorouracil-withdrawal group using flow cytometry.

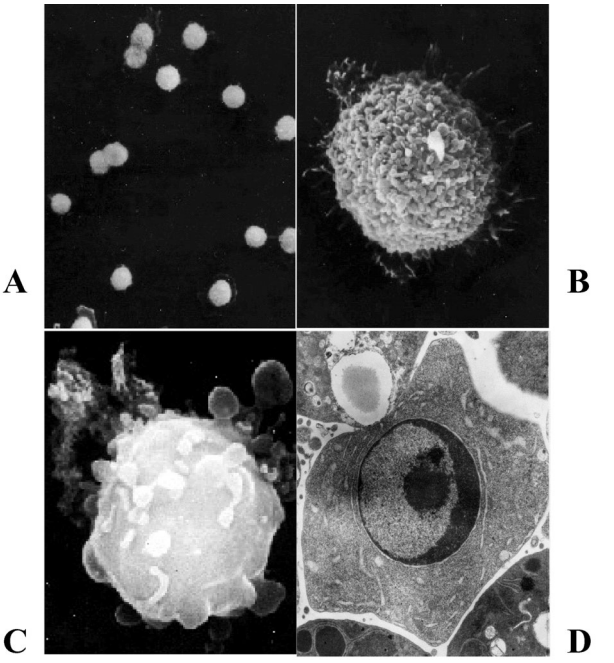

Fig. 2. A. MGC-803 cells treated by 5-fluorouracil for 12 h showing spherical shape (scanning electron microscope × 600); B. MGC-803 cells treated by 5-fluorouracil for 12 h showing surface villi edema (scanning electron microscope × 5000); C. MGC-803 cells treated by 5-fluorouracil-withdrawal for 48 h showing surface blebbing (scanning electron microscope × 7000); D. MGC-803 cells nuclear treated by 5-fluorouracil-withdrawal for 48 h showing condensation chromatin (transmission electron microscope × 8000).

tion were excised from the gels, cut into 1-2 mm^2 slices with a blade, decoloured with 100 µl 50% acetonitrile prepared from 25 mmol/l ammonium bicarbonate, and dried in a vacuum concentrator. The tryptic peptides were first extracted using 5% TFA at 40°C for one hour, then 2.5% TFA and 50% acetonitrile at 30°C for one hour. The extracted solutions were mixed in an Eppendorf tube, and dried with a vacuum concentrator. After the peptide mixture was solubilized with 0.5% TFA, the peptide mass analysis of the peptide mixture was performed by means of MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) analysis. One µl of 0.01g/l peptide mixture was added onto the MALDI target, followed closely by 1 µl of α-cyano-4-hydroxycinnamic acid (10 g/l in 1:1 ACN : 0.1% w/v TFA) matrix solution. The mass spectra were externally calibrated with insulin and matrical peaks.

Database Searching

Database searching was performed using the internet available program Mascot (http://www.matrixscience.com/). The search parameters were used as follows: Database: SWISS-PROT; species of protein origin: human or mammalia; S-carbamidomethyl-derivative (Cys-CAM) as cysteines, allowed maximum peptide mass error of 0.1-1 Da, more than four peptide mass hits required for protein match, 1-2 enzymatic missed cleavages, methionine in oxidized form, and protein mass restricted to a range of 30%.

Results

Determination of Time Point of Apoptosis Initiation Induced by 5-Fluorouracil in MGC-803 Cells

