Surg Today (2003) 33:847–853
DOI 10.1007/s00595-003-2620-5

SURGERY TODAY

Significance of Apoptosis Induced by Tumor Necrosis Factor-α and/or Interferon-γ Against Human Gastric Cancer Cell Lines and the Role of the p53 Gene

TAKAMI FUKUI, KOUJI MATSUI, HIROKI KATO, HIROSHI TAKAO, YASUYUKI SUGIYAMA, KATSUYUKI KUNIEDA, and SHIGETOYO SAJI

Department of Tumor and General Surgery, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500-8705, Japan

Abstract

Purpose. The expression of the p53 gene in target cells plays an important role in the induction of apoptosis by tumor necrosis factor (TNF)-α and interferon (IFN)-γ. We herein present a study suggesting the existence of a caspase-independent pathway from p53 via insulin-like growth factor binding protein 3 (IGF-BP3) which acts as an apoptosis induction mechanism.

Methods. MKN-45 (wild-type p53) or MKN-28 (mutant-type p53) was cultured with TNF-α or IFN-γ either alone or together. After 24 and 48 h, the apoptotic index (AI) was determined by Hoechst staining and then compared. To clarify whether the mechanism of apoptosis is induced by TNF-α and/or IFN-γ, apoptosis-related genes were examined by a cDNA on microarray analysis and a Western blot analysis.

Results. (1) The AI for MKN-45 increased at 48 h in the presence of TNF-α or IFN-γ alone. (2) In the case of combination treatment using TNF-α and IFN-γ, the AI for MKN-45 was higher than those in the groups with a single treatment. (3) A cDNA microarray analysis showed that IGF-BP3, the TNF superfamily, and caspase 1 were all upregulated after treatment with the combination of TNF-α and IFN-γ. (4) A Western blot analysis of MKN-45 showed a reaction with an anti-IGF-BP3 antibody.

Conclusions. These results suggest that the induction mechanism underlying apoptosis induced by TNF-α and IFN-γ might therefore involve the caspase-independent pathway via IGF-BP3.

Key words Apoptosis · Tumor necrosis factor-α · Interferon-γ · p53 gene · Human gastric cancer cell line · Insulin-like growth factor binding protein 3

Reprint requests to: T. Fukui
Received: August 5, 2002 / Accepted: January 21, 2003

Introduction

Apoptosis or programmed cell death is an essential event for normal development and tissue homeostasis of multicellular organisms. The biological response in a cancer microenvironment is considered to be lower in apoptotic cells that are phagocytosed by macrophages,[1] and less likely to induce cachexia than in the case of necrotic cells. From the above point of view, we have been investigating the assumption that low-dose cancer chemotherapy[2] and/or antitumor cytokines might be able to demonstrate a superior induction of apoptosis. Among the various antitumor cytokines that inhibit tumor growth, tumor necrosis factor (TNF)-α produced by antigen-activated macrophages and T lymphocytes shows cytotoxic activity, induces heat shock proteins, inhibits tumor angiogenicity, and activates antitumor immunity. However, TNF-α at low doses also exerts contradictory biological effects in rare cases such as the promotion of neovascularization and tumor growth.[3] On the other hand, interferon (IFN)-γ produced by antigen-activated T lymphocytes and natural killer (NK) cells exerts direct effects such as an antiviral effect and the inhibition of cancer cell growth, or indirect effects such as the inhibition of tumor growth by the potentiation of macrophages and the NK cell activity, and also the regulation of the immune response.[4]

Regarding its relationship with apoptosis, TNF-α activates caspase-8 via TNFR-1-associated death domain protein/Fas-associating protein with the death domain (TNFR1/TRADD)[5,6] and it enhances apoptosis by activating caspase-3 and the Bcl-2 family of proteins located downstream.[7,8] In this study, using a cDNA microarray analysis, we investigated the antitumor effects of TNF-α and/or IFN-γ against human gastric cancer cell lines regarding the rate of tumor growth, apoptosis inducibility, and an up- or downregulation of apoptosis-related genes. We found the expression of the p53 gene in target cells to be an important factor for apoptosis induc-

tion by TNF-α and/or IFN-γ, and our findings thus suggested the existence of a caspase-independent pathway from p53 via insulin-like growth factor binding protein 3 (IGF-BP3).

