# Difference of Apoptosis-associated Gene Expression using DNA Microarray Analysis in Gastric Cancer Cell Lines according to p53 Status induced by Low-dose CDDP+5FU, or TNF $\alpha$ +IFN $\gamma$

# Difference of Apoptosis-associated Gene Expression using DNA Microarray Analysis in Gastric Cancer Cell Lines according to p53 Status induced by Low-dose CDDP＋5FU, or TNFα＋IFNγ

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Anticancer drugs; fluorouracil（5FU）＋cisplatin（CDDP）or cytokines; tumor necrosis factor（TNF）α＋ interferon（IFN）γ, characteristically induced apoptosis in gastric cancer cell lines. To elucidate each of the apoptotic pathways, we investigated the apoptosis-associated genes in p53 wild-or mutant-type gastric cancer cells using a cDNA microarray. MKN45（p53 wild type）or MKN28（p53 mutant type）was treated with either 5FU＋CDDP or TNFα＋ IFNγ. The apoptotic index（AI）was determined by Hoechst staining. 5FU＋CDDP induced a higher degree of apoptosis in MKN45 than in MKN28. In microarray analysis, anticancer drug-induced apoptosis was associated with the up-regulation of some genes such as jun B proto-oncogene（jun B）. The Fas-associated via death domain（FADD）gene was markedly down-regulated. Caspase3 and prefoldin genes were up-regulated in MKN45. TNFα＋ IFNγ also induced a higher degree of apoptosis in MKN45 than in MKN28. Although we found that the junB gene was up-regulated, FADD gene expression was unchanged. Expression of the insulin-like growth factor binding protein 2（IGFBP2）was markedly down-regulated. For confirmation, the expression of selected genes was analyzed by RT-PCR. It was suggested that Fas directly activates caspase3, and that transcription factors, including molecular chaperones, play an important role in 5FU＋CDDP-induced apoptosis. On the other hand, it was suggested that IGFBP2 is associated with the apoptotic pathway by TNFα＋IFNγ. The p53 status relates to the induction of apoptosis. It should be possible to use microarray analysis in order to elucidate the apoptotic pathway in detail.

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Key words：cDNA microarray, Apoptosis, Gene expression, CDDP＋5FU, TNFα ＋ IFNγ

## INTRODUCTION

In cancer chemotherapy, there are two conflicting concepts. One is that the maximum blood or plasma concentration（C（max））of drugs must be used for effective chemotherapy, and the other is that chemotherapy requires long-term administration because of the absence of total cell killing[1]. In cancer chemotherapy for gastric carcinoma, low-dose administration of 5FU and CDDP has been widely used. Cisplatin is widely recognized as a DNA-damaging agent and the current thinking is that DNA plastination is an essential first step in the cytotoxic activity of the drug. However, the mechanism（s）whereby these DNA adducts kill cells is not fully understood[2]. Antitumor effects consist not only of DNA-damaging but also other actions, such as inhibition of angiogenesis and induction of apoptosis. Anticancer drugs commonly used in cancer therapy can induce tumor cell death by apoptosis.[3] Numerous signals modulate cell death in apoptosis. Recently, a proposed model for receptor-dependent and-independent signaling pathways in low-dose 5FU and CDDP-induced apoptosis in gastric cells was reported[4].

Cytokines are also thought to regulate apoptosis. For example, interferon（INF）γ periodically promotes or inhibits apoptosis[5]. Although we verified that IFNγ and tumor necrosis factor（TNF）α synergistically induce apoptosis in gastric cancer cells, the mechanism（s）has not been elucidated.

Microarray analysis has recently been developed as a highly efficient method of gene expression profiling[6,7]. To investigate the difference between the signaling pathway of the apoptosis induced by anticancer drugs（5FU and CDDP）and that of the apoptosis induced by cytokines（IFN γ and TNF α）, we analyzed the gene expression patterns in apoptotic cells using the microarray technique with gastric cancer cell lines, MKN45（p53 wild type）and MKN28（p53 mutant type）. The microarray results were confirmed by a semi-quantitative reverse transcriptase polymerase chain reaction.

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## MATERIALS AND METHODS

***Cell lines and medium*** The human gastric cancer cell lines, MKN45 (p53 wild type) and MKN28 (p53 mutant type), were maintained in RPMI1640 supplemented with 10% fetal bovine serum and antibiotic/antimyotic solution (Sigma, St. Louis, MO, USA) in 5 % $CO_2$ and 95% air at 37℃. Experiments were performed using the cells in the log phase.