As shown in Figure 1, the apoptotic rate of 5-fluorouracil-treated cells, 5-fluorouracil-withdrawal cells and control cells were analyzed by means of flow cytometry. Compared with the control cells, the apoptotic rates of 5-fluorouracil- withdrawal cells at 3 and 6 hours showed no difference, while at 12, 24, 48 and 72 hours the rates remarkably increased by 4.50-fold, 4.77-fold, 9.03-fold and 5.64-fold, respectively. In 5-fluorouracil-treated group, the apoptotic rates of MGC-803 cells treated by 5-fluorouracil for 3, 6 and 12 hours showed no difference, while for 24, 48 and 72 hours the rates remarkably increased by 3.42-fold, 5.41-fold and 4.50-fold, respectively. Under electron microscope, MGC-803 cells treated by 5-fluorouracil for 12 hours had no apoptotic characteristic change, showing only spherical shape (Figure 2A) and surface villi edema (Figure 2B). There was no change in cell nuclear and cytoplasma under scanning electron microscopy. While MGC-803 cells treated by 5-fluorouracil for 12 hours, and continuously cultured till to 72 hours, showed unique morphological features of apoptosis including cell membrane blebbing (Figure 2C), chromatin condensation, nuclear fragmentation and

apoptotic bodies (Figure 2D). Taken together, these results demonstrated 12 hours was the time point of apoptosis initiation induced by 5-fluorouracil in MGC-803 cells. At this time point, these cells showed irreversible apoptosis even after the 5-fluorouracil-withdrawal appeared. So this special setpoint can be considered as the phase for the apoptosis initiation.

Protein Expressions Associated with Apoptosis Initiation Induced by 5-Fluorouracil

Two-dimensional electrophoresis of proteins extracted from MGC-803 cells treated or not treated with 5-fluorouracil was performed to find proteins associated with the apoptosis initiation (Figure 3A, B). Electrophoresis of the same samples was performed three times under the same conditions. The gels stained with Coomassie Brilliant Blue G-250 were analysed using ImageMaster 2D Elite 3.01. 1032±61 spots in MGC-803 cells not treated with 5-fluorouracil, and 1051±49 spots in MGC-803 cells treated with 5-fluorouracil were detected respectively. With one of the MGC-803 cell gels free from 5-fluorouracil as the reference gel, 80.41% and 78.53% of the spots could be matched with the MGC-803 cells not treated with 5-fluorouracil and MGC-803 cells treated with 5-fluorouracil gels respectively. Differential analysis of protein expressions indicated, during apoptosis initiation, that 22 spots were differentially expressed with more than twofold alterations, including 14 upregulated spots and 8 downregulated spots.

Identification of Protein Associated with Apoptosis Initiation by Peptide Mass Fingerprinting

The proteins associated with the apoptosis initiation were excised from 2D gels. After in-gel digestion, 22 mass spectrums of proteins were acquired by peptide mass fingerprinting. The mass spectrum of spot 876 is presented in Figure 4. Database search parameters used are listed in Table 1, and the search result in Figure 5 using Mascot. It reveals the score of spots 876 is 156. Because protein scores greater than 60 are considered significant ($P < 0.05$), spot 876 is identified to glyceraldehyde 3-phosphate dehydrogenase. By this way, 22 proteins associated with the apoptosis initiation were identified, as shown in Table 2.