Materials and Methods

Human Gastric Cancer Cell Lines and Tissue Culture

The MKN-45 cell line established from human metastatic liver tumor (round to spindle-shaped adherent cells, expressing the wild-type p53 gene)[9] and MKN-28 from human lymph node metastatic tumor (adherent cells that show paving stone-like growth and expressing the mutant p53 gene)[10] were cultured in RPMI-1640 medium containing 10% inactivated fetal calf serum, 100 IU/ml penicillin G, and 50 μg/ml streptomycin at 37°C in 5% CO_2.

Recombinant Cytokines

Recombinant TNF-α, IFN-γ, and IGF-BP3 were purchased from R&D Systems Minneapolis, MN, USA, ENDOGEN (Woburn, MA, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively.

Cell Growth

MKN-45 or MKN-28 cells (1×10^4) were cultured with the medium containing 100 ng/ml TNF-α or IFN-γ alone or both, and the number of viable cells was counted every day for 10 days using trypan blue stain.

Induction of Apoptosis

After 1×10^6 MKN-45 or MKN-28 cells were cultured with 50, 100, and 200 ng/ml TNF-α and/or IFN-γ for 24 or 48 h, the rate of apoptosis (Apoptotic Index, AI) was determined by Hoechst staining.[11] In the case of combination treatment with TNF-α and IFN-γ, the following treatment groups were prepared and the AI values were examined after 24 and 48 h of culturing: 100 ng/ml TNF-α was administered simultaneously in combination with 50, 100, or 200 ng/ml IFN-γ. The cells were exposed to TNF-α only (50, 100, 200 ng/ml), IFN-γ only (50, 100, 200 ng/ml), and in combination TNF-α (100 ng/ml) and IFN-γ (100 ng/ml).

cDNA Microarray Analysis

After MKN-45 was cultured with 100 ng/ml TNF-α and IFN-γ for 48 h, total RNA and mRNA were extracted. On the other hand, MNK-45 was also cultured without the cytokines as a control group, and total RNA and mRNA were extracted using the same procedure. The treatment and control groups were labeled with Cy5 and Cy3, respectively, and subjected to hybridization.[12] The results were analyzed by global normalization. (1) The expression ratios of Cy5 and Cy3 were converted to log ratios (base: 2) and the medians were calculated. (2) The median was subtracted from the log ratio (base: 2). (3) Log values >2.0 or <-0.2 were selected as the cutoff values and changes in the expression level of apoptosis-related genes detected by cDNA microarray analysis were compared.

Protein Expression Level Determined by Western Blotting

MKN-45 was cultured with TNF-α or IFN-γ alone at doses of 50, 100, and 200 ng/ml or in combination with 100 ng/ml TNF-α and IFN-γ. After culturing 24, 36, 48, and 60 h, the treated cells were reacted with an anti-IGF-BP3 antibody and the protein expression level was determined based on the antigen–antibody reaction. Each relative protein expression with a Western blotting analysis was analyzed by densitometry using the ATTO Densito graph 3.02 (ATTO, Tokyo, Japan). Statistically, the measured values are presented as the mean ± standard error (SE) and the significance of differences was analyzed by the Mann-Whitney *U*-test, with $P < 0.05$ regarded as significant.

Results

Anti-Tumor Effects of TNF-α and IFN-γ

On day 10 of culture, MKN-45 treated with IFN-γ alone or with combination of TNF-α and IFN-γ showed almost no growth. The MKN-45 growth was also moderately inhibited in the presence of TNF-α alone. In the case of MKN-28 treated with a combination of TNF-α and IFN-γ, a moderate growth inhibition was observed, but the groups treated with TNF-α or IFN-γ alone showed almost no inhibition (Fig. 1).

Induction of Apoptosis

The rate of apoptotic MKN-45 cells determined by Hoechst staining markedly increased in the case of combination treatment with TNF-α and IFN-γ in comparison with single treatment. The nucleus of an apotic cell exhibited fragmented chromatin and was stained pale blue (Fig. 2). The AI values for MKN-45 after 24 h treatment with TNF-α alone at 50, 100, and 200 ng/ml were 2.79% ± 2.76%, 4.64% ± 4.78%, and 4.68% ± 4.55%, and were higher than that in the control group ($P = 0.0472, 0.0283, 0.0163$). The AI values after 48 h treatment at 50, 100, and 200 ng/ml were 9.22% ±

