Jpn. J. Cancer Res. 88, 49–55, January 1997

Expression of Fas Antigen and Its Mediation of Apoptosis in Human Gastric Cancer Cell Lines

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Fas, a member of the tumor necrosis factor receptor/nerve growth factor receptor family, induces apoptosis by crosslinking with Fas ligand or anti-Fas antibody in a variety of cultured cells. We examined the expression of Fas antigen and its mediation of apoptosis in six human gastric carcinoma cell lines. Flow cytometric analysis and western blotting revealed relatively high expression of Fas antigen in MKN-74 (wild-type *p53* gene) and MKN-45 (wild-type), followed by MKN-1 (mutated), MKN-7 (mutated) and KATO-III (deleted). MKN-28 (mutated) showed minimal expression of the antigen. The expression was apparently enhanced by interferon-γ, except for MKN-1 and MKN-28. Anti-Fas antibody (100 ng/ml) induced nuclear fragmentation characteristic of apoptosis. Apoptosis occurred in a delayed fashion and the apoptotic index at 72 h was approximately 60% in MKN-74, 35% in MKN-45, and 20% in MKN-1 and KATO-III. A DNA ladder was noted in MKN-74 at 72 h. Expression levels of P53 and $P21^{Waf1}$ did not change for up to 48 h in MKN-74. The biological effects did not correlate with endogenous Bcl-2 expression. These results indicated that a) Fas antigen is variably expressed in human cultured gastric carcinoma cells, b) the protein transduces an apoptotic signal which leads to delayed cell death, and c) susceptibility to the antibody correlates well with the expression level of Fas antigen.

Key words: Apoptosis — Fas — Human gastric cancer cell line

Apoptosis is a distinct form of cell death, different from necrosis,[1–4)] by which cells are actively eliminated from normal and neoplastic tissue. Apoptosis plays a major role during development, embryogenesis, regulation of the immune system, and carcinogenesis, as well as in the maintenance of tissue homeostasis.[5)] Various protein molecules or oncogenes and suppressor genes are involved in the process of apoptosis, including *p53, myc, ras, c-fos, bcl-2, bax,* $p21^{waf1}$ and the Fas/Fas ligand system.[5–8)]

Fas is a 45 kDa cell surface protein, which is expressed in thymocytes, activated T and B cells, normal liver, heart and lung, as well as in several tumors.[9, 10)] Fas belongs to the TNFR (tumor necrosis factor receptor)/NGFR (nerve growth factor receptor) family, which includes CD27, CD30, and CD40.[11)] Repeating cysteine-rich extracellular domains characterize the members of this family. Fas and TNFR also contain a unique cytoplasmic region, termed the death domain, that is essential for initiating a cytolytic response.

The binding of agonistic anti-Fas antibody or Fas ligand to Fas antigen results in transduction of a cytolytic signal into the cell, followed by apoptosis. This was well examined in a variety of leukemia/lymphoma cell lines *in vitro*.[12–14)] Fas-mediated apoptosis has been shown to be induced within a few hours after anti-Fas antibody treatment. This mode of apoptosis is considered to be affected by Bcl-2, and to occur in a cell-cycle-independent manner.[15, 16)]

On the other hand, little is known about the expression or the role of the Fas antigen in epithelial tumor cells, including human gastric cancer. In this study, we examined the expression of Fas antigen in six human cultured gastric cancer cell lines. P53 and $P21^{Waf1}$ levels were also analyzed during the process of apoptosis induced by anti-Fas antibody. To our knowledge, this is the first report to demonstrate that expression of Fas antigen mediates signal transduction for apoptosis in human gastric carcinoma cell lines.

MATERIALS AND METHODS

Cell lines Six cell lines derived from human gastric cancer were used. Table I summarizes histological type and *p53* gene status, which has been reported elsewhere.[17)] All the cells were cultured in RPMI 1640 (Cosmo Bio, Tokyo) containing 10% fetal bovine serum, 2 m*M* glutamine and 100 U/ml penicillin in a humidified 5% CO_2 incubator at 37°C. Cells were passaged twice weekly and routinely examined for *mycoplasma* contamination.