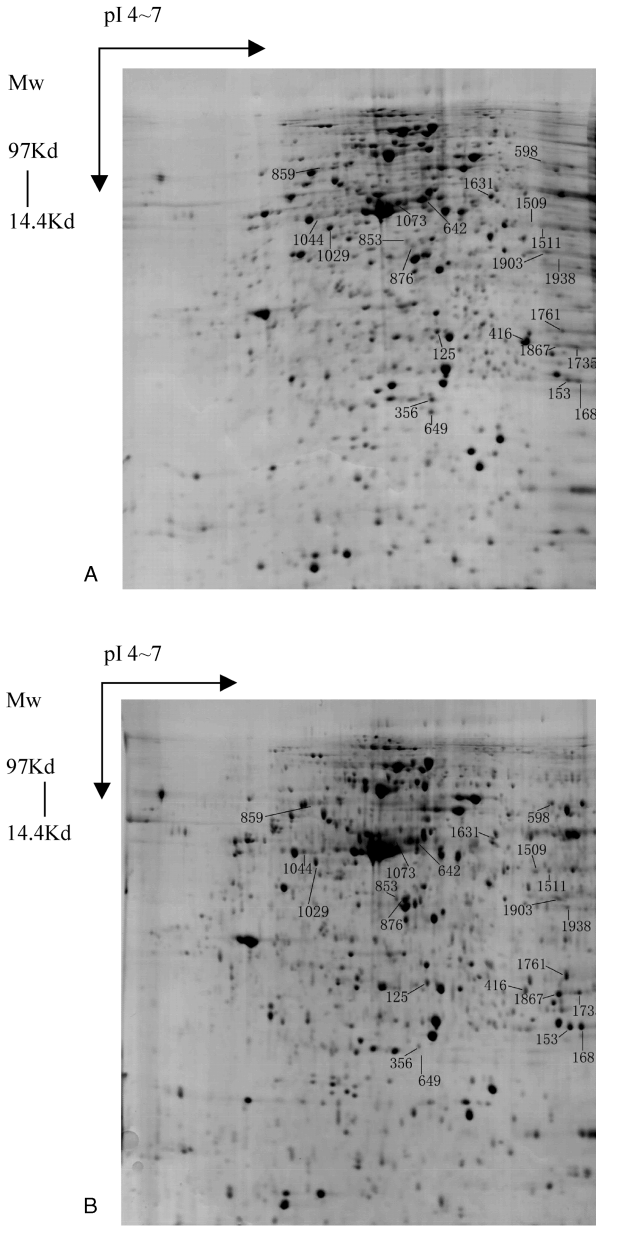

Fig. 3. Maps of MGC-803 cells proteins during the apoptosis initiation induced by 5-fluorouracil A. Cells without 5-fluorouracil; B. Cells treated with 5-fluorouracil for 12 h.

Discussion

There is a subtle balance between apoptosis and the intracellular defense against apoptosis. Once the balance is disturbed, cells would irreversibly initiate to undergo the execution of apoptosis (8). At the same time, cell apoptosis was regulated by the cellular genetic background in which it is immersed, and external enviroment. Therefore, different apoptosis inducers and different tumor cells have different apoptosis-initiation phases. In the present study, human gastric cancer MGC-803 cells at logarithm growth during apoptosis-initiation induced by 5-fluorouracil in MGC-803 cells were determined with 5-fluorouracil-withdrawal, several proteins associated with apoptosis initiation induced by 5-fluorouracil in MGC-803 cells were screened by means of comparative proteomics analysis. These proteins are involved in

Table 1. Database searching parameters of spot 876.

Type of search	Peptide Mass Fingerprint
Database	SwissProt
Taxonomy	Homo sapiens (human)
Enzyme	Trypsin
Modifications	Carbamidomethyl (C), Oxidation (M)
Mass values	Monoisotopic
Protein Mass	Unrestricted
Peptide Mass Tolerance	±0.1 Da
Peptide Charge State	1+
Max Missed Cleavages	1
Number of queries	28