***Drug effects*** The anticancer drugs 5-fluorouracil (5FU, Kyowa, Tokyo, Japan) and cisplatin (CDDP) (Randa, Nihonkayaku, Tokyo, Japan) were prepared. The cultured cells were treated with a mixture of 5-FU (100$\mu$ g/ml) and CDDP (20$\mu$ g/ml) in RPMI1640 and incubated for 24h at 37℃[8]. In the same way, the effects of cytokines were examined using IFN $\gamma$ (ENDOGENE, Woburn, USA) (100ng/ml) and TNF $\alpha$ (R&D Systems, Inc. Minneapolis, USA) (100ng/ml).

***Hoechst staining*** The cells treated with either anticancer drugs or cytokines were fixed with 1 % glutaraldehyde in phosphate-buffered saline (PBS) for 30 min, washed twice with PBS and stained with Hoechst33258. A thousand cells were observed and the proportion of normal cells and those presenting apoptotic morphology (fragmented or shrunken nuclei) were counted under fluorescence microscopy as the apoptotic index (AI). We performed three independent experiments and two different researchers observed the results.

***RNA isolation*** Total RNA was extracted from the treated and untreated cells using the modified acid guanidium/phenol/chloroform method. In addition, mRNA was purified from total RNA using the Oligotex™-dT30 〈Super〉 mRNA Purification kit (TaKaRa Shuzo Co., Kyoto, Japan).

***Preparation of probe and array hybridization*** mRNA from the treated cells 2 $\mu$ g was labeled by incorporating Cy 3-dUTP (Amersham Pharmacia Biotech, Piscataway, USA) during random-primed reverse transcription. With Cy 5-dUTP (Amersham Pharmacia Biotech, Piscataway, USA), we labeled the same quantity of mRNA from untreated cells. This labeling procedure was performed using the RNA Fluorescence Labeling Core Kit (M-MLV Version) Ver. 2.0 (TaKaRa Shuzo Co., Kyoto, Japan). Briefly, after the reaction mixtures containing the Oligo dT (18) primer were heated at 70℃ for 5 min, the labeling was carried out at 42℃ for 1 h. Labeled mRNA was purified by column chromatography, and applied to the microarray. After hybridization at 65℃ for 16 h, the slides were washed in washing buffer. We used the IntelliGene™ Human Apoptosis CHIP Version1.1 (TaKaRa Biomedicals, Shiga, Japan) in which 164 human apoptosis-related genes were spotted onto glass plates. A list of these genes is available on TaKaRa's Web site (http://www.takara-bio.co.jp).

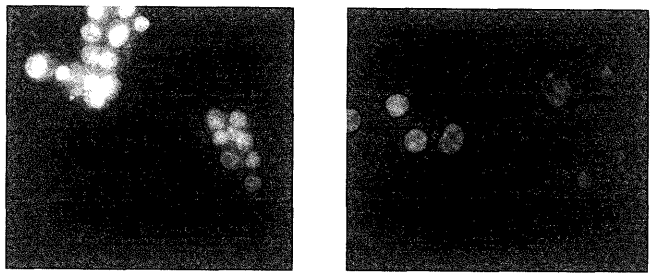
***Data analysis*** These slides were scanned on a ScanArray® 4000 (Microarray Analysis System) (GSI LUMONICS, Billerica, USA) confocal laser scanner, and the images were analyzed using Quant Array® (Quantative microarray software) (GSI LUMONICS, Billerica, USA). Data were normalized by using the results for house keeping genes ($\beta$-actin, Uba80 mRNA for ubiquitin, tubulin $\alpha$2, and glyceraldehydes-3-phosphate dehydrogenase (GAPDH)). We further classified genes for which the expression level increased or decreased by two-fold or more, and sorted them using Microsoft Excel[9]. Moreover, we set the lowest raw data value arbitrarily.

***Semi-quantative RT-PCR*** Based on previously published studies[6,9,10] and database information, PCR primers were designed for the following genes; GAPDH, junB, FAS-associated via death domain (FADD), insulin-like growth factor-binding protein2 (IGFBP2) and prefoldin. The number of amplifications was determined as 28 cycles and Table 1 shows the annealing temperature (Table 1). The data were normalized using GAPDH.