Flow cytometric analysis The gastric cancer cells (5×10^5) were preincubated in the presence or absence of 50 JRU/ml interferon-γ (IFN-γ: Shionogi, Osaka) for 24 h.

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Table I. Human Gastric Cancer Cell Lines

Cell line	Histology	*p53* status[a]	Fas expression[b]	
			FACSort	western blotting
MKN-1	adenosquamous	mutated	++	++
MKN-7	well	mutated	+	+
MKN-28	well	mutated	±	±
MKN-45	poorly	wild type	++	++
MKN-74	well	wild type	++	++
KATO-III	scirrhous	complete deletion	+	±

a) *p53* gene status has been reported elsewhere.[17]
b) Expression levels are graded as (−) to (++).

The cells were incubated with 1 μg of anti-Fas monoclonal antibody (clone DX2; Pharmingen, San Diego, CA) for 30 min at 4°C, and washed twice with phosphate-buffered saline (PBS). Next, the cells were incubated with 1 : 20 dilution of FITC-labeled anti-mouse κ antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) for 30 min on ice. Finally, the cells were washed twice with PBS and resuspended in 1ml of PBS. Fluorescence was quantified using a FACSort (Becton Dickinson, San Jose, CA).

Cell viability and detection of apoptotic cells Approximately 5×10^4 cells were seeded in 28 cm^2 dishes. The cells were incubated with 50 JRU/ml IFN-γ in fresh medium for 24 h, followed by washing twice with PBS. Then, the cells were incubated with 100 ng/ml anti-Fas antibody in fresh medium for 12, 24, 48 and 72 h. After exposure to anti-Fas antibody, the cells were trypsinized and harvested. Cell viability was counted by trypan blue staining. Apoptotic cells were assessed morphologically by staining with Hoechst 33258 using cells fixed with Clarke fixative (ethanol : acetic acid = 3 : 1). Apoptotic index (AI) was defined as follows: AI = 100×apoptotic cells / 200 cells. We also conducted Wright and Giemsa staining for morphological observation of nuclei.

DNA fragmentation The cultured cells (5×10^5) were exposed to 100 ng/ml anti-Fas antibody for 12, 24, 48, and 72 h. Then, the cells were trypsinized, washed twice with PBS, and suspended in lysis buffer (25 m*M* Tris-HCl pH 8.0, 5 m*M* EDTA, 25 m*M* NaCl, 0.5% SDS, 100 μg/ml proteinase K). After incubation overnight at 37°C, DNA was extracted with phenol/chloroform and precipitated with isopropanol according to standard protocols. Finally, DNA was dissolved in TE buffer. One microgram of DNA was analyzed by electrophoresis on 2% agarose gel containing 0.5 μg/ml ethidium bromide and visualized under ultraviolet (UV) illumination.

Western blotting The cells were washed in PBS and solubilized in lysis buffer (50 m*M* Tris-HCl pH 7.4, 125 m*M* NaCl, 0.1% NP-40, 5 m*M* NaF, 1 m*M* PMSF, 1 ng/ml leupeptin, 10 ng/ml soybean trypsin inhibitor, 1 ng/ml aprotinin, 10 ng/ml *N*-tosyl-L-phenylalanyl chloromethyl ketone) for 60 min on ice. Lysates were centrifuged at 2,500*g* for 5 min. The protein concentration was determined by means of the Bradford protein assay (Bio-Rad Lab., Richmond, CA) using bovine serum albumin as the standard. Thirty micrograms of protein was resolved by electrophoresis on 12% polyacrylamide gels, electrotransferred to a polyvinylidene difluoride filter (Millipore, Bedford, MA), then blotted with mouse monoclonal antibody for Bcl-2 (1:50 dilution; clone: 124; Dako, Copenhagen, Denmark), P53 (1:200 dilution; clone: 1801; NOVO, Newcastle, UK) and P21^{Waf1} (1:25 dilution; clone 2G12; Pharmingen, San Diego, CA). Blots were developed with peroxidase-labeled anti-mouse IgG (1:2000 dilution; MBL Co., Nagoya) using an enhanced chemiluminescence system (ECL Detection System; Amersham, Bucks, UK).

