PSK enhances the efficacy of docetaxel in human gastric cancer cells through inhibition of nuclear factor-κB activation and survivin expression

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Abstract. Docetaxel, a member of the taxane family, induces antitumor effects in patients with advanced gastric cancer. However, toxicity at therapeutic doses can be severe, resulting in discontinuation of therapy. It is possible that dose reduction due to adverse events may decrease the cytotoxic efficacy of docetaxel. PSK, a protein-bound polysaccharide, has been used as a chemoimmunotherapy agent in the treatment of cancer in Asia for over 30 years. In the present study, we investigated the enhancing effects of PSK on the cytotoxicity of docetaxel in human gastric cancer through non-immunological actions both *in vitro* and *in vivo*. The sensitization effects of PSK on docetaxel were evaluated by MTT assay using human gastric cancer cell lines *in vitro*. In addition, to elucidate the molecular mechanism, we analyzed the activation of NF-κB and the subsequent production of the antiapoptotic molecule survivin in combined treatment with docetaxel and PSK. Accordingly, TMK-1 xenograft growth in SCID mouse was used to evaluate the *in vivo* efficacy, and the survivin expression in xenografts was also investigated by immunohistochemistry. *In vitro*, PSK enhanced docetaxel-induced growth inhibition in TMK-1 cells. The docetaxel-induced NF-κB activation was inhibited by adding PSK in a dose-dependent manner. Furthermore, the expression of survivin, which is transcriptionally regulated by NF-κB, was also inhibited by treatment with PSK. In SCID mouse, PSK significantly inhibited growth of TMK-1 subcutaneous xenografts in combination with low-dose docetaxel, and decreased the docetaxel-induced survivin expression in TMK-1 xenografts. Our data suggest that PSK enhanced the efficacy of docetaxel against human gastric cancer both *in vitro* and *in vivo*, at least in part, by downregulating NF-κB activation and survivin expression induced by low-dose docetaxel.

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Introduction

Gastric cancer remains a major public health problem, and is the second most common type of malignancy worldwide (1). The chemotherapeutic agent docetaxel, which belongs to the taxane family, shows significant cell-killing activity in a variety of tumor cells, including gastric cancer (2). Docetaxel is a unique anticancer agent that poisons mitotic spindles by stabilizing microtubules, thus inhibiting their depolymerization to free tubulin (3). Docetaxel-based chemotherapy has recently been shown to improve survival in patients with metastatic gastric cancer and has become the standard treatment in such cases (4,5). However, docetaxel is cytotoxic and can induce myelosuppression and granulocytopenia, including neutropenia, which limits the dose of docetaxel that can be administered (6). As it has been reported that low or moderate doses of docetaxel have no significant antitumor activity in patients with pancreatic cancer (7), there is interest in developing ways to reduce the dose of docetaxel without affecting its antitumor activity to obtain clinical benefits and for the rational design of combination strategies that may improve therapeutic results.

Nuclear factor κB (NF-κB), a ubiquitous transcription factor, is known for its role in immunity, inflammation, embryonal development, regulation of cell growth and apoptosis (8,9). NF-κB suppresses apoptosis by inducing expression of a number of antiapoptotic proteins, including intracellular inhibitors of apoptosis, such as c-IAP, Bcl-xL and survivin (10,11). The role of NF-κB in chemoresistance is well established (12-14), and accumulating evidence suggests that elevated expression of Bcl-xL and survivin confers resistance to apoptosis induced by taxanes and other anticancer drugs (15-17). It is possible that dose reduction

due to adverse events may decrease the cytotoxic efficacy of docetaxel by activation of these antiapoptotic factors.

PSK, a protein-bound polysaccharide obtained from the mycelial culture of *Colorius versicolor* (Fr.) Quel, also known as *Krestin*®, has been used in the treatment of cancer in Asia for over 30 years. Several randomized clinical trials have demonstrated that PSK has great potential in adjuvant cancer therapy, with positive results in the treatment of gastric, colorectal, breast and lung cancers (18-22). These studies suggested the efficacy of PSK as an immunomodulator of biological responses. In addition, PSK has also been shown to suppress NF-κB activity induced by taxanes in several experimental studies, and to enhance apoptosis induced by low-dose docetaxel by downregulation of NF-κB activity (23,24). These findings suggest that combination therapy with PSK could reduce the dose of docetaxel without impairing cytotoxic efficacy by suppression of antiapoptotic proteins transcriptionally regulated by NF-κB.

However, the mechanisms of the enhancement by PSK remain to be clarified, and the *in vivo* clinical translation potential of combination treatment for gastric cancer has not been fully established in an animal model. Therefore, the present study was performed to determine the general applicability of the sensitization effect of PSK on docetaxel, and to determine the *in vivo* efficacy of combining docetaxel with PSK against TMK-1 human gastric cancer xenograft in a SCID mouse model.

Materials and methods

Cell culture. Six human gastric cancer cell lines, MKN28, MKN45, NUGC3, KATO III, AZ521 and TMK-1, were obtained from the American Type Culture Collection (Rockville, MD, USA). The cell lines were maintained in RPMI-1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Iwaki, Tokyo, Japan), 2 mM glutamine (Nissui Pharmaceutical), 10 U/ml penicillin-streptomycin (Gibco-BRL, CA, USA). Cell culture was maintained in 37˚C in a humidified atmosphere containing 5% (v/v) CO_2. All experiments were performed within 30 passages of the frozen stocks from which the cells were periodically recovered.

Reagents. PSK was generously donated by Kureha Chemical Industry (Tokyo, Japan), and docetaxel was donated by Sanofi-Aventis (Paris, France).

Antibody. As primary antibodies, we obtained mouse monoclonal anti-Bcl-xL antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse monoclonal anti-c-IAP1 antibody (Santa Cruz Biotechnology), and mouse monoclonal anti-survivin 6E4 antibody (Cell Signaling Technology, Beverly, MA, USA).