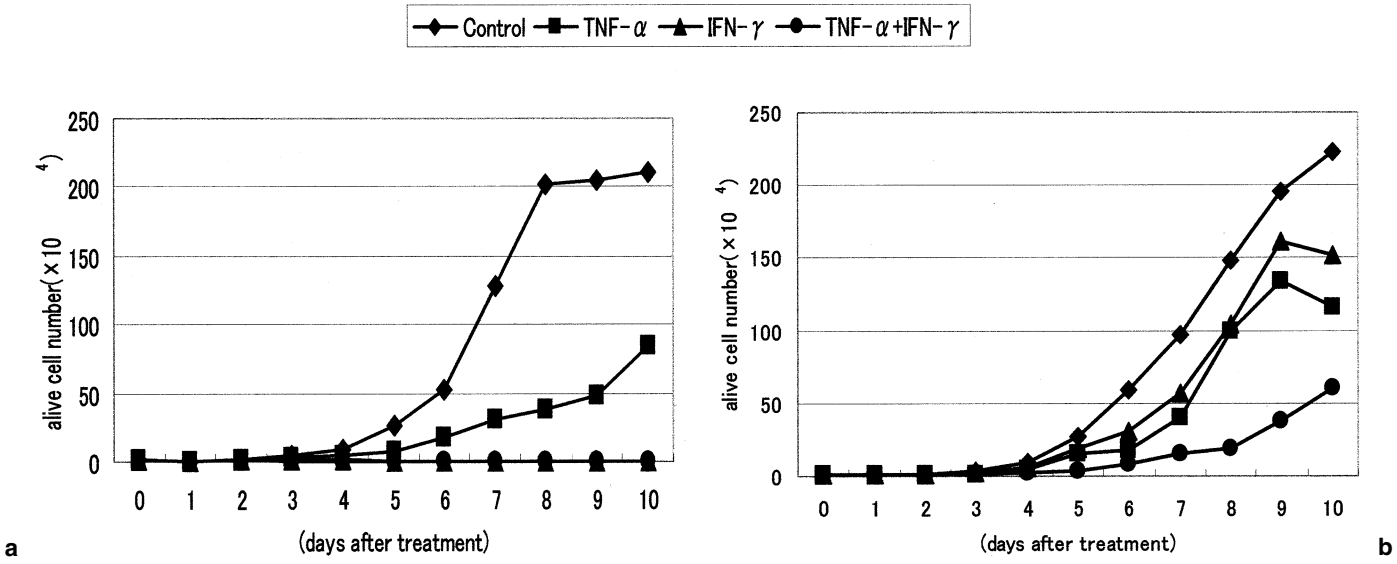

Fig. 1a,b. Growth inhibition of human gastric cancer cell lines by tumor necrosis factor (*TNF*)-α and interferon (*IFN*)-γ. **a** MKN-45 (wild-type p53). **b** MKN-28 (mutant-type p53)

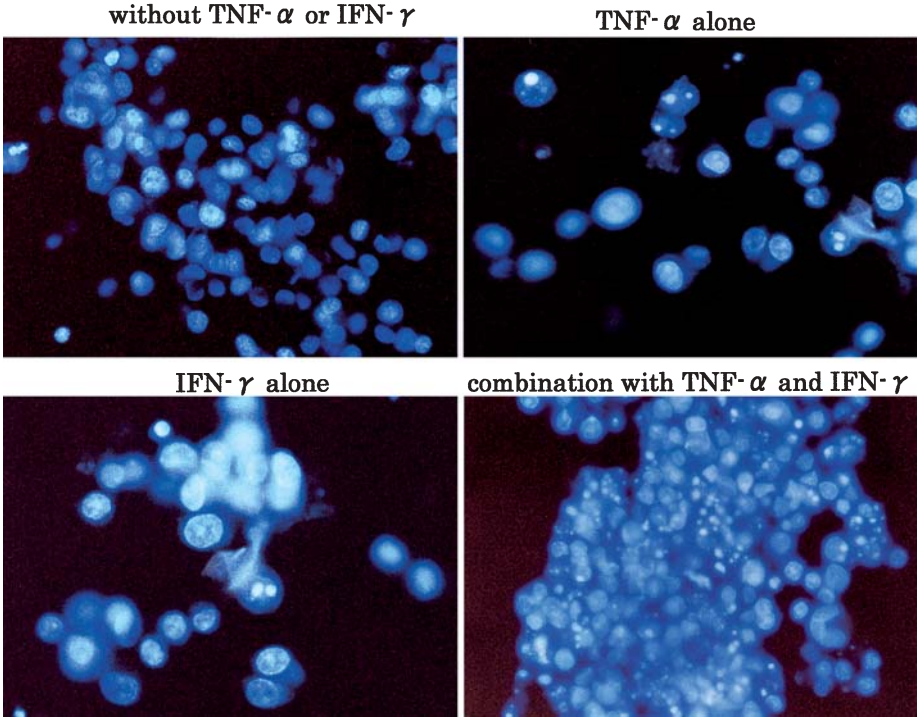

Fig. 2. The degrees of apoptosis induction by TNF-α and/or IFN-γ in a human gastric cancer cell line MKN-45 (Hoechst staining, ×400). The number of positive Hoechst-stained cells of MKN-45 showed a marked increase in the combination use with TNF-α and IFN-γ in comparison with their single use