RESULTS

Expression of Fas antigen in human gastric cancer cell lines First, we examined expression levels of Fas antigen by FACSort and western blotting. As shown in Fig. 1, Fas antigen was variably expressed in all 6 cell lines examined. MKN-74 and MKN-45 showed high expression levels, followed by MKN-1, MKN-7 and KATO-III, in contrast to the minimal expression in MKN-28. The cell lines were treated with INF-γ at the dose of 50 JRU/ml, which did not affect the viability of the cells examined, as shown in Fig. 2. INF-γ apparently enhanced the expression of the antigen in MKN-7, MKN-45, MKN-74, KATO-III. The enhanced expression levels of these four cells were comparable to the reported level of the Jurkat E-6 lymphoid cell line, which is sensitive to anti Fas antibody-induced apoptosis.[17] On the other hand, INF-γ had no or little effect on MKN-1 and MKN-28.

Western blot analysis showed higher expression of Fas antigen in MKN-1, MKN-45 and MKN-74, followed by MKN-7, MKN-28 and KATO-III (Fig. 3). The results

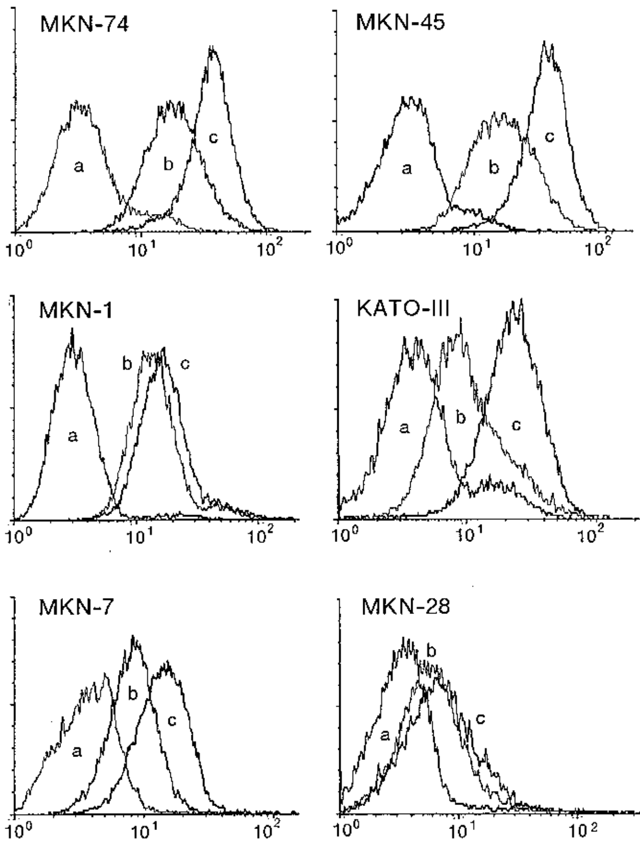

Fig. 1. Flow cytometric analysis of Fas antigen in the six cell lines. Background reactivity was that of cells stained with FITC-conjugated second antibody only. a, Background, b, basal level without IFN-γ treatment, c, enhanced expression of Fas antigen by treatment with 50 JRU/ml IFN-γ for 24 h.

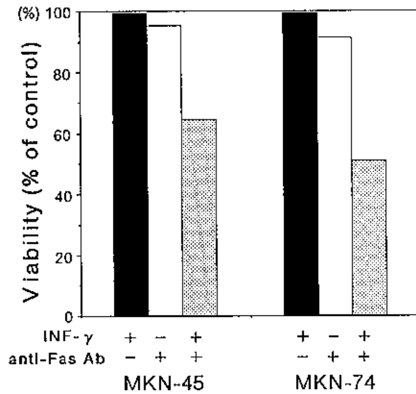

Fig. 2. Effect of IFN-γ on the viability of MKN-45 and MKN-74 cells. Cells were incubated for 24 h with only 50 JRU/ml IFN-γ, with only 100 ng/ml anti-Fas antibody, and with both.

