

## Antitumor Activity of Peptide Fraction from Traditional Korean Soy Sauce

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**Abstract** Antitumor activities of a peptide fraction isolated from traditional Korean soy sauce (SSP) were investigated *in vitro* and *in vivo* using cancer cell lines and F9 teratocarcinoma-bearing BALB/c mice. SSP exerted a dose-dependent antiproliferative effect on P388D1 mouse lymphoma, F9 mouse teratocarcinoma, and DLD-1 human colon cancer cells with IC<