3.57%, 12.42% ± 2.53%, and 10.28% ± 2.42%, thus indicating significant increases compared with those at 24 h (P = 0.0283, 0.0472, 0.0758), respectively, but no dose dependency was observed. In contrast, the AIs for MKN-28 were less than 5% throughout the observation period (Fig. 3a).

In the case of treatment with IFN-γ alone at 50, 100, and 200 ng/ml, the AIs for MKN-45 were 2.2% ± 0.86%, 2.46% ± 1.10%, and 3.06% ± 1.05%, and were higher than in the control group at 24 h (P = 0.0758, 0.0472, 0.0283). At 48 h of treatment with IFN-γ alone at 50, 100, and 200 ng/ml, the AIs significantly increased to 8.32% ± 4.67%, 11.08% ± 4.34%, and 10.92% ± 3.66%, and were higher than that in the control group (P = 0.0163, 0.009, 0.009), respectively, but no dose dependency was observed. In contrast, the AIs for

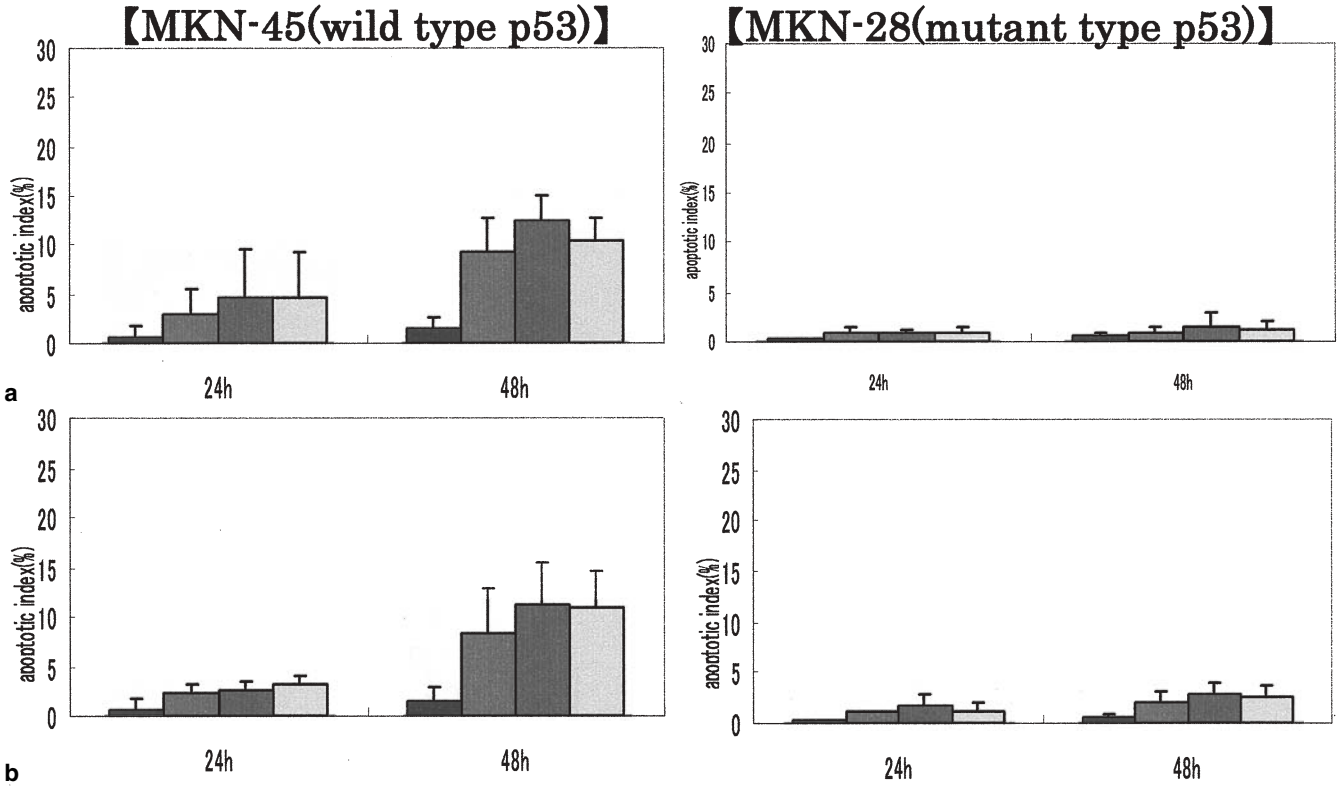

Fig. 3a,b. Comparison of the apoptotic index (AI) of gastric cancer cell lines treated by TNF-α or IFN-γ in a dose-dependent manner. **a** TNF-α. **b** IFN-γ. *Bars* form left to right: *Black bars*, without TNF-α or IFN-γ; *dark shading*, 50 ng/ml; *medium shading*, 100 ng/ml; *light shading*, 200 ng/ml

MKN-28 were less than 4% throughout the observation period (Fig. 3b).

In the cases of combination treatment with TNF-α and IFN-γ, the AI for MKN-45 at 48 h of culture (36.6% ± 1.17%) was significantly higher than those in the groups treated with TNF-α (12.42% ± 2.53%) or IFN-γ (11.08% ± 4.34%) alone ($P < 0.05$), showing an effect of both TNF-α and IFN-γ. There were significant differences among the groups treated with TNF-α (100 ng/ml) alone, IFN-γ (100 ng/ml) alone, or of both ($P = 0.009$). In the case of combination treatment, on the other