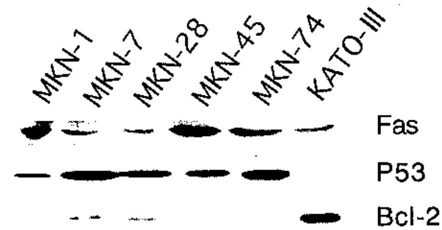

Fig. 3. Expressions of Fas, P53 and Bcl-2 in six gastric cancer cell lines by western blotting.

coincided well with those obtained by FACSort, except for KATO-III, in which a lower expression level was noted in the western blot, in contrast to the relatively higher level by FACSort.

Table I summarizes the expression of Fas antigen in the six cell lines examined; the highest expression was noted in MKN-74 and MKN-45, both of which carry the wild-type *p53* gene.[18] On the other hand, apparent Fas antigen expression was also found in MKN-1 and MKN-7, carrying the mutated *p53* gene, and in KATO-III with the *p53* gene deleted. In fact, KATO-III did not express P53 (Fig. 3). Thus, Fas expression did not necessarily correlate with the *p53* gene status or the histological type of cells.

Induction of cell death with anti-Fas antibody treatment As described above, a single treatment whth 50 JRU/ml INF-γ did not affect cell viability. Consecutive treatment with INF-γ and anti-Fas antibody, however, clearly decreased the viability of the cells examined.

To determine the susceptibility of the six cell lines to anti-Fas-mediated apoptosis, cells were initially cultured in the presence of INF-γ for 24 h, and then treated with 100 ng/ml anti-Fas antibody for 12, 24, 48 and 72 h. The experiments were repeated three times. The number of viable cells gradually decreased in a time-dependent manner (data not shown). Susceptibility to 100 ng/ml anti-Fas antibody was highest for MKN-74, followed by MKN-45, KATO-III, and MKN-1, in that order. MKN-7 and MKN-28 were highly resistant to the antibody. Thus, susceptibility correlated fairly well with enhanced expression level of the Fas antigen.

Morphological examination demonstrated the occurrence of apoptosis of the cells, even at 12 h. The apoptotic cells revealed fragmented nuclei by Hoechst 33258 staining (Fig. 4A). Giemsa staining showed a few cells with condensation of heterochromatin, but lacking nuclear fragmentation (Fig. 4B); this has been considered to be an early stage of apoptosis. Cells with fragmented nuclei increased gradually in all the cell lines, except for MKN-28, in which apoptotic cells were hardly detected.

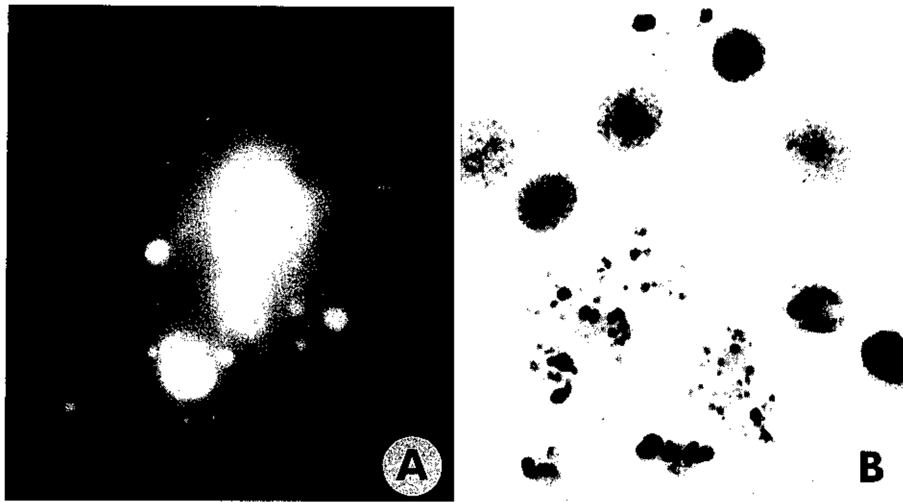

Fig. 4. Morphological analysis of MKN-74 cells treated with 100 ng/ml anti-Fas antibody for 48 h. Cells were stained with (A) Hoechst 33258 and (B) Wright and Giemsa. Cells with fragmented and condensed nuclei corresponding to apoptotic cells. A, ×1000, B, ×300.