Cell growth assay. The sensitivities of human gastric cancer cell lines to docetaxel and PSK, alone or in combination, were determined by MTT colorimetric assay. Briefly, cell lines were seeded at a density of $1x10^4$/well in 96-well plates in RPMI-1640 medium and incubated for 24 h at 37˚C in a humidified environment containing 5% CO_2. The cells were then further incubated with docetaxel and/or PSK at the indicated concentrations. After incubation for 48 h, MTT solution was added to each well at 250 μg/ml. The cells were incubated for 3 h and lysed in dimethyl sulfoxide, and the absorbance was analyzed using a spectrophotometer (EAR 340 AT; SLT, Vienna, Austria) at a wavelength of 540 nm. Cell proliferation was assessed in triplicate.

Electrophoretic mobility shift assay. Electrophoretic mobility shift assay was performed as described previously (25). Briefly, nuclear proteins of cancer cells were extracted with nuclear and cytoplasmic extraction reagents (NE-PER; Pierce Biotechnology, Rockford, IL, USA), in accordance with the manufacturer's recommendations. Protein concentration was determined using Advanced Protein Assay Reagent (Cytoskeleton, Denver, CO, USA) with bovine serum albumin as a reference. Double-stranded NF-κB consensus oligonucleotides (5'-AGTGAGGGGACTTTCCCAGGC-3', 5'-TCAA CTCCCCTGAAAGGGTCCG-3'; Promega, Madison, WI, USA) were end-labeled with [γ-^{32}P]-adenosine triphosphate (3000 Ci/mmol at 10 mCi/ml; NEN Life Science Products, Boston, MA, USA) using T4 polynucleotide kinase. Binding reactions that contained 10 μg of nuclear protein extracts and 10^5 cpm of oligonucleotides were performed for 30 min at room temperature in binding buffer consisting of 10 mM HEPES (pH 7.6), 50 mM KCl, 1 mM EDTA, 0.4% Ficoll, 1 mM DTT, 0.125 mM PMSF and 0.05 mg/ml poly(dI-dC). Reaction products were separated on 4% polyacrylamide gels and analyzed by autoradiography. The specificity of the DNA and protein complex was confirmed by competition with a 50-fold excess of unlabeled NF-κB oligonucleotides. For supershift analysis, 1 μg of anti-NF-κB antibodies (p50 and p65; Santa Cruz Biotechnology) was added to the reaction mixtures for 30 min at room temperature before the addition of a radiolabeled probe.

Western blot analysis. Cell lysates were prepared using standard methods. The protein concentration of each sample was measured using a Bio-Rad protein assay kit II (Bio-Rad, Richmond, CA, USA). For SDS-PAGE, aliquots of 30 μg of proteins from each sample were subjected to electrophoresis on 10-15% polyacrylamide gels. Proteins were transferred electrophoretically onto polyvinylidene difluoride membranes with a tank transfer system (Bio-Rad), then blocked with buffer containing 5% skim milk and 0.1% Tween-20 in Tris-buffered saline (TBST) at room temperature for 1 h. All primary antibodies were diluted in TBST containing 5% skim milk. The membranes were incubated overnight with primary antibody at 4˚C, washed (4x10 min) with washing buffer (TBST), followed by incubation with horseradish peroxidase-conjugated secondary antibody (0.02 μg/ml in TBST) as appropriate for 1 h at room temperature, and then washed (4x10 min) with washing buffer. Detection of chemiluminescence was performed with ECL Western blot detection kits (Amersham, Little Chalfont, UK) in accordance with the manufacturer's instructions.

Animal study with docetaxel/PSK combination. Seven-week-old female severe-combined immunodeficient mice (CB17 SCID) were obtained from Charles River Laboratories Japan

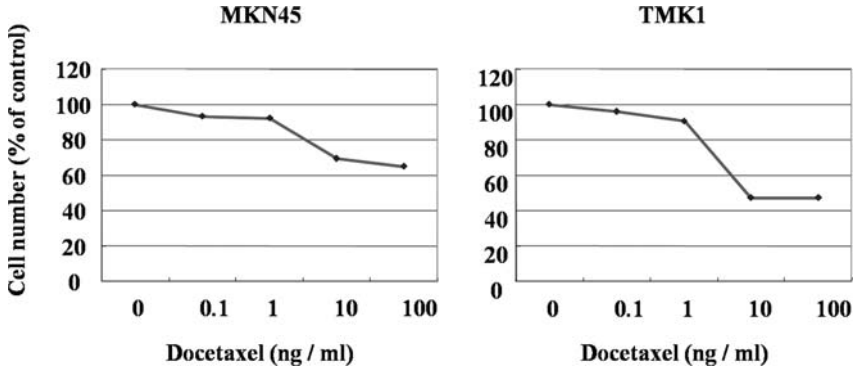

Figure1. The sensitivity of docetaxel on the growth of human gastric cancer cells (MKN45, TMK-1) was quantified by MTT assay. Cells seeded in 96-well plates ($1x10^4$ cells/well) were treated with different concentrations of docetaxel (0.1-100 ng/ml) for 48 h. Results are expressed as the percentage of growth of control cell lines. Docetaxel induced proliferative activity in a dose-dependent manner in both MKN45 and TMK-1 cells. The 10% growth inhibition was observed at 1 ng/ml. The results are the mean of three independent experiments.

(Yokohama, Japan). We selected the SCID mouse, which is lacking in functional T and B lymphocytes and immunoglobulins, as a xenograft model in this study to preclude the non-specific immunological effects of PSK. TMK-1 cells ($1x10^7$ cells), suspended in 50 μl of Matrigel (Becton-Dickinson, NJ, USA), were implanted subcutaneously in the flank of each mouse on the first day. Docetaxel was administered intravenously at various concentrations (0-20.0 mg/kg) on the 14th and 21st days after tumor implantation. PSK (50 mg/kg) was also administered intraperitoneally 3 times a week from the 7th to the 28th day. The animals were carefully monitored, and the tumors were measured twice a week until 6 weeks after injection. The tumor volume (V) was calculated according to the formula $V=AB^2/2$, where A is the greatest diameter and B the diameter perpendicular to A. The subcutaneous tumors were harvested on the 42nd day.