hand, the AI value for MKN-28 slightly increased to 5.75% ± 2.52%, but it was significantly lower than that for MKN-45 (Fig. 4). When 100 ng/ml TNF-α was concurrently administered with 50, 100, or 200 ng/ml IFN-γ, the AI value for MKN-45 increased with the increase in the IFN-γ concentration at 24 h of culture, but it slightly decreased in the case of combination treatment with 200 ng/ml IFN-γ at 48 h.

In the case of MKN-28, the AI value was maintained at the quantification limit within the established cytokine dose. The above findings suggested that 100 ng/ml might be the optimum IFN-γ concentration for apoptosis induction.

cDNA Microarray Analysis

Changes in expression levels of apoptosis-related genes were analyzed with a cDNA microarray using MKN-45, which expresses the wild-type p53 gene. Upregulation of the TNF superfamily, IGF-BP3, caspase 1, caspase 7, and tumor protein 53, and downregulation of caspase 6 and caspase 9 were observed in cultures containing both TNF-α and IFN-γ.

Western Blot Analysis

The level of protein reacting with anti-IGF-BP3 antibody was found to increase by a Western blot analysis at 24 and 60 h of culture with both TNF-α and IFN-γ (Fig. 5). A quantitative analysis study by densitometry showed a high level of the culture with both TNF-α and IFN-γ at 24 and 60 h.

Discussion

In this study, TNF-α and IFN-γ were found to induce apoptosis, and the number of apoptotic cells significantly increased in the presence of IFN-γ alone or both

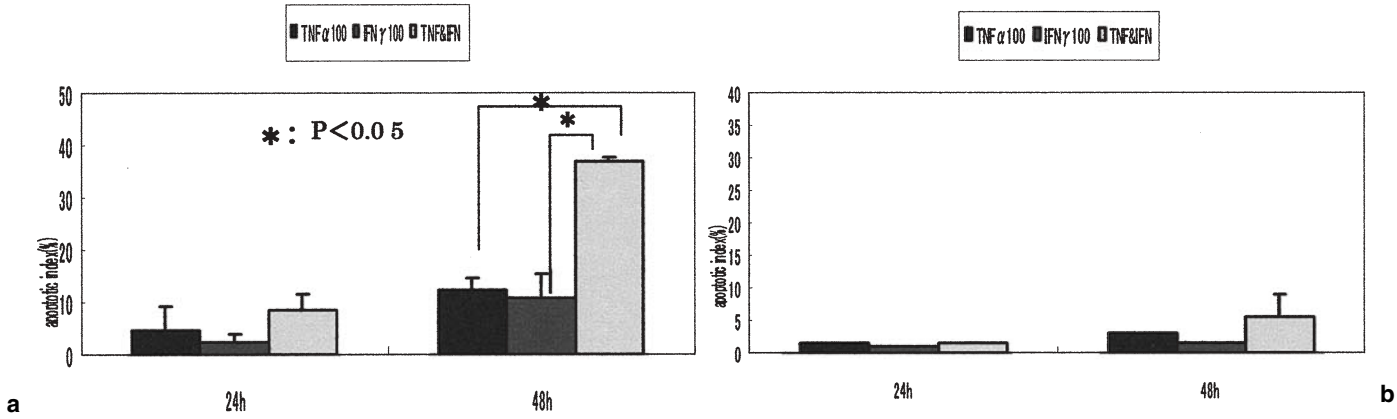

Fig. 4a,b. Comparison of the AI values of MKN-45 or MKN-28 treated by the simultaneous administration of TNF-α and/or IFN-γ either alone or in combination. **a** MKN-45. **b** MKN-28