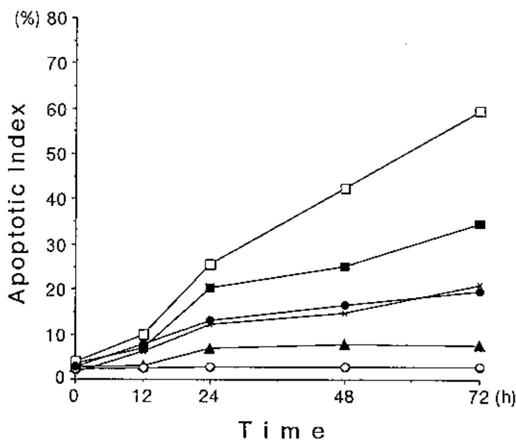

Fig. 5. Apoptotic indices induced by anti-Fas antibody treatment. MKN-1 (×), MKN-7 (▲), MKN-28 (○), MKN-45 (■), MKN-74 (□) and KATO-III (●) cells were incubated with 100 ng/ml anti-Fas antibody for 12, 24, 48 and 72 h. Apoptotic index was determined as described in "Materials and Methods." Data are average values of three independent experiments.

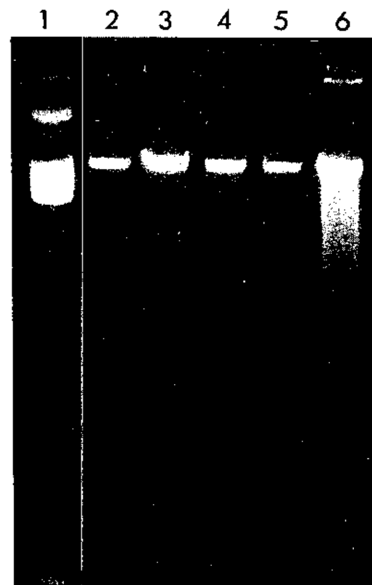

Fig. 6. Detection of DNA laddering on agarose gel electrophoresis. MKN-74 cells were incubated with or without 100 ng/ml anti-Fas antibody for 0 (lane 2), 12 (lane 3), 24 (lane 4), 48 (lane 5), and 72 h (lane 6). A 123 bp DNA ladder was used as a molecular marker (lane 1). A DNA ladder is noted only in lane 6 (72 h).

Fig. 5 shows the time course of the AI, i.e., the number of cells with fragmented nuclei per total cells. The AI was highest for MKN-74, followed by MKN-45, MKN-1, KATO-III, and MKN-7, in that order. At 72 h after anti-Fas antibody treatment, approximately 60% of MKN-74 cells, 35% of MKN-45 cells, and 20% of MKN-1 and KATO-III cells showed apoptosis. Thus, AI is correlated inversely with cell viability. A DNA ladder on agarose gel was clearly demonstrated in MKN-74 cells at 72 h (Fig. 6), in contrast to its absence in the case of MKN-45.

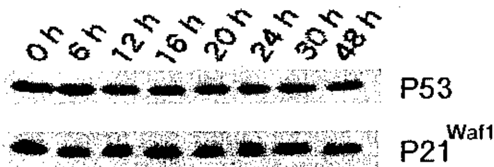

Fig. 7. Western blot analysis of the expression levels of P53 and P21^{Waf1} in MKN-74 cells after anti-Fas antibody treatment.

Expression of Bcl-2, P53 and P21^{Waf1} Bcl-2 was most highly expressed in KATO-III cells (Fig. 3). Lower levels of expression were noted in MKN-7 and MKN-28, in contrast to the almost complete absence of expression in MKN-1, MKN-45 and MKN-74 cells. The expression levels did not correlate with the apoptotic index after treatment with anti-Fas antibody.

Further, we examined levels of P53 and P21^{Waf1} after anti-Fas antibody treatment, using MKN-74 at 6, 12, 16, 20, 24, 30 and 48 h. No difference was detected in the expression levels of P53 and P21^{Waf1} after anti-Fas antibody treatment (Fig. 7).