We also investigated the expression of survivin in tumors in our xenograft models. Briefly, TMK-1 cells ($1x10^7$ cells) were injected into the flank of mice, and animals were divided into four groups (saline/saline, PSK/saline, docetaxel/saline, PSK/docetaxel) (n=7, each group). The animals were injected intraperitoneally with PSK (50 mg/kg) dissolved in saline or with saline alone as a control 3 times per week after tumor cell injection. Docetaxel (5 mg/kg) or vehicle was injected intravenously on the 14th (day 0) and 21st (day 7) days after tumor cell injection. The tumors were resected on day 0 (4 h after injection of docetaxel), days 1 and 7 for immunohistochemical analysis. Procedures involving animals and their care were conducted in accordance with national and international laws and policies and were approved by our institutional review board.

Immunohistochemistry. Tumor tissue specimens were fixed with 10% buffered formalin and embedded in paraffin. Briefly, sections 4 μm thick were mounted on poly-l-lysine-coated glass slides, air-dried and deparaffinized through a graded series of xylene and ethanol solutions. For antigen retrieval, sections were pretreated in 10 mM citrate buffer (pH 6.0), and autoclaved for 10 min at 120˚C before immunohistochemical staining with the primary antibody. Endogenous peroxidase activity was blocked with peroxidase block solution provided with the EnVision kit for 10 min, and the slides were rinsed/washed with phosphate-buffered saline (PBS). The slides were incubated overnight at 4˚C with mouse monoclonal antibody (1:100). Immunostaining was performed using the EnVision method (Dako, Glostrup, Denmark) according to the manufacturer's instructions. The slides were developed with diaminobenzidine and counter-stained with hematoxylin.

Statistical analysis. Statistical analysis was performed using Student's t-test. Statistical significance was set at $P<0.05$.

Results

PSK enhances docetaxel-induced growth inhibition in TMK-1 cells. First, the effects of docetaxel on the growth of human gastric cancer cell lines were quantified after 48 h of incubation at concentrations from 0.1 to 100 ng/ml. Docetaxel showed a dose-dependent inhibitory effect on the growth of both MKN45 and TMK-1 cancer cell lines (Fig. 1). The concentration corresponding to 10% growth inhibition (1 ng/ml) was used in subsequent combined treatment experiments.

Next, to investigate whether PSK affects the susceptibility of cells to docetaxel, MKN45 and TMK-1 cells were cultured in the presence of docetaxel (1 ng/ml) with or without various concentrations of PSK (Fig. 2). Taking its bioavailability into consideration, the maximum concentration of PSK used in the present study was 500 μg/ml. PSK dose-dependently enhanced the cytotoxic effect of docetaxel in TMK-1 cells, although PSK alone showed no significant effect on cell proliferation at a concentration of 500 μg/ml. In contrast, PSK showed no significant effect on the cytotoxicity of docetaxel in MKN45 cells.

PSK inhibits docetaxel-induced activation of NF-κB in TMK-1 cells. Electrophoretic mobility gel shift assays (EMSA) were performed to determine whether treatment with docetaxel and PSK affects NF-κB activation. As shown in Fig. 3A, nuclear extracts from untreated TMK-1 cells showed a low level of NF-κB-binding activity; this basal constitutive NF-κB activity has been described previously (26). Docetaxel alone enhanced NF-κB nuclear translocation in TMK-1 cells. Furthermore, the docetaxel-induced NF-κB activation was

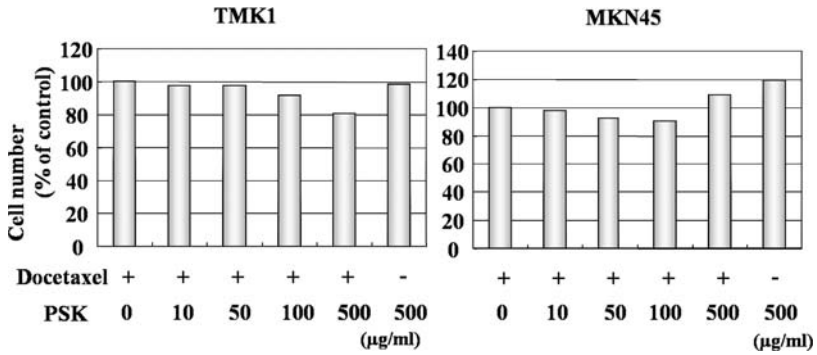

Figure 2. Gastric cancer cell lines (MKN45, TMK-1) were treated with 1 ng/ml docetaxel and increasing concentrations of PSK for 48 h. Cell viability was assessed by MTT assay. Results are expressed as the percentage of cancer cells treated with docetaxel alone. The results are the mean of three independent experiments. PSK alone had no significant effect on cell proliferation at a concentration of 500 ng/ml. However, PSK enhanced the docetaxel-induced growth inhibition of TMK-1 cell in a dose-dependent manner. In MKN45 cells, PSK had no significant response to the cytotoxic effects of docetaxel.