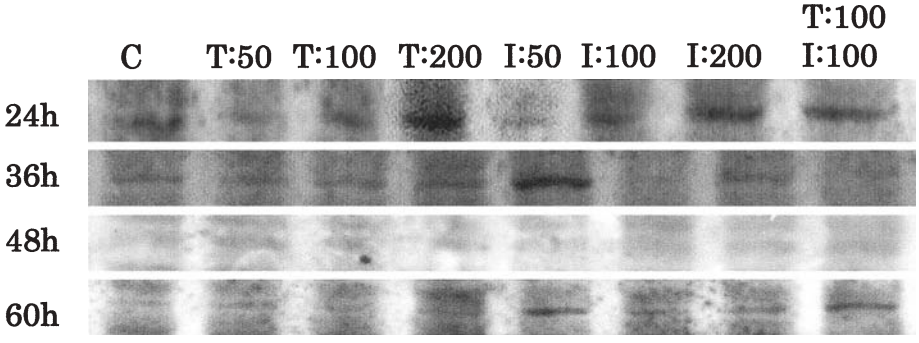

Fig. 5. Western blot analysis of insulin-dependent growth factor binding protein 3 (IGF-BP3). The level of protein reacting with anti-IGF-BP3 antibody was increased at 24 and 60 h after simultaneous combination with TNF-α and IFN-γ. *C*, without treatment; *T*, TNF-α treatment; *I*, IFN-γ treatment

IFN-γ and TNF-α. The interaction between TNF-α and IFN-γ in apoptosis induction in various cancer cells has been reported,[13–20] and the mechanism of that IFN-γ has also been reported to promote TNF-α-induced apoptosis[21] and trigger other types of apoptosis.[22,23] In this study of TNF-α and IFN-γ using gastric cancer cell lines, the combination treatment with TNF-α and IFN-γ significantly promoted apoptosis in comparison with that induced by single treatment. Although apoptosis of gastric cancer cell lines was promoted by the combination treatment with TNF-α and IFN-γ, there was a significant difference in the degree of apoptosis between MKN-45 and MKN-28.

On comparison of this finding with the p53 gene expression pattern in the two cell lines, TNF-α and IFN-γ both alone and in combination significantly promoted apoptosis in MKN-45 expressing wild-type p53, but not in MKN-28 expressing mutant-type p53, thus suggesting the existence of an apoptosis induction pathway via the p53 gene.[24,25] The activation of caspase in TNF-α-induced apoptosis has been extensively investigated and its involvement has been demonstrated in many studies.[26–29] Generally, caspase 8 is thought to play an important role for TNF-α-induced apoptosis because it directly activates caspase 3.[11] The activation of caspase 8-like protease (IETDase), caspase 3-like protease (DEVDase), and caspase 3 has been observed in the induction of Hepa 1–6 (murine hepatoma) apoptosis.[13] Therefore, cytotoxic activity may be induced by the TNF-α-dependent apoptosis pathway. In contrast, there are still many aspects of the IFN-γ-induced caspase-dependent apoptosis pathway that remain to be clarified, but the terminal caspase activation pathway involved in the progression of inflammation and apoptosis has been elucidated. The Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway is considered to play an important role in the induction of caspase 1 in the IFN-γ-induced apoptosis pathway.[30,31] For cell death induced by combination treatment with IFN-γ and TNF-α, the involvement of a caspase-independent pathway via p53-induced IGF-BP3 and c-myc-dependent pathway has been suggested. In this study, we performed an apoptosis-related cDNA microarray analysis and the presence of the caspase-independent pathway via IGF-BP3 was confirmed.[32]