DISCUSSION

We confirmed the expression of Fas antigen in all six human gastric carcinoma cell lines at various levels, by both FACSort and western blotting. The two methods showed similar expression levels of Fas antigen in each of the six cell lines, except for KATO-III, and in the latter case, the discrepancy was not large.

It is well documented that INF-γ upregulates Fas mRNA and enhances Fas antigen on the cell surface in normal and neoplastic cells.[9, 10] This is also consistent with the previous report that INF-γ-treated colon cancer cell line HT-29 cells acquired sensitivity to anti-Fas antibody.[9] In this study, we have confirmed the enhancement of Fas expression by INF-γ treatment in 4 of 6 cell lines, though MKN-1 and MKN-28 did not show a distinct effect. The precise mechanism involved remains to be elucidated.

Anti-Fas antibody variably reduced the cell viability of the cell lines examined, apparently due to induction of apoptosis, which was confirmed morphologically and biochemically. All the cell lines except for MKN-28 showed nuclear fragmentation, which is a morphological characteristic of apoptosis. Induction of apoptosis was relatively high in MKN-74 and MKN-45, in which apoptotic cells showed a similar morphology. A DNA ladder on agarose gel was apparent with the extract from MKN-74, but not with that from MKN-45. This might be partly due to delayed and gradual occurrence of apoptosis. It is unlikely that fragmented DNAs maintain their form in the culture medium. With progressive degradation of the fragmented DNAs into smaller molecules, the DNA ladder might be hardly detectable. A smaller number of apoptotic cells with optimal DNA cleavage might also result in the absence of the DNA ladder. In fact, no ladder was detected in the case of MKN-74 at 48 h, when the AI was higher than that of MKN-45 at 72 h.

It has been reported that inherent susceptibility to anti-Fas-induced apoptosis does not necessarily correlate with the expression level of this protein. This phenomenon has been well documented in both hematopoietic cells and non-hematopoietic cells, other than gastric carcinomas.[19] In this study, the AI was highest for MKN-74, followed by MKN-45 and KATO-III, although almost equal levels of expression were noted among the three cell lines. AI was less than 10% in MKN-7, which expressed lower levels of the antigen. Thus, susceptibility to anti-Fas antibody correlated relatively well with the enhanced expression level of this protein in the gastric cancer cell lines.

Of particular interest is the correlation between anti-Fas antibody susceptibility and *p53* gene status. Anti-Fas antibody-induced apoptosis occurred more readily in MKN-74 and MKN-45, which carry the wild-type *p53* gene, than in the other cell lines. Owen-Schaub *et al.* have demonstrated that transfection of wild-type *p53* gene upregulates the expression of cell surface Fas antigen in human lung cancer cell lines.[17] Thus, wild-type *p53* gene might partly correlate with higher expression of Fas antigen, resulting in higher susceptibility to anti-Fas antibody-induced apoptosis. Tamura *et al.* investigated the role of p53 protein in the induction of the Fas-mediated apoptosis using human cultured colon cancer cell, WiDr, which carries a mutation of the *p53* gene at codon 273.[20] They successfully transfected wild-type *p53* gene using a non-inducible promoter and confirmed the expression of wild-type p53 mRNA in the transfected clones, which were apparently more susceptible to anti-Fas-induced apoptosis than their parental cells, expressing mutant-type p53 mRNA. These findings suggested that wild-type *p53* gene might upregulate Fas gene expression. In the present study, we could not confirm a relationship between Fas expression and *p53* gene status. In fact, KATO-III carrying no normal *p53* gene and MKN-7 carrying the mutated *p53* gene, expressed relatively high levels of Fas antigen, the levels being similar to those of MKN-45 and MKN-74.