***Statistic analysis*** The measured values are presented as the mean ± standard deviation (SD) and the significance of differences was analyzed by the Mann-Whitney U test, regarding $p<0.05$ as significant.

Table 1 Panel of primers used for polymerase chain reaction

| gene | primers | | annealing temperature |
|---|---|---|---|
| GAPDH | 5'-CAACAGCCTCAAGATCATCAGC-3' | sense | 60℃ |
| | 5'-TTCTAGACGGCAGGTCAGGTC-3' | antisense | |
| junB | 5'-CCAGTCCTTCCACCTCGACGTTTACAAG-3' | sense | 58℃ |
| | 5'-GACTAAGTGCGTGTTTCTTTTCCACAGTAC-3' | antisense | |
| FADD | 5'-GTGCGGGAGTCACTGAGAAT-3' | sense | 56℃ |
| | 5'-GTCAGCCACCAGGTTCATAT-3' | antisense | |
| IGFBP 2 | 5'-ACTTGTGAGAAGCGCCGGGA-3' | sense | 56℃ |
| | 5'-GCTGCCCGTTCAGAGACATC-3' | antisense | |
| prefoldin | 5'-ACAGGGACCACGAGGTCATC-3' | sense | 56℃ |
| | 5'-TTAGCAGTAGCCTGAGCTGC-3' | antisense | |



MKN45 MKN28

Fig. 1 Microscopic findings of apoptotic cells in which apoptosis was induced by anticancer drugs (5FU+CDDP), evaluated by Hoechst 33258 immunostaining against MKN45 and MKN28 cells.

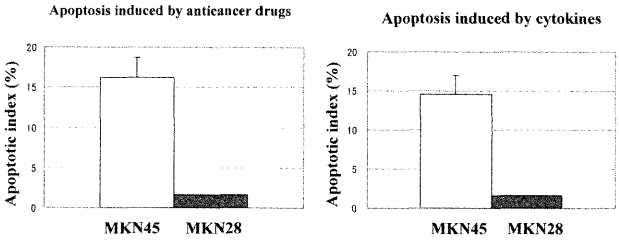



Fig. 2 Comparison of the apoptotic index (AI) of MKN45 (p 53；wild type) and MKN28 (p53；mutant type) cells treated with anticancer drugs (5FU+CDDP) or cytokines (TNFα+IFNγ).

## Results

***Either anticancer drugs or cytokines induced apoptosis in gastric cancer cell lines***

We confirmed with Hoechst staining that the apoptotic index was higher in cells treated with 5FU and CDDP than in those treated with 5FU or CDDP alone (data not shown). After the cells were cultured in the presence of 5FU (100μg/ml) and CDDP (20μg/ml), they were stained with the Hoechst stain (Fig. 1) and the apoptotic index (AI) was calculated. Although the AI for MKN45 (p53 wild type) cells was 16±2.5%, that for MKN28 (p53 mutant type) cells was only 1.6±0.1% ($p<0.05$) (Fig. 2-a). The synergism of IFN γ and TNF α in terms of the induction of apoptosis was also confirmed with Hoechst staining. MKN45 and MKN28 cells had AIs of 14.6±2.4% and 1.6±0.1%, respectively ($p<0.05$) (Fig. 2-b).

***Microarray analysis***

Genes were considered to be up-or down-regulated if the fold change in their expression level was at least 2.0 or greater. We investigated 164 apoptosis-associated genes using the IntelliGene™ Human Apoptosis CHIP (TaKaRa). When the cell lines were treated with anticancer drugs, 9 genes were up-regulated and 20 genes were down-regulated in MKN 45 cells. In MKN28 cells, 14 genes were up-regulated and 15 were down-regulated (Fig. 3). In both MKN45 and MKN28 cells, the up-regulated genes were the jun B proto-oncogene (jun B), the v-jun avian sarcoma virus 17 oncogene homolog and the retinoblastoma-binding protein gene. The down-regulated genes were the Fas (TNFRSF6)-associated via death domain (FADD), topoisomerase (DNA) II α (17kD) and cyclin-dependent kinase-like 1 genes. On the other hand, in MKN45 cells, the up-regulated genes were the profoldin 5, caspase3, superoxide dismutase1 and glutathione peroxidase 1 genes, which is the characteristic gene expression pattern for MKN45. Mitogen-activated protein kinase kinase1, 6 and mitogen-activated protein kinase 14 were down-regulated. In MKN28 cells, the up-regulated genes were the TRAF family member-associated NFκB activator and insulin-like growth factor 2 (somatomedin A) genes. The apoptosis inhibitor 4 (survivin) and cell division cycle2, 25B, 25C genes were down-regulated (Table 2).