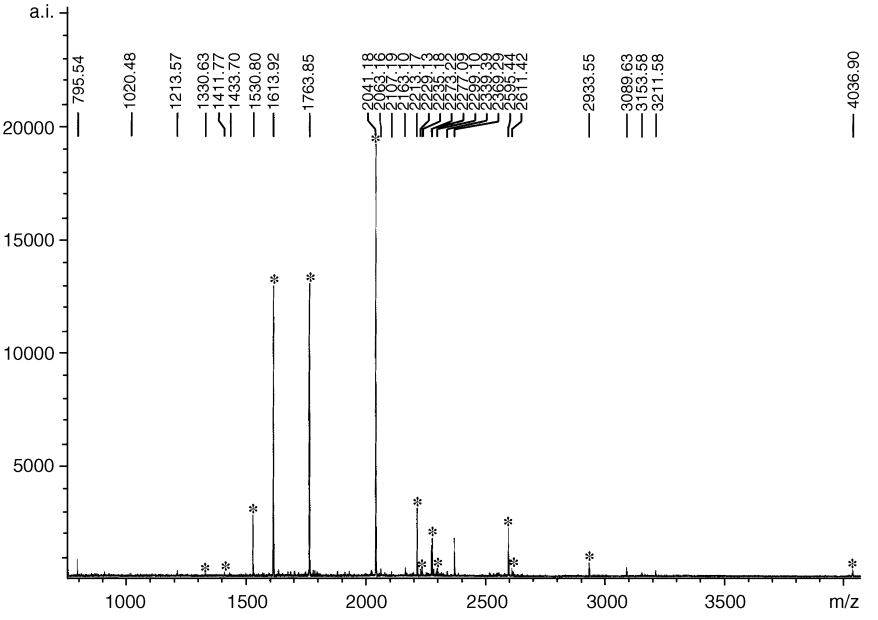

Fig. 4. Peptide mass fingerprint spectrum of protein spot 876 * matching peptide.

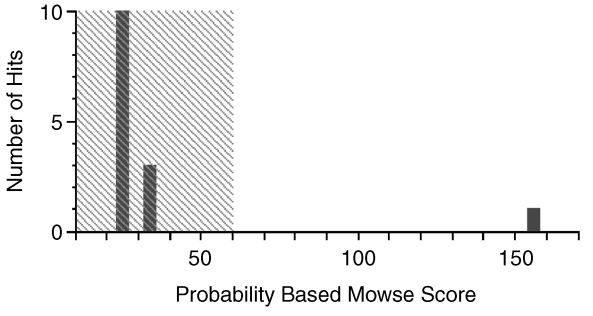

Fig. 5. Database searching results of the PMF data from spot 876.

metabolism, oxidation, cytoskeleton, signal transduction and other aspects. According to their roles in cell apoptosis, they may be divided into two kinds: molecules indicating a role in the response against cell apoptosis, and contributing to defend against apoptosis and protect cells from apoptosis induced by external apoptosis triggers, and another kind of proteins promoting the occurrence of cell apoptosis.

Five proteins related to metabolism and biological oxidation were upregulated at the apoptosis-initiation phase, and they protected the cells from apoptosis by antagonising apoptosis-initiation. When the expression of these proteins was blocked, the cells apoptosis would be initiated. These proteins include glyceraldehyde 3-phosphate dehydrogenase (GAPDH), alpha enolase, ATP synthase D chain, Metallothionein (MTs) and Peroxiredoxin 2 (Prx II). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a well-known glycolytic enzyme that plays a key role in energy metabolism of cells, especially in tumor cells. It was reported that GAPDH mRNA increased in tumor cells

Table 2. Identification of proteins associated with the apoptosis initiation by Mascot in Swiss-port

Spot	Swiss-port number	Protein name (fold)#	Protein alterations	Score Coverage (%)	Sequence
876	P04406	Glyceraldehyde 3-phosphate dehydrogenase	+23.4	156	55%
1735	P06733	Alpha enolase	+2.73	178	52%
1511	075947	ATP synthase D chain, mitochondrial	+3.16	104	63%
598	042152	Metallothionein	+4.52	83	100%
125	P80292	Metallothionein-II E	-2.23	70	75%
1044	P32119	Peroxiredoxin 2	+13.43	104	35%
1938	Q06830	Peroxiredoxin 1	+15.38	117	55%
1867	P30044	Peroxiredoxin 2	+3.79	88	37%
1903	P46405	40S ribosomal protein S12	+9.51	78	51%
1631	Q9NTW7	Zinc finger protein 338	+18.5	63	37%
649	075469	Orphan nuclear receptor PXR	-8.74	60	27%
642	P20694	Cellular nucleic acid binding protein (CNAP)	-8.64	80	53%
168	P02570	Action, cytoplasmic (Beta action)	+4.02	136	38%
356	P08670	Vimentin	-3.11	71	25%
859	P08670	Vimentin	-2.34	146	33%
153	P26038	Moesin	+3.91	66	27%
416	P05783	Cytoskeletal 18	-4.88	77	26%
1029	Q9UL16	Nasopharyngeal epithelium specific protein 1	-3.27	83	17%
1059	P27797	Calreticulin precursor (CRP55)	+20.17	71	19%
1073	Q9NQ38	Serine protease inhibitor Kazal-type 5 precursor	-3.43	61	16%
1761	P24605	Phospholipase A2 homolog 2	+3.18	76	66%
853	Q9BX26	Synaptonemal complex protein 2	+8.77	68	20%