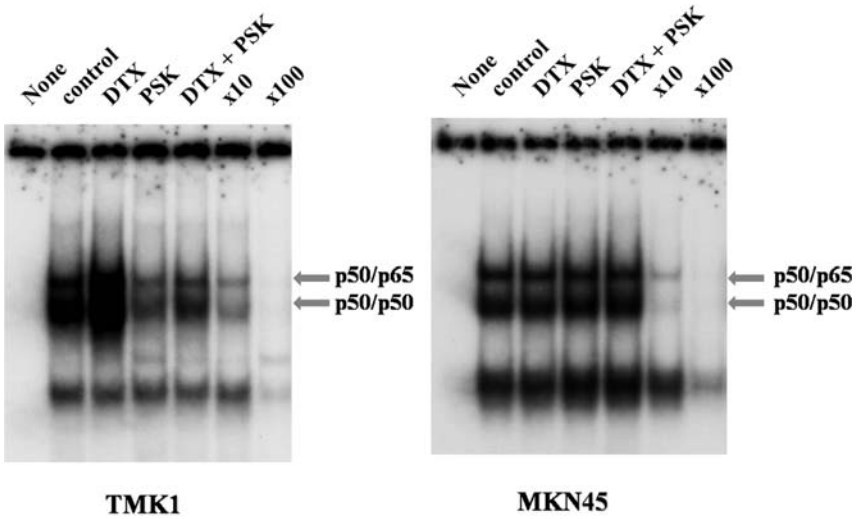

Figure 3. The effect of PSK on docetaxel-induced NF-κB-binding activity in gastric cancer cell lines (MKN45, TMK-1). Cells were serum-deprived for 24 h then treated with docetaxel (1 ng/ml) and different concentrations of PSK for 4 h and nuclear extracts from the cells were analyzed by EMSA. Lane 1, control; lane 2, docetaxel alone (1 ng/ml); lane 3, PSK (50 μg/ml) alone; lane 4-6, docetaxel + PSK (50, 100, 500 μg/ml), lane 7; lane 8. In TMK-1 cells, PSK inhibits docetaxel-induced NF-κB-binding activity in a dose-dependent manner. The involvement of NF-κB activation in the docetaxel and PSK treatment was not seen in MKN45 cells.

	Enhancement of cytotoxic efficacy of DTX	Suppression of NF-κB activation
MKN28	(-)	(-)
MKN45	(-)	(-)
NUGC3	(+)	(+)
KATO III	(-)	(-)
AZ521	(-)	(-)
TMK1	(+)	(+)

Figure 4. PSK enhances the cytotoxic efficacy of low-dose docetaxel and inhibits NF-κB activation induced by docetaxel in 2/6 of human gastric cancer cell lines (NUGC3 and TMK1).

inhibited in a dose-dependent manner by addition of PSK. This inhibition was also observed when the cells were exposed to PSK alone. In contrast, no involvement of NF-κB activation was observed in MKN45 cells treated with docetaxel and PSK (Fig. 3).

The specificity of the detected DNA-protein complexes was determined by supershift assays identifying both p50/p65 heterodimers and p50/p50 homodimers (data not shown).

Effects of combined treatment with low-dose docetaxel and PSK in the other gastric cancer cell lines. In addition, we investigated whether PSK enhances the cytotoxic efficacy of low-dose docetaxel and inhibits NF-κB activation induced by docetaxel in the other cell lines, MKN28, NUGC3, KatoIII and AZ521. As shown in Fig. 4, NUGC3 cells showed the same results as TMK-1 cells (data not shown). Briefly, the combined effects of docetaxel + PSK treatment were confirmed in 2/6 of human gastric cancer cell lines examined.

Docetaxel induces antiapoptotic gene products and PSK decreases docetaxel-induced expression of survivin in TMK-1 cells. NF-κB regulates expression of antiapoptotic proteins,

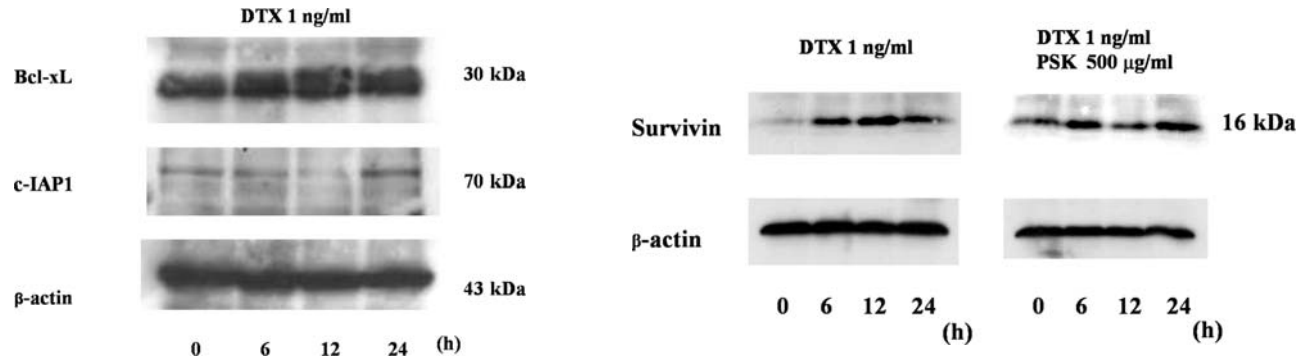

Figure 5. (A) Docetaxel induced upregulation of Bcl-xL and c-IAP1 expression in a time-dependent manner in TMK-1 cells. Cells were serum-deprived for 24 h, then treated with docetaxel (1 ng/ml) for the incubated time. Cell lysates were prepared and analyzed by Western blotting using antibodies against specific gene products. Equal protein loading was evaluated by ß-actin. (B) The effect of PSK upon docetaxel-induced survivin expression. Cells were serum-derived for 24h then treated with docetaxel (1 ng/ml), PSK (500 μg/ml), or both for the incubated time and lysates were used for Western blot analysis of survivin, or ß-actin. Docetaxel enhanced survivin expression in TMK-1 cells and PSK suppressed survivin expression induced by docetaxel.