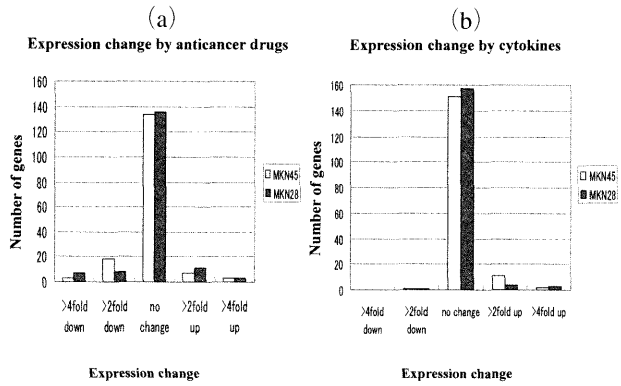



Fig. 3 Summary of the changes in gene expression before and after the treatment with anticancer drugs (a) or cytokines (b) analyzed using a cDNA microarray. (The expression of genes was either unchanged or increased or decreased by at least2-, or 4-fold)

Although cytokines (TNFα+IFNγ) induced apoptosis on a level equal to that induced by anticancer drugs (Fig. 3), we found different gene expression patterns. In MKN45 cells, 14 genes were up-regulated and only one gene (insulin-like growth factor binding protein 2) was markedly down-regulated. The expression of this gene (IGFBP2) was unchanged when apoptosis was induced by anticancer drugs. The FADD gene, which was down-regulated in anticancer drug-induced apoptosis, exhibited an unchanged expression pattern when cytokines were used to induce apoptosis. In MKN28 cells, the syndecan gene was down-regulated markedly (Table 3).

***RT-PCR***

To confirm the expression data from the cDNA microarray studies, we analyzed, by RT-PCR, the expression of 4 genes (junB, FADD, IGFBP2, and prefoldin) (Fig. 4). We found that for both the up-regulated and down-regulated genes, both methods detected similar patterns of expression.

## DISCUSSION

Antitumor effects in cancer chemotherapy have previously been thought to primarily involve the damaging of DNA. Cur-

Table 2 Changes in gene expression between the groups of up-regulated or down-regulated genes after co-culture with anticancer drugs

| MKN45 | |
|---|---|
| **gene name** | **fold change** |
| **4-fold up-regulated genes** | |
| v-jun avian sarcoma virus 17 oncogene homolog | 6.3 |
| tumor necrosis factor receptor superfamily, member 6 | 5.9 |
| jun B proto-oncogene | 4.7 |
| **2-fold up-regulated genes** | |
| retinoblastoma-binding protein 6 | 3.6 |
| glutathione peroxidase 1 | 3.2 |
| caspase 3, apoptosis-related cysteine protease | 2.4 |
| prefoldin 5 | 2.3 |
| myeloid cell lekemia sequence 1 (BCL2-related) | 2.2 |
| superoxide dismutase 1, solube (amyotrophic lateral sclerosis 1 (adult)) | 2.2 |
| **4-fold down-regulated genes** | |
| growth factor receptor-bound protein 2 | 7.2 |
| growth factor receptor-bound protein 10 | 6.9 |
| Fas (TNFRSF6) -associated via death domain | 6.8 |
| ectodermal-neurl cortex (with BTB-like somain) | 5.3 |
| v-akt murine thymoma viral oncogene homolog 1 | 5.1 |
| mitogen-activated protein kinase kinase 6 | 5.0 |
| **2-fold down-regulated genes** | |
| retonblastoma-binding protein 4 | 3.7 |
| retonblastoma 1 (including osteosarcoma) | 3.6 |
| mitogen-activated protein kinase kinase 1 | 3.3 |
| nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p10 | 3.2 |
| E2F transcription factor 3 | 3.1 |
| mitogen-activated protein kinase 14 | 3.0 |
| topoisomerase (DNA) II alpha (170kD) | 3.0 |
| cyclin-dependent kinase-like 1 (CDC2-related kinase) | 3.0 |
| cell division cycle 10 (homologous to SDS10 of S. cerevisiae) | 3.0 |
| cell division cycle 25A | 2.7 |
| BCL2-like 2 | 2.6 |
| cyclin-dependent kinase 4 | 2.3 |
| BCL2/adenovirus E1B 19kD-interacting protein 3 | 2.2 |
| menage a trois 1 (CAK assembly factor) | 2.1 |
| **MKN28** | |
| **gene name** | **fold change** |
| **4-fold up-regulated genes** | |
| insulin-like growth factor 2 (somatomedin A) | 5.3 |
| retinoblastoma-binding 6 | 4.8 |
| retinoblastoma-binding 2 | 4.4 |
| **2-fold up-regulated genes** | |
| BCL2-antagonist/killer 1 | 4.0 |
| retinoic acid receptor, beta | 3.7 |
| jun B proto-oncogene | 3.5 |
| E2F transcription factor 3 | 2.9 |
| adenosine monophosphate deaminase 2 (isoform L) | 2.7 |