MGC-803 cells treated by 5-fluorouracil for 12 hours *vs*. MGC-803 cells

including human cervical carcinoma, hepatocellular, and human prostate cancer cells (9, 10, 19). GAPDH antisense oligodeoxynucleotides could induce apoptosis by blocking the GAPDH expression in human cervical carcinoma (10). GAPDH is also a unique target of nitric oxide, which is implicated in transmission signals of apoptosis (22). Alpha enolase, a key glycolytic enzyme. Recently, autoantibodies against alpha-enolase were detected in the patients with cancer-associated retinopathy syndrome and in healthy subjects using synthetic peptides covering the entire sequence of human alpha-enolase (1). ATP synthase D chain, mitochondrial, also known as the F (1) F (0)-type ATP synthase, is a key enzyme in cellular energy interconversion. It can use a proton gradient and the associated membrane potential to synthesize ATP. It is also able reverse and hydrolyze ATP to generate a proton gradient. Oligomycin and apoptolidin, inhibitors of ATP-synthase, can suppress the TNF-induced apoptosis (20, 21). The overexpression of metallothionein (MTs) was recently found to induce apoptosis by reinforces of DNA repair, decreasing of DNA topoisomerase activity, inhibition of NF-B, reverse of caspase-8 and Jun N-termal kinase, and participate in the tumor resistance (12, 23). Peroxiredoxin 2 (Prx II), a new family member of highly conserved antioxidant enzymes, can regulate the signal transduction pathways that utilize c-Abl, caspases, nuclear factor-kappa B (NF-kappa B) and activator protein-1 (AP-1) to influence cell growth and apoptosis (2). Overexpressed Prx II inhibits cisplatin-induced apoptosis, thereby contributing to chemoresistance of tumor cells (5). Antisense of human peroxiredoxin II enhances radiation-induced apoptosis (18). Peroxiredoxin 1 and Peroxiredoxin 5 have similar biological properties.

Other upregulated proteins may influence MGC-803 cells apoptosis-initiation induced by 5-fluorouracil. Zinc finger protein 338, a member of the zinc finger protein family C_2H_2 (2Cys-2His). Zinc finger protein is a negative regulator of tumor necrosis factor (TNF)-induced signaling pathways by blocking both TNF-induced activation of c-Jun N-terminal kinase, NF-kappa B and processing of the receptor-associated caspase-8 leading to apoptosis

(4, 13). Phospholipases A2 homolog 2, and phospholipases A2 induce cell apoptosis *via* a mechanism involving cytochrome c-dependent apoptotic signaling cascade or tumor necrosis factor and TNF-related apoptosis-inducing ligand (TRAIL) (7, 14, 17). Actin, cytoskeletal filaments, action reorganization triggered signal transduction mediated by Ras and Rho, family members of G protein related to Rac, affect cell apoptosis (11, 27). Moesin, membrane-organizing extension spike protein, serves as an "ankyrin", closely links some specific proteins to cytoskeleton filament, and actualizes cell rigidity and ductility. It can accelerate apoptosis by inhibiting the activation of NF-Kappa B (6).

Serine protease inhibitor Kazal-type 5 precursor is downregulated in MGC-803 cells during the initiation phase of apoptosis induced by 5-fluorouracil. Due to the decrease of serine protease inhibitor, execution function of cell apoptosis through serine protease pathway was reinforced, and cells apoptosis was promoted (26).

Although other proteins expressed differentiately in MGC-803 cells during apoptosis-initiation phase induced by 5-fluorouracil, their meaning was still unclear. Many gene coding products were implicated in the occurrence and regulation of cell apoptosis, so further analyses are in progress to screen and identify these proteins so as to obtain apoptosis-initiation correlated proteins of greater importance.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 30270079) ,the Scientific Foundation of Innovative Research Team in Shandong University, the Natural Science Foundation of Shandong Province (No. Y2004C03) and the Science Foundation for Young Scholars of Shandong Province (No.2004BS02007), P.R.China.