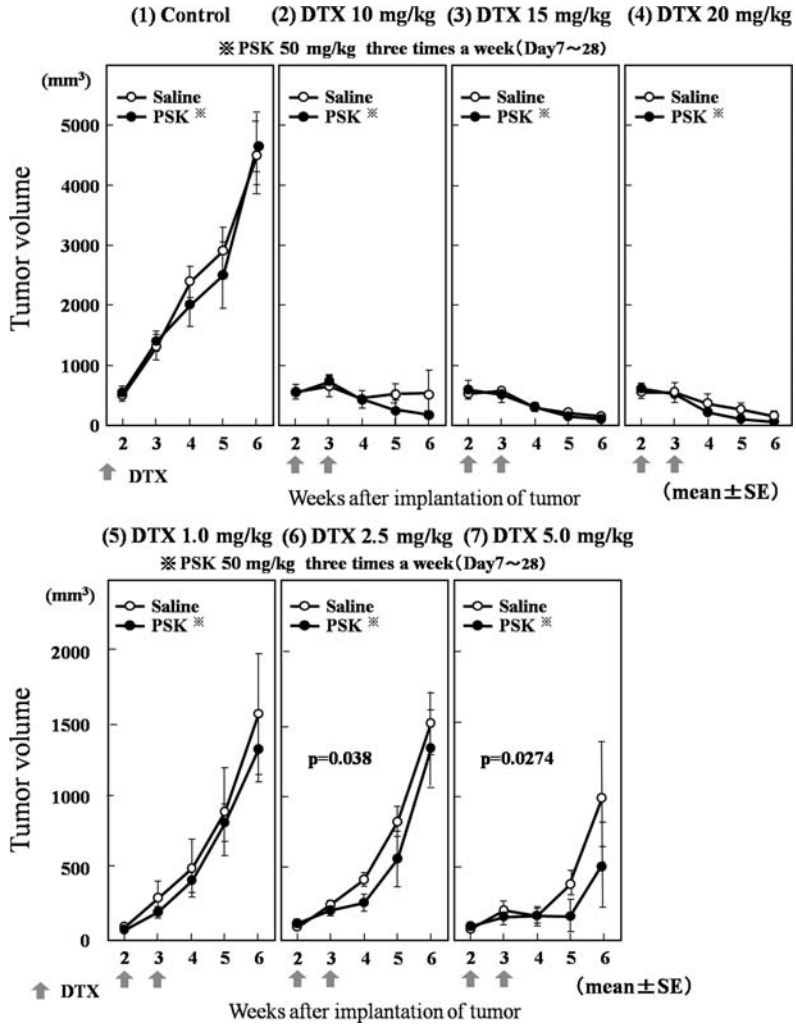

Figure 6. Effects of treatment with docetaxel and PSK on the growth of TMK-1 human gastric cancer xenografts. Well-established s.c. TMK-1 ($1x10^7$ cells) xenografts in SCID mice were treated by intra-peritoneal injections of PSK (50 mg/kg) or saline (control) and intraveouns injections of docetaxel at the different concentrations (0-20.0 mg/kg). Points, mean (n=7); bars, SE.

such as IAP-1, Bcl-xL. To determine whether docetaxel activates cIAP-1 and Bcl-xL expression, Western blotting analyses were performed using TMK-1 cells based on the data from MTT assay and EMSA. Docetaxel induced the activity of these antiapoptotic proteins in a time-dependent manner (Fig. 5A).

Subsequently, we investigated the effects of combination treatment on the expression of survivin, which belongs to the

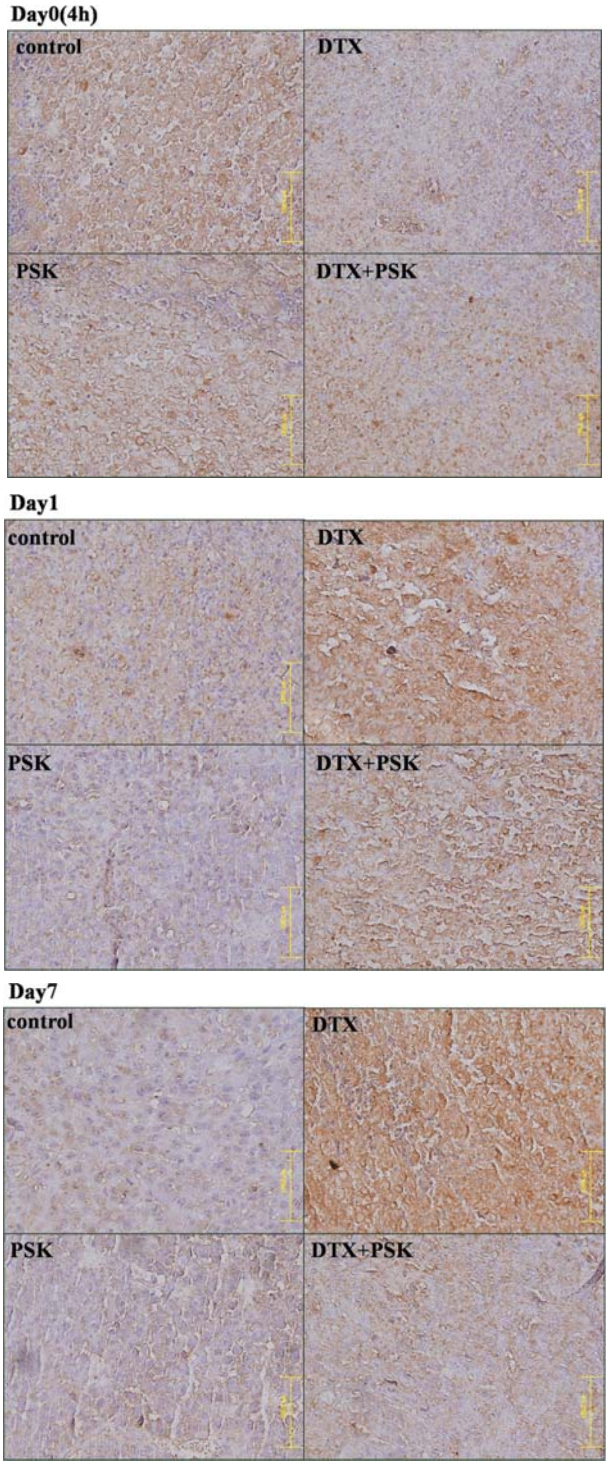

Figure 7. Effect of docetaxel and PSK on survivin expression in TMK-1 xenografts was investigated by immunohistochemistry. Animals were divided into four groups (saline/saline, PSK/saline, docetaxel/saline, PSK/docetaxel) (n=7, each group). (A) Day 0: 4 h after injection of docetaxel; (B) day 1: 1 day after injection of docetaxel; (C) day 7: 7 days after injection of docetaxel PSK suppressed the expression of survivin, which was enhanced by docetaxel on days 1 and 7. Original magnification, x200 (A-D).