| | |
|---|---|
| TRAF family menophosphate deaminase 2 （isoform L） | 2.7 |
| CDC6（cell bivision cycle 6, S cerevisiae）homolog | 2.4 |
| death-associated protein 6 | 2.4 |
| jun D proto-oncogene | 2.3 |
| v-jun avian sarcoma virus 17 oncogene homolog | 2.0 |
| lymphotoxin bera receptor（TNFR superfamily, member 3 | 2.0 |
| **4-fold down-regulated genes** | |
| cell division cycle 25C | 7.6 |
| cell division cycle 25B | 4.8 |
| retinoblastoma-like 2 （p130） | 4.5 |
| polo（Drosophia）-like kinase | 4.3 |
| topoisomerase（DNA）Ⅱ alpha（170kD） | 4.1 |
| DNA-damage-induclble transcript 3 | 4.1 |
| **2-fold down-regulated genes** | |
| apoptosis inhibitor 4 （survivin） | 3.1 |
| cyclin G1 | 2.7 |
| wee 1 +（S. pombe）homolog | 2.6 |
| CASP 8 and FADD-like apoptosis regulator | 2.6 |
| hest shock protease inhibited via death domain | 2.4 |
| serine protease inhibitor, Kunitz type, 2 | 2.3 |
| Fas（TNFRSF6）-associated via death domain | 2.1 |
| cell division cycle 2, G1 to S and G2 to M | 2.1 |
| cyclin-dependent kinase-like 1 （CDC2-related kinase） | 2.1 |

Table 3 Changes in gene expression between the groups of up-regulated or down-regulated genes after co-culture with cytokines

| **MKN45** | |
|---|---|
| **gene name** | **fold change** |
| **4-fold up-regulated genes** | |
| apoptosis inhibitor 2 | 26.2 |
| v-rel avian reticuloendotheliosis viral oncogene homolog B（nuclear factor of kappa light polypeptide gene enhancer in B-cells 3） | 18.6 |
| **2-fold up-regulated genes** | |
| caspase 7, apoptosis-related cysteine protease | 3.3 |
| tumor necrosis factor receptor superfamily, member 6 | 3.0 |
| TNF receptor-associated factor 2 | 3.0 |
| jun B proto-oncogene | 2.8 |
| nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 （p105） | 2.7 |
| BCL2-antagonist/killer 1 | 2.6 |
| mitogen-activated protein kinase kinase 1 | 2.5 |
| TRAF interacting protein | 2.4 |
| apoptosis inhibitor 1 | 2.2 |
| cell division cycle 25A | 2.2 |
| E2F transcription factor 5, p130-binding | 2.0 |
| **4-fold down-regulated genes** | |
| none | |
| **2-fold down-regulated genes** | |
| insulin-like growth factor binding protein 2 （36kD） | 2.1 |

| MKN28 | |
|---|---|
| **gene name** | **fold change** |
| **4-fold up-regulated genes** | |
| apoptosis inhibitor 2 | 27.2 |
| CASP8 and FADD-like apoptosis regulator | 5.2 |
| v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3) | 4.2 |
| **2-fold up-regulated genes** | |
| jun B proto-oncogene | 3.9 |
| tumor necrosis factor receptor superfamily, member 10 | 3.4 |
| tumor necrosis factor receptor superfamily, member 10 | 3.4 |
| apoptosis inhibitor 1 | 2.2 |
| tumor protein p53-binding protein, 2 | 2.2 |
| **4-fold down-regulated genes** | |
| none | |
| **2-fold down-regulated genes** | |
| syndecan 1 | 2.6 |