Tamura *et al.* also suggested that anti-Fas antibody-induced apoptosis might be incidentally linked to a *p53*-dependent apoptotic pathway.[20] *p53*-dependent apoptosis has been demonstrated to be initiated by DNA damage, such as radiation, UV, and DNA-damaging agents.[21, 22] We could not find any increase of P53 and P21^{Waf1}

expression in MKN-74 carrying wild-type *p53* gene for up to 48 h. This might imply that wild-type *p53* gene is not necessarily essential for induction of Fas-mediated apoptosis in human gastric carcinoma cell lines. Ni *et al.* found that anti-Fas antibody killed mice without *p53* gene as efficiently as wild-type mice, indicating that *p53* gene is not involved in the apoptotic pathway mediated by Fas.[23] In order to clarify the mechanism of Fas-mediated apoptosis, further study should focus on the intracellular signalling pathway mediated by Fas. Recent studies have demonstrated the involvement of several Fas "death-domain"-associated proteins and cysteine proteases of the interleukin-1β-converting enzyme (ICE)-family in Fas signal transduction.[24, 25] To date, nothing is known concerning interaction between *p53* and ICE in Fas-mediated apoptosis.

Expression of Bcl-2 has been shown to inhibit DNA fragmentation induced by a variety of stimuli, including anti-Fas, in cultured cell lines.[15, 26] Levels of Bcl-2 appear to be correlated with the biological effects of Fas in human malignant B-cells.[27] Transgenic mice expressing human *bcl-2* gene product could avoid anti-Fas antibody-induced fulminant hepatic failure due to massive apoptosis.[16] Our study, however, did not suggest an involvement of Bcl-2 levels in Fas-mediated apoptosis in the gastric cancer cell lines examined.

ACKNOWLEDGMENTS

We wish to thank Mr. Norihisa Itaki (First Department of Pathology, Tottori University) and Mrs. Misaki Kato (Gene Research Center, Tottori University) for their technical advice. This work was supported in part by a Grant-in-Aid for Cancer Research (No. 08265103) from the Ministry of Education, Science, Sports and Culture, Japan.

(Received September 13, 1996/Accepted October 31, 1996)

REFERENCES

1) Kerr, J. F. R., Wyllie, A. H. and Currie, A. A. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer,* **26,** 239–257 (1972).
2) Kerr, J. F. R., Winterford, C. M. and Harmon, B. V. Apoptosis. Its significance in cancer and cancer therapy. *Cancer,* **73,** 2013–2026 (1994).
3) Majno, G. and Joris, I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am. J. Pathol.,* **146,** 3–15 (1995).
4) Wyllie, A. H., Morris, R. G., Smith, A. L. and Dunlop, D. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. *J. Pathol.,* **142,** 67–77 (1984).
5) Steller, H. Mechanisms and genes of cellular suicide. *Science,* **267,** 1445–1449 (1995).
6) Shimamura, A. and Fisher, D. E. p53 in life and death. *Clin. Cancer Res.,* **2,** 435–440 (1996).
7) Bargou, R. C., Daniel, P. T., Mapara, M. Y., Bommert, K., Wagener, C., Kallinich, B., Royer, H. D. and Dörken, B. Expression of the *bcl-2* gene family in normal and malignant breast tissue: low bax-α expression in tumor cells correlates with resistance towards apoptosis. *Int. J. Cancer,* **60,** 854–859 (1995).
8) Nagata, S. and Golstein, P. The Fas death factor. *Science,* **267,** 1449–1456 (1995).
9) Yonehara, S., Ishii, A. and Yonehara, M. A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. *J. Exp. Med.,* **169,** 1747–1756 (1989).
10) Watanabe-Fukunaga, R., Brannan, C. I., Itoh, N., Yonehara, S., Copeland, N. G., Jenkins, N. A. and Nagata, S. The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. *J. Immunol.,* **148,** 1274–1279 (1992).
11) Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S-I., Sameshima, M., Hase, A., Seto, Y. and Nagata, S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell,* **66,** 233–243 (1991).
12) Trauth, B. C., Klas, C., Peters, A. M. J., Matzku, S., Möller, P., Falk, W., Debatin, K.-M. and Krammer, P. H. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science,* **245,** 301–305 (1989).
13) Owen-Schaub, L. B., Yonehara, S., Crump III, W. L. and Grimm, E. A. DNA fragmentation and cell death is selectively triggered in activated human lymphocytes by Fas antigen engagement. *Cell. Immunol.,* **140,** 197–205 (1992).
14) Miyawaki, T., Uehara, T., Nibu, R., Tsuji, T., Yachie, A., Yonehara, S. and Taniguchi, N. Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. *J. Immunol.,* **149,** 3753–3758 (1992).
15) Itoh, N., Tsujimoto, Y. and Nagata, S. Effect of *bcl-2* on Fas antigen-mediated cell death. *J. Immunol.,* **151,** 621–627 (1993).
16) Lacronique, V., Mignon, A., Fabre, M., Viollet, B., Rouquet, N., Molina, T., Porteu, A., Henrion, A., Bouscary, D., Varlet, P., Joulin, V. and Kahn, A. Bcl-2 protects from lethal hepatic apoptosis induced by an anti-Fas antibody in mice. *Nat. Med.,* **2,** 80–86 (1996).
17) Owen-Schaub, L. B., Radinsky, R., Kruzel, E., Berry, K. and Yonehara, S. Anti-Fas on nonhematopoietic tumors: levels of Fas/APO-1 and *bcl-2* are not predictive of biological responsiveness. *Cancer Res.,* **54,** 1580–1586 (1994).
18) Mattar, R., Yokozaki, H., Yasui, W., Ito, H. and Tahara, E. *P53* gene mutation in gastric cancer cell lines. *Oncology (Life Sci. Adv.),* **11,** 127–132 (1992).