p53RE has been confirmed to be located in the first and second introns on the genome and the expression of the IGF-BP3 gene is induced by normal p53.[33] Regarding the function of IGF-BP3, it acts as a negative factor for cell growth or a proapoptotic factor.[34] It has been reported that when a normal p53 gene is expressed in human colon carcinoma cell line EB1, biologically active IGF-BP3 is secreted in the culture supernatant and cell growth is thereby inhibited due to apoptosis induction. In addition, the possibility that the specific binding of IGF-BP3 to IGF-1 inhibits the growth of human breast cancer cell line Hs578T and fibroblasts has also been reported.[35,36] In our study, in which apoptosis of MKN-45 was induced in the presence of both TNF-α and IFN-γ, an upregulation of the IGF-BP3 gene expression compared with that in the nontreated control group was observed by cDNA microarray analysis and an increase in the protein expression level was also confirmed by Western blotting using the anti-IGF-BP3 antibody, thus suggesting the involvement of IGF-BP3 in apoptosis induced by TNF-α and IFN-γ. The elucidation of the caspase-independent apoptosis induction pathway mainly involving IGF-BP3 is expected to lead to a new approach to anticancer therapy.

Conclusions

Using human gastric cancer cell lines MKN-45 (wild-type p53) and MKN-28 (mutant-type p53), the antitumor effects of treatment with TNF-α or IFN-γ alone or in combination were evaluated based on the degree of cell growth inhibition and the induction of apoptosis, and their mechanisms were analyzed by a cDNA microarray analysis and a Western blot analysis. Our results suggest that the cell growth inhibition was due to apoptosis induction by TNF-α and the promotion of apoptosis in the presence of both TNF-α and IFN-γ. In addition, apoptosis induction was also observed in target cells expressing the wild-type p53 gene, thus suggesting the presence of a caspase-independent pathway via IGF-BP3.

References

1. Schulze-Osthoff K, Ferrari D, Los M, Wesselborg S, Peter ME. Apoptosis signaling by death receptors. Eur J Biochem 1998;254: 439–59.
2. Saji S, Sugiyama Y, Kunieda K. Pre- and/or post-operative immunochemotherapy for advanced digestive cancer (in Japanese). Gan To Kagaku Ryoho (Jpn J Cancer Chemother) 1997;24:239–49.
3. Yoshida S, Ono M, Shono T, Izumi H, Ishibashi T, Suzuki H, et al. Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. Mol Cell Biol 1997;17:4015–23.
4. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol 1997;15:563–91.
5. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathway. Cell 1996;84:299–308.
6. Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 1996;85: 803–15.
7. Stennicke HR, Jurgensmeier JM, Shin H, Deveraux Q, Wolf BB, Yang X, et al. Pro-caspase-3 is a major physiologic target of caspase-8. J Biol Chem 1998;273:27084–90.
8. Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, et al. Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-xL prevents this release but not tumor necrosis factor-R1/Fas death. J Biol Chem 1999;274:1156–63.
9. Jiang XH, Wong BC, Lin MC, Zhu GH, Kung HF, Jiang SH, et al. Functional p53 is required for triptolide-induced apoptosis and AP-1 and nuclear factor-kappaB activation in gastric cancer cells. Oncogene 2001;20:8009–18.
10. Fan XM, Wong BC, Wang WP, Zhou XM, Cho CH, Yuen ST, et al. Inhibition of proteasome function induced apoptosis in gastric cancer. Int J Cancer 2001;93:481–8.
11. Ohnishi K, Ota I, Takahashi A, Yane K, Matsumoto H, Ohnishi T. Transfection of mutant p53 gene depresses X-ray- or CDDP-induced apoptosis in a human squamous cell carcinoma of the head and neck. Apoptosis 2002;7:367–72.
12. Manos EJ, Jones DA. Assessment of tumor necrosis factor receptor and fas signaling pathways by transcriptional profilling. Cancer Res 2001;61:433–8.
13. Sasagawa T, Hlaing M, Akaike T. Synergistic induction of apoptosis in murine hepatoma Hepa1-6 cells by IFN-γ and TNF-α. Biochem Biophys Res Commun 2000;272:674–80.
14. Enzinger C, Wirleitner B, Bock G, Baier-Bitterlich G, Fuchs D. Influence of cytokines tumor necrosis factor-alpha and interferon-gamma on signaling cascades associated with apoptosis in rat PC12 cells. Neurosci Lett 2001;316:157–60.
15. Suk K, Kim S, Kim YH, Kim KA, Chang I, Yagita H, et al. IFN-gamma/TNF-alpha synergism as the final effector in autoimmune diabetes: a key role for STAT1/IFN regulatory factor-1 pathway in pancreatic beta cell death. J Immunol 2001;166:4481–9.
16. Matsumura R, Umemiya K, Goto T, Nakazawa T, Ochiai K, Kagami M, et al. Interferon gamma and tumor necrosis factor alpha induce Fas expression and anti-Fas mediated apoptosis in a salivary ductal cell line. Clin Exp Rheumatol 2000;18:311–8.
17. Ruegg C, Yilmaz A, Bieler G, Bamat J, Chaubert P, Lejeune FJ. Evidence for the involvement of endothelial cell integrin α V β3 in the disruption of the tumor vasculature induced by TNF and IFN-γ. Nat Med 1998;4:408–14.
18. Riccioli A, Starace D, D'Alessio A, Starace G, Padula F, De Cesaris P, et al. TNF-alpha and IFN-gamma regulate expression and function of the Fas system in the seminiferous epithelium. J Immunol 2000;165:743–9.
19. Luttmann W, Dauer E, Schmidt S, Marx O, Hossfeld M, Matthys H, Virchow JC Jr. Effects of interferon-gamma and tumour necrosis factor-alpha on CD95/Fas ligand-mediated apoptosis in human blood eosinophils. Scand J Immunol 2000;51:54–9.
20. Chang-Min K, Weon-Seon H, Jhin-Oh L, Tae-Woong K, Young-Whan K, Jae-Kwan S, et al. Enhancement of cytotoxicity of cisplatin in vitro by recombinant human tumor necrosis factor and/or recombinant human interferon-α,-β, and -γ. Jpn J Cancer Res 1989;80:904–9.
21. Sveinbjornsson B, Rushfeldt C, Smedsrod OB, Seljelid R. Cytotoxic effect of cytokines on murine colon carcinoma cells involves TNF-mediated apoptosis. Biochem Biophys Res Commun 1997;240:376–81.