References

1. Adamus, G., Amundson, D., Seigel, G.M. and Machnicki, M. Anti-enolase-alpha autoantibodies in cancer-associated retinopathy: epitope mapping and cytotoxicity on retinal cells. *J. Autoimmun.* 11: 671-677, 1998.
2. Butterfield, L.H., Merino, A., Golub, S.H. and Shau, H. From cytoprotection to tumor suppression:the multifactorial role of peroxiredoxins. *Antioxid. Redox. Signal.* 1: 385-402, 1999.
3. Chen, C., Jia, J., Zhang, M., Meng, Y., Kong, D., Pan, X. and Yu, X. Proteomic analysis on multi-drug resistant cells HL-60/DOX of acute myeloblastic leukemia. *Chinese J. Physiol*. 48: 115-120, 2005.
4. Chen, D., Li, X., Zhai, Z. and Shu, H.B. A novel zinc finger protein interacts with eceptor-interacting protein (RIP) and inhibits tumor necrosis factor (TNF)-and IL1-induced NF-Kappa B activation. *J. Biol. Chem*. 277: 15985-15991, 2002.
5. Chung, Y.M., Yoo, Y.D., Park, J.K., Kim, Y.T. and Kim, H.J. Increased expressed of peroxiredoxin II confers resistance to cisplatin. *Anticancer Res.* 21: 1129-1133, 2001.
6. de Bruin, E.C., Meersma, D., de Wilde, J., den Otter, I., Schipper, E.M., Medema, J.P. and Peltenburg, L.T. A serine protease is involved in the initiation of DNA damage-induced apoptosis. *Cell Death Differ*. 10: 1204-1212, 2003.
7. Fasanmade, A.A., Owuor, E.D., Ee, R.P., Qato, D., Heller, M. and Kong, A.N. Quinacrine induces cytochrome c-dependent apoptosis signaling in human cervical carcinoma cells. *Arch. Pharm. Res.* 24: 126-135, 2000.
8. Gonzalez, V.M., Fuertes, M.A., Alonso, C., and Perez, J.M. Is cisplatin-induced cell death always produced by apoptosis? *Mol. Pharmacol*. 59: 657-663, 2001.
9. Kim, J.W., Kim, S.J., Han, S.M., Paik, S.Y., Hur, S.Y., Kim, Y.W., Lee, J.M. and Namkoong, S.E. Increased glyceraldehyde-3-phosphate dehydrogenase gene expression in human cervical cancers. *Gynecol. Oncol*. 71: 266-269, 1998.
10. Kim, J.W., Kim, T.E., Kim, Y.K., Kim, Y.W., Kim, S.J., Lee, J.M., Kim, I.K. and Namkoong, S.E. Antisense oligodeoxynucleotide of glyceraldehyde-3-phosphate dehydrogenase gene inhibits cell proliferation and induce apoptosis in human cervival carcinoma cell line. *Antisense Nucleic Acid Drug.* 9: 507-513, 1999.
11. Kim, J.Y., Kim, H., Jeun, S.S., Rha, S.J., Kim Y.H., Ko, Y.J., Won, J., Lee, K.H., Rha, H.K. and Wang, Y.P.. Inhibition of NF-KappaB activation by merlin. *Biochem. Biophys. Res. Commun.* 296: 1295-1302, 2002.
12. Koo, M.S., Kwo, Y.G., Park, J.H., Choi, W.J., Billiar, T.R. and Kim, Y.M. Signaling and function of caspase and C-Jun N-terminal kinase in cisplatin-induced apoptosis. *Mol. Cell.* 13: 194-201, 2002.
13. Lademann, U., Kallunki, T. and Jaattela, M. A20 Zinc finger protein inhibits TNF-induced apoptosis and stress response early in the signalling cascades and independently of binding to TRAF2 or 14-3-3 proteins. *Cell Death Differ*. 8: 265-272, 2001.
14. Liu, Y., Hong, Y., Androphy, E.J. and Chen, J.J. Rb-independent induction of apoptosis by bovine papllomavirus type1 E7 in response to tumor nencrosis factor alpha. *J. Biol. Chem*. 275: 30894-30900, 2000.
15. Makin, G. Targeting apoptosis in cancer chemotherapy. *Expert Opin Ther Targets.* 6: 73-84, 2002.
16. Malliri, A., van der, Kammen, R.A., Clark, K., van der, Valk. M., Michiels, F. and Collard, J.G. Mice deficicent in the Rac activator Tiaml are resistant to Ras-induced skin tumor. *Nature* 417: 867-871, 2002.
17. Martin, S.S. and Leder, P. Human MCF10A mammary epithelial cells undergo apoptosis following actin depolymerzation that is independent of attachment and rescued by Bcl-2. *Mol. Cell. Biol*. 21: 6529-6536, 2001.
18. Park, S.H., Chung, Y.M., Lee, Y.S., Kim, H.J., Kim, J.S., Chae, H.Z. and Yoo, Y.D. Antisense of human peroxiredoxin II enhances radiation-induce cell death. *Clin. Cancer Res.* 6: 4915-4920, 2000.
19. Rondinelli, R.H., Epner, D.E. and Tricoli, J.V. Increased glyceraldehyde-3-phosphate dehydrogenase gene expression in late pathological stage human prostate cancer. *Prostate Cancer Prostatic Dis.* 1: 66-72, 1997.
20. Salomon, A.R., Voehringer, D.W., Herzenberg, L.A. and Khosla, C. Apoptolidin, a selective cytotoxic agent, is an inhibitor of F0F1-ATPase. *Chem. Biol*. 8: 71-80, 2001.
21. Shchepina, L.A., Pletjushkina, O.Y., Avetisyan, A.V., Bakeeva, L.E., Fetisova, E.K., Izyumov, D.S., Saprunova, V.B., Vyssokikh, M.Y., Chernyak, B.V. and Skulachev, V.P. Oligomycin, inhibitor of F0 part of H^+-ATP-systhase, suppresses the TNF-induced apoptosis. *Oncogence* 21: 8149-8157, 2002.
22. Sirover, M.A. New insights into an old protein:the functional dirersity of mammalian glyceraldehydes-3-phosphate dehydrogenase. *Biochim. Biophys. Acta*. 1432: 159-184, 1999.
23. Tekur, S. and Ho, S.M. Ribozyme-mediated downregulation of human metallthionein II (α) induces apoptosis in human prostate