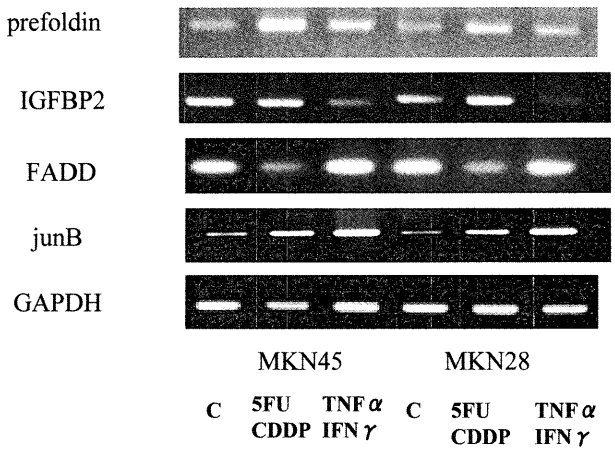



Fig. 4 Comparison of mRNA expression of prefoldin, IGFBP 2, FADD and jun B genes obtained from MKN45 or MKN28 cells treated with 5FU+CDDP or TNFα+IFNγ. C:control (untreated)

rently, events such as the inhibition of angiogenesis and induction apoptosis have become the index of antitumor effects. Furthermore, it was reported that low-dose 5FU+CDDP therapy was useful in a clinical setting. It was assumed that low-dose CDDP therapy might be an important modulator of 5FU cytotoxicity[11], but the exact mechanism (s) underlying this modulation has not been elucidated in terms of molecular biology.

On the other hand, with progress in the area of the molecular requirements for the induction of apoptosis, we found various reports on apoptotic pathways. Simone reported that strong induction of the CD95 ligand (CD95-L) and caspase activity was found in chemosensitive tumor cells (Hodgkin, Ewing's sarcoma, colon carcinoma and small cell lung carcinoma) but not in tumor cells which responded poorly to drug treatment (breast carcinoma and renal carcinoma) using CDDP and/or doxorubicin[12]. This may indicate that the same anticancer drug can induce apoptosis in different cancer cells via different pathways. Therefore, we showed that low-dose 5FU+CDDP or TNFα+IFNγ induced different apoptotic pathways in terms of p53 status using cDNA microarray analysis.

***Characteristics of apoptosis induced by 5FU+CDDP***

The junB, v-jun and FADD genes exhibited the same expression change in both MKN45 and MKN28 cells, which suggested that these genes were not associated with the p53 status or the apoptotic index. MacLaren et al[13] reported that v-jun-induced apoptosis was not associated with stabilization of p53 and did not utilize FADD or caspase8.

***Characteristics of gene expression change and apoptotic index***

***Cells treated with 5FU+CDDP***

Apoptosis was induced in MKN45 cells to a level ten times higher than in MKN28 cells, and microarray analysis indicated that the caspase3 gene was up-regulated in MKN45 cells. Kim et al[4] hypothesized that in treatment with low doses of 5FU+CDDP the induction of Fas did not activate capase 8 but directly activated caspase3. We found that the FADD gene was down-regulated and the expression of the caspase8 gene unchanged, which was not inconsistent with hypothesis proposed by Kim et al.. We also found that using MKN45 and MKN28 cells, the gene for prefoldin, one of the molecular chaperones associated with c-myc[14], and that for glutathione peroxidase 1 were up-regulated in MKN45 alone. Concerning the molecular chaperones and transcription factors, it was reported that the serine/threonine kinase Akt was inactivated after topotecan treatment and Akt inactivation might be im-

portant for topotecan-induced apoptosis in tumor cells[15]. In addition, Sato et al[16]. found that Akt could bind to Hsp90 (one of the molecular chaperones) in vivo and the formation of the Akt-Hsp90 complex stabilized the Akt kinase activity and protected cells from undergoing apoptosis by preventing phospho-Akt dephosphorylation. Although we have not found association between prefoldin and apoptosis, our study suggested that transcription factors might play an important role in the apoptosis induced by the treatment with 5FU+CDDP and prefoldin might be involved. The up-regulation of the glutathione peroxidase 1 gene in MKN45 alone leads us to believe that there is a p53-dependent apoptotic pathway via mitochondria.