19) Westendorf, J. J., Lammert, J. M. and Jelinek, D. F. Expression and function of Fas (APO-1/CD95) in patient myeloma cells and myeloma cell lines. *Blood,* **85,** 3566–3576 (1995).
20) Tamura, T., Aoyama, N., Saya, H., Haga, H., Futami, S., Miyamoto, M., Koh, T., Ariyasu, T., Tachi, M., Kasuga, M. and Takahashi, R. Induction of Fas-mediated apoptosis in p53-transfected human colon carcinoma cells. *Oncogene,* **11,** 1939–1946 (1995).
21) Haapajarvi, T., Kivinen, L., Pitkanen, K. and Laiho, M. Cell cycle dependent effects of U.V.-radiation on p53 expression and retinoblastoma protein phosphorylation. *Oncogene,* **11,** 151–159 (1995).
22) Rosselli, F., Ridet, A., Soussi, T., Duchaud, E., Alapetite, C. and Moustacchi, E. p53-dependent pathway of radio-induced apoptosis is altered in Fanconi anemia. *Oncogene,* **10,** 9–17 (1995).
23) Ni, R., Tomita, Y., Matuda, K., Ichihara, A., Ishimura, K., Ogasawara, J. and Nagata, S. Fas-mediated apoptosis in primary cultured mouse hepatocytes. *Exp. Cell Res.,* **215,** 332–337 (1994).
24) Enari, M., Hug, H. and Nagata, S. Involvement of an ICE-like protease in Fas-mediated apoptosis. *Nature,* **375,** 78–81 (1995).
25) Los, M., Van-de-Craen, M., Penning, L. C., Schenk, H., Westendorp, M., Baeuerle, P. A., Dröge, W., Krammer, P. H., Fiers, W. and Schulze-Osthoff, K. Requirement of an ICE/CED-3 protease for Fas/APO-1-mediated apoptosis. *Nature,* **375,** 81–83 (1995).
26) Jaattela, M., Benedict, M., Tewari, M., Shayman, J. A. and Dixit, V. M. Bcl-X and Bcl-2 inhibit TNF and Fas-induced apoptosis and activation of phospholipase A_2 in breast carcinoma cells. *Oncogene,* **10,** 2297–2305 (1995).
27) Mapara, M. Y., Bargou, R., Zugck, C., Döhner, H., Ustaoglu, F., Jonker, R. R., Krammer, P. H. and Dörken, B. Apo-1 mediated apoptosis or proliferation in human chronic B lymphocytic leukemia: correlation with *bcl-2* oncogene expression. *Eur. J. Immunol.,* **23,** 702–708 (1993).