inhibitor of apoptosis protein (IAP) family, induced by docetaxel. Docetaxel activated survivin expression in a time-dependent manner, and the maximum level was reached after 24 h of exposure. PSK suppressed this survivin expression activated by docetaxel (Fig. 5B).

Effects of PSK on antitumor activity of docetaxel in human gastric cancer xenograft models. The impressive results of the *in vitro* studies prompted us to examine the *in vivo* effects in a TMK-1 xenograft model in SCID mice that lack an intact adaptive immune repertoire.

We investigated the ability of PSK to suppress tumor growth with different concentrations of docetaxel in the xenograft model (Fig. 6). The results indicated that PSK (50 mg/kg) alone had no significant effect on tumor growth. Docetaxel markedly suppressed tumor growth at a range of doses (10, 15, 20 mg/kg), but there were no significant differences in tumor growth between docetaxel alone and docetaxel + PSK. Therefore, we reduced the doses to below 10 mg/kg to determine the suboptimal dose of docetaxel; i.e., 0.2, 1, 2.5 and 5 mg/kg. At 6 weeks after tumor implantation, the combination of docetaxel + PSK (50 mg/kg) significantly blocked tumor development as compared to treatment with docetaxel alone at dose of 2.5 and 5 mg/kg. No toxicity was observed for any of the dosage groups; there were no changes in animal weight or body condition over the course of the experiment (data not shown).

Effects of PSK and DTX on survivin expression in TMK-1 gastric cancer xenografts. Immunohistochemical analysis was performed to examine the effects of docetaxel and PSK on survivin expression in TMK-1 gastric cancer xenografts (Fig. 7). A docetaxel dose of 5 mg/kg was chosen as the suboptimal dose in this experiment. On day 0, there were no significant differences in survivin expression among the four groups. On day 1, slight constitutive expression of survivin was detected in small parts of the tumors of control mice. PSK alone did not significantly affect survivin expression, while docetaxel (5 mg/kg) alone markedly enhanced survivin expression. Importantly, PSK suppressed the expression of survivin, which was enhanced by docetaxel. Furthermore, the effects of PSK on survivin expression were more marked on day 7.

Discussion

In the present study, we found that combined treatment with low-dose docetaxel and PSK enhanced growth inhibition of cancer cell lines and xenografts compared with low-dose docetaxel alone. We also showed that low-dose docetaxel activated NF-κB, which then upregulated the antiapoptotic molecules cIAP-1, Bcl-xL and survivin in gastric cancer cells. However, PSK suppressed both docetaxel-induced NF-κB activity and the expression of survivin *in vitro*. In a human gastric cancer xenograft model, administration of PSK significantly enhanced the cytotoxic efficacy of docetaxel, and this was correlated with suppression of survivin expression induced by docetaxel in tumor tissues.

PSK, an oral biological response modifier, has a completely different mechanism of action from cytotoxic chemotherapy agents. The actions of PSK include immunological effects, such as the induction of interleukin-2 and interferon-γ, thereby stimulating lymphokine-activated killer cells, enhancing natural killer cells, and neutralizing immunosuppressive cytokines associated with surgical invasion or tumor aggressiveness (27). Therefore, PSK is considered suitable for

concurrent use with chemotherapeutic agents as postoperative adjuvant treatment. In fact, Nakazato *et al* demonstrated that combination therapy with PSK as adjuvant chemotherapy with mitomycin C and oral fluorouracil significantly prolonged survival after curative gastrectomy in a large prospective trial of patients with gastric cancer (20). Moreover, PSK also has direct effects on cancer cells, including apoptosis, suppression of tumor infiltration and augmentation of HLA class I expression (23). The present study indicated another advantage of PSK, i.e., it has non-immunological effects through suppression of NF-κB and antiapoptotic proteins in combined treatment with chemotherapeutic agents. We also demonstrated the suppression of survivin expression by administration of PSK in combination with docetaxel *in vivo* using SCID mice, which lack an adaptive immunological response.

The transcription factor NF-κB has been shown to regulate the expression of numerous genes, including survival factors and cell growth regulatory molecules. Under certain conditions, cytotoxic drugs such as taxane, cisplatin, 5-fluorouracil and anthracyclines can have antiapoptotic effects by activating the stress-induced NF-κB survival pathway. Our results also indicated that low-dose docetaxel induced NF-κB activation in gastric cancer cells. This activation of NF-κB is likely due to some type of stress, oxidative or inflammatory, and is regarded as a contributing factor in the resistance of the tumor to chemotherapy and apoptosis (28-31). In the present study, we demonstrated that PSK reduced NF-κB activation induced by docetaxel in gastric cancer cells. This result was consistent with the findings reported by Zhang *et al* (23) and Yamasaki *et al* (24).