22. Tamura T, Ueda S, Yoshida M, Matsuzaki M, Mohri H, Okubo T. IFN-γ induces ice gene expression and enhances cellular susceptibility to apoptosis in the U937 leukemia cell line. Biochem Biophys Res Commun 1996;229:21–6.
23. Koshiji M, Adachi Y, Taketani S, Takeuchi K, Hioki K, Ikehara S. Mechanisms underlying apoptosis induced by combination of 5-fluorouracil and IFN-γ. Biochem Biophys Res Commun 1997; 240:376–81.
24. Kagawa S, Fujiwara T, Hizuta A, Yasuda T, Zhang WW, Roth JA, et al. p53 expression overcomes p21WAF1/CIP1-mediated G1 arrest and induces apoptosis in human cancer cells. Oncogene 1997;15:1903–9.
25. Lanni JS, Lowe SW, Licitra EJ, Liu JO, Jacks T. p53-independent apoptosis induced by paclitaxel through an indirect mechanism. Proc Natl Acad Sci USA 1997;94:9679–83.
26. Baker SJ, Reddy EP. Modulation of life and death by the TNF receptor superfamily. Oncogene 1998;17:3261–70.
27. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3:673–82.
28. Lin Y, Devin A, Rodriguez Y, Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 1999;13;2514–26.
29. Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, et al. Targeted disruption of the mouse Caspase8 gene ablates cell death induction by the TNF receptors, Fas/Apol, and DR3 and is lethal prenatally. Immunity 1998;9:267–76.
30. Chin YE, Kitagawa M, Kuida K, Flavell RA, Fu XY. Activation of the STAT signaling pathway can cause expression of caspase 1 and apoptosis. Mol Cell Biol 1997;17:5328–37.
31. Lee KY, Anderson A, Madani K, Rosen GD. Loss of STAT1 expression confers resistance to IFN-induced apoptosis in ME180 cells. FEBS Lett 1999;459:323–6.
32. Yasuoka Y, Naomoto Y, Yamatsuji T, Takaoka M, Kimura M, Uetsuka H, et al. Combination of tumor necrosis factor alpha and interferon alpha induces apoptotic cell death through a c-myc-dependent pathway in p53 mutant H226br non-small-cell lung cancer cell line. Exp Cell Res 2001;271:214–22.
33. Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR, et al. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 1995;377:646–9.
34. Anwar A, Zahid AA, Scheidegger KJ, Brink M, Delafontaine P. Tumor necrosis factor-alpha regulates insulin-like growth factor-1 and insulin-like growth factor binding protein-3 expression in vascular smooth muscle. Circulation 2002;105:1220–5.
35. Valentinis B, Bhala A, DeAngelis T, Baserga R, Cohen P. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. Mol Endocrinol 1995;9:361–7.
36. Oh Y, Muller HL, Lamson G, Rosenfeld RG. Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. Cell surface binding and growth inhibition. J Biol Chem 1993;268:14964–71.