***Cells treated with TNF α+IFN γ***

Although the junB gene was also up-regulated in cells treated with 5FU+CDDP, this expression change may be directly associated with the drugs used but not with apoptosis. Treatment with 5FU+CDDP resulted in the down-regulation of the gene for FADD, but the treatment with TNFα+IFNγ had no effect on the expression of FADD. We suggest that the cell-surface receptor is related to this differentiation. Moreover, it was observed that the gene for apoptosis inhibitor1, apoptosis inhibitor 2, v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cell 3), mitogen-activated protein kinase kinase 1 and E2F transcriptional factor 5 p103 binding were up-regulated, and only the IGFBP2 gene was down-regulated. These findings document the importance of apoptotic induction by cytokines. IGFBP2, one of the IGFBP1-7 proteins that bind insulin growth factors (IGFs) Ⅰ, Ⅱ and regulate their expression, has a stronger affinity for IGF-Ⅱ than for IGF-Ⅰ[17]. It has been confirmed that p53 RE is located in the first and second introns on the genome and expression of the IGFBP3 gene is increased by wild-type p53[18]. Furthermore, it was reported that IGFBP2 expression was associated with proliferation in colon cancer cell lines, whereas IGFBP3 was associated with differentiation.[19], and that circulating IGFBP2 levels markedly increased in prostate cancer, whereas IGFBP3 levels decreased[20]. Our result shows that the down-regulation of IGFBP2 is a novel and important event in the apoptotic induction mechanism.

## CONCLUSIONS

Our findings may indicate that the Fas pathway directly activating caspase 3 is more important than the pathway via FADD and caspase 8 in 5FU+CDDP-induced apoptosis, and that the transcription factors, including molecular chaperones, play an important role in apoptosis. On the other hand, it was suggested that IGFBP2 is associated with the apoptosis induced by TNFα+IFNγ. Moreover, the p53 status relates to the induction of apoptosis.

The molecular mechanism in apoptosis is not as simple as a specific initiator leading to a specific pathway. It seems that many signaling molecules are modulated in apoptosis. Because of this complicated network, we suggested that microarray should be used to determine the expression of a large number of genes in a single experiment. It would be useful to further investigate the expression of apoptotic genes in detail using the microarray, such that apoptosis-associated genes are spotted as a network, to provide new information in the cancer treatment field.

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# 胃癌細胞株におけるp53発現と抗癌剤（5FU＋CDDP）及びサイトカイン（TNFα＋IFNγ）によるアポトーシス誘導の特徴 －マイクロアレイでの検討—

胃癌細胞株を用いて，p53発現の有無と抗癌剤及びサイトカインにより誘導されるアポトーシスの特徴をcDNAマイクロアレイを用いた遺伝子発現の変化から検討した。ヒト胃癌細胞株MKN45（p53wild）とMKN28（p53mutant）を用い，low doseの5FU＋CDDP或いはTNFα＋IFNγと接触させて誘導される遺伝子発現をマイクロアレイを用いて解析し，up-regulate及びdown-regulateした遺伝子を同定した。さらに，up-regulate及びdown-regulateした数種類の遺伝子に対しRT-PCRを施行し，発現の有無をmRNAレベルで確認した。抗癌剤で誘導されるアポトーシスにおいて，MKN45，MKN28で共通にup-regulateしたものとしてjunBなどがあり，共通にdown-regulateしたのはFADDであった。アポトーシスが高頻度に誘導されたMKN45にのみ特徴的にup-regulateしたのはcaspase3，prefoldinであった。また，caspase8の発現変化は認めなかった。一方，サイトカインによるアポトーシスで特徴的であったのは，抗癌剤によるアポトーシスでは共通にdown-regulateしていたFADDに変化がなく，IGFBP2がdown-regulateしていることであった。low doseの5FU＋CDDPで誘導されるアポトーシスでは，転写因子prefoldinが何らかの役割を果たしている可能性が示唆され，サイトカインにより誘導されるアポトーシスではIGFBP2の低発現が重要であると思われた。また，複雑なネットワークをもつアポトーシス経路を解明する上で，多数の遺伝子の変化を一度に解析しうるcDNAマイクロアレイは，有用なツールであり，多彩な新知見が得られる可能性が示唆された。