NF-κB is generally presumed to control the expression of the IAP and Bcl-2 families, and these antiapoptotic molecules block the proapoptotic signals at the step where external stimuli activate caspase (32). Previous studies indicated that the IAP survivin is expressed in embryonic and fetal organs, but its expression has not been reported in differential normal tissues. In contrast, survivin is overexpressed in most human tumor types (33), including gastric cancer (34), and high-level survivin expression was shown to be associated with resistance to chemotherapy and poor prognosis of carcinomas of the lung, breast, colon, esophagus and stomach (35-39). Several studies have demonstrated the correlation between docetaxel resistance and upregulation of survivin expression in gastric cancer (40-42). The results of the present study provided *in vitro* and *in vivo* evidence that low-dose docetaxel induced the expression of survivin protein and PSK inhibited this docetaxel-induced survivin expression in TMK-1 cells and xenografts in SCID mice. These results support the suggestion that survivin may be central for not only intrinsic resistance but also acquired resistance to docetaxel. Therefore, targeting survivin is a rational approach to overcome docetaxel resistance in cancer cells (43).

In summary, the results presented here should prompt further exploration of the potential therapeutic value of combined treatment docetaxel and PSK through inhibition of NF-κB and survivin. Our results indicated the usefulness of PSK through non-immunological effects in combined treatment with anticancer agents for gastric cancer in addition to conventional immunomodulatory effects. We found efficacy of combined treatment in 2 of 6 human gastric cancer cell lines. Therefore, ~30% of patients with advanced gastric cancer could show clinical benefits of combined treatment with PSK, which can reduce the dose of docetaxel without impairing antitumor activity and also alleviate the harmful side effects of the chemotherapy.

References

1. Pisani P, Parkin DM, Bray F and Ferlay J: Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer 83: 18-29, 1999.
2. Kretzschmar A, Köhne CH and Dörken B: Docetaxel in treatment of other solid tumors. Med Klin (Munich) 92 (Suppl. 4): 16-22, 1997.
3. Bissery MC, Guénard D, Guéritte-Voegelein F and Lavelle F: Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. Cancer Res 51: 4845-4852, 1991.
4. Haller DG and Misset JL: Docetaxel in advanced gastric cancer. Anticancer Drugs 13: 451-460, 2002.
5. Tsutani Y, Ohara M, Suzuki T, Minami K, Miyahara E, Kameda A and Noso Y: Docetaxel and S-1 as a first-line treatment in patients with advanced or recurrent gastric cancer. Anticancer Res 29: 2775-2779, 2009.
6. Bissett D, Setanoians A, Cassidy J, Graham MA, Chadwick GA, Wilson P, Auzannet V, Le Bail N, Kaye SB and Kerr DJ: Phase I and pharmacokinetic study of taxotere (RP 56976) administered as a 24-hour infusion. Cancer Res 53: 523-527, 1993.
7. Okada S, Sakata Y, Matsuno S, Kurihara M, Sasaki Y, Ohashi Y and Taguchi T: Phase II study of docetaxel in patients with metastatic pancreatic cancer: a Japanese cooperative study. Cooperative group of docetaxel for pancreatic cancer in Japan. Br J Cancer 80: 438-443, 1999.
8. Pahl HL: Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18: 6853-6866, 1999.
9. Baldwin AS Jr: The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol 14: 649-683, 1996.
10. Lee R and Collins T: Nuclear factor-kappaB and cell survival: IAPs call for support. Circ Res 88: 262-264, 2001.
11. Lee JU, Hosotani R, Wada M, Doi R, Kosiba T, Fujimoto K, Miyamoto Y, Tsuji S, Nakajima S, Nishimura Y and Imamura M: Role of Bcl-2 family proteins (Bax, Bcl-2 and Bcl-X) on cellular susceptibility to radiation in pancreatic cancer cells. Eur J Cancer 35: 1374-1380, 1999.
12. Wang CY, Cusack JC Jr, Liu R and Baldwin AS Jr: Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nat Med 5: 412-417, 1999.
13. Wang CY, Mayo MW and Baldwin AS Jr: TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. Science 274: 784-787, 1996.
14. Wang CY, Mayo MW, Korneluk RG, Goeddel DV and Baldwin AS Jr: NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680-1683, 1998.
15. Mita AC, Mita MM, Nawrocki ST and Giles FJ: Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. Clin Cancer Res 14: 5000-5005, 2008.
16. Tu Y, Renner S, Xu F, Fleishman A, Taylor J, Weisz J, Vescio R, Rettig M, Berenson J, Krajewski S, Reed JC and Lichtenstein A: BCL-X expression in multiple myeloma: possible indicator of chemoresistance. Cancer Res 58: 256-262, 1998.
17. Xing H, Weng D, Chen G, Tao W, Zhu T, Yang X, Meng L, Wang S, Lu Y and Ma D: Activation of fibronectin/PI-3K/Akt2 leads to chemoresistance to docetaxel by regulating survivin protein expression in ovarian and breast cancer cells. Cancer Lett 261: 108-119, 2008.
18. Sakamoto J, Morita S, Oba K, Matsui T, Kobayashi M, Nakazato H and Ohashi Y: Meta-analysis group of the Japanese Society for cancer of the colon rectum: efficacy of adjuvant immunochemotherapy with polysaccharide K for patients with curatively resected colorectal cancer: a meta-analysis of centrally randomized controlled clinical trials. Cancer Immunol Immunother 55: 404-411, 2006.
19. Oba K, Teramukai S, Kobayashi M, Matsui T, Kodera Y and Sakamoto J: Efficacy of adjuvant immunochemotherapy with polysaccharide K for patients with curative resections of gastric cancer. Cancer Immunol Immunother 56: 905-911, 2007.