and Dvarian Cancer cell lines. *Mol. Carcinog-en*. 33: 44-55, 2002.

24. Uetsuka, H., Haisa, M., Kimura, M., Gunduz, M., Kaneda, Y., Ohkawa, T., Takaoka, M., Murata, T., Nobuhisa, T., Yamatsuji, T., Matsuoka, J., Tanaka, N. and Naomoto, Y. Inhibition of inducible NF-kappaB activity reduces chemoresistance to 5-fluorouracil in human stomach cancer cell line. *Exp. Cell Res*. 289: 27-35, 2003.
25. Voss, T., Ahorn, H., Haberl, P., Dohner, H. and Wilgenbus, K. Correlation of clinical data with proteomics profiles in 24 patients with B-cell chronic lymphocytic leukemia. *Int. J. Cancer* 91: 180-186, 2001.
26. Wan, J., Wang, J., Cheng, H., Yu, Y., Xing, G., Oiu, Z., Qian, X. and He, F. Proteomic analysis of apoptosis initiation induced by all-trans retinoic acid in human acute promyelocytic leukemia cells. *Electrophoresis* 22: 3026-3037, 2001.
27 Wilson, H.A., Allred, D.V., O'Neill, K. and Bell, J.D. Activities and interactions among phospholipases A2 during thapsigargin-induced S49 cell death . *Apoptosis* 5: 389-396, 2000.