20. Nakazato H, Koike A, Saji S, Ogawa N and Sakamoto J: Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. Study group of immunochemotherapy with PSK for gastric cancer. Lancet 343: 1122-1126, 1994.
21. Iino Y, Yokoe T, Maemura M, Horiguchi J, Takei H, Ohwada S and Morishita Y: Immunochemotherapies versus chemotherapy as adjuvant treatment after curative resection of operable breast cancer. Anticancer Res 15: 2907-2911, 1995.
22. Hayakawa K, Mitsuhashi N, Saito Y, Takahashi M, Katano S, Shiojima K, Furuta M and Niibe H: Effect of krestin (PSK) as adjuvant treatment on the prognosis after radical radiotherapy in patients with non-small cell lung cancer. Anticancer Res 13: 1815-1820, 1993.
23. Zhang H, Morisaki T, Nakahara C, Matsunaga H, Sato N, Nagumo F, Tadano J and Katano M: PSK-mediated NF-kappaB inhibition augments docetaxel-induced apoptosis in human pancreatic cancer cells NOR-P1. Oncogene 22: 2088-2096, 2003.
24. Yamasaki A, Shoda M, Iijima H, Nagai S, Wada J, Suzuki H, Chikazawa N, Tasaka T, Kameda C, Tanaka H, Ikebe M, Jo E, Sato N, Nakamura M, Sekine F, Morisaki T and Katano M: A protein-bound polysaccharide, PSK, enhances tumor suppression induced by docetaxel in a gastric cancer xenograft model. Anticancer Res 29: 843-850, 2009.
25. Funaki H, Shimizu K, Harada S, Tsuyama H, Fushida S, Tani T and Miwa K: Essential role for nuclear factor kappaB in ischemic preconditioning for ischemia-reperfusion injury of the mouse liver. Transplantation 74: 551-556, 2002.
26. Sasaki N, Morisaki T, Hashizume K, Yao T, Tsuneyoshi M, Noshiro H, Nakamura K, Yamanaka T, Uchiyama A, Tanaka M and Katano M: Nuclear factor-kappaB p65 (RelA) transcription factor is constitutively activated in human gastric carcinoma tissue. Clin Cancer Res 7: 4136-4142, 2001.
27. Kato M, Hirose K, Hakozaki M, Ohno M, Saito Y, Izutani R, Noguchi J, Hori Y, Okumoto S, Kuroda D, *et al*: Induction of gene expression for immunomodulating cytokines in peripheral blood mononuclear cells in response to orally administered PSK, an immunomodulating protein-bound polysaccharide. Cancer Immunol Immunother 40: 152-156, 1995.
28. Bian X, McAllister-Lucas LM, Shao F, Schumacher KR, Feng Z, Porter AG, Castle VP and Opipari AW Jr: NF-kappa B activation mediates doxorubicin-induced cell death in N-type neuroblastoma cells. J Biol Chem 276: 48921-48929, 2001.
29. Chuang SE, Yeh PY, Lu YS, Lai GM, Liao CM, Gao M and Cheng AL: Basal levels and patterns of anticancer drug-induced activation of nuclear factor-kappaB (NF-kappaB), and its attenuation by tamoxifen, dexamethasone, and curcumin in carcinoma cells. Biochem Pharmacol 63: 1709-1716, 2002.
30. Yeh PY, Kuo SH, Yeh KH, Chuang SE, Hsu CH, Chang WC, Lin HI, Gao M and Cheng AL: A pathway for tumor necrosis factor-alpha-induced Bcl10 nuclear translocation. Bcl10 is up-regulated by NF-kappaB and phosphorylated by Akt1 and then complexes with Bcl3 to enter the nucleus. J Biol Chem 281: 167-175, 2006.
31. Aggarwal BB, Shishodia S, Takada Y, Banerjee S, Newman RA, Bueso-Ramos CE and Price JE: Curcumin suppresses the paclitaxel-induced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. Clin Cancer Res 11: 7490-7498, 2005.
32. LaCasse EC, Baird S, Korneluk RG and MacKenzie AE: The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 17: 3247-3259, 1998.
33. Ambrosini G, Adida C and Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3: 917-921, 1997.
34. Zhu XD, Lin GJ, Qian LP and Chen ZQ: Expression of survivin in human gastric carcinoma and gastric carcinoma model of rats. World J Gastroenterol 9: 1435-1438, 2003.
35. Monzó M, Rosell R, Felip E, Astudillo J, Sánchez JJ, Maestre J, Martín C, Font A, Barnadas A and Abad A: A novel anti-apoptosis gene: re-expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancers. J Clin Oncol 17: 2100-2104, 1999.
36. Tanaka K, Iwamoto S, Gon G, Nohara T, Iwamoto M and Tanigawa N: Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. Clin Cancer Res 6: 127-134, 2000.
37. Kawasaki H, Altieri DC, Lu CD, Toyoda M, Tenjo T and Tanigawa N: Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. Cancer Res 58: 5071-5074, 1998.
38. Kato J, Kuwabara Y, Mitani M, Shinoda N, Sato A, Toyama T, Mitsui A, Nishiwaki T, Moriyama S, Kudo J and Fujii Y: Expression of survivin in esophageal cancer: correlation with the prognosis and response to chemotherapy. Int J Cancer 95: 92-95, 2001.
39. Li YH, Wang C, Meng K, Chen LB and Zhou XJ: Influence of survivin and caspase-3 on cell apoptosis and prognosis in gastric carcinoma. World J Gastroenterol 10: 1984-1988, 2004.
40. Wang T, Wei J, Qian X, Ding Y, Yu L and Liu B: Gambogic acid, a potent inhibitor of survivin, reverses docetaxel resistance in gastric cancer cells. Cancer Lett 262: 214-222, 2008.
41. Meng H, Tanigawa N, Hao CY, Dai DJ, Lu CD and Ji JF: Chemoresponse to docetaxel correlates with expression of the survivin splicing variants in patients with gastric cancer. Hepatogastroenterology 54: 1934-1940, 2007.
42. Meng H, Lu CD, Dai DJ, Sun YL and Tanigawa N: Elevated expression level of wild-type survivin in gastric cancer promotes in vitro docetaxel-resistance. Zhonghua Yi Xue Za Zhi 84: 2060-2063, 2004.
43. Qiao L and Wong BC: Targeting apoptosis as an approach for gastrointestinal cancer therapy. Drug Resist Updat 12: 55-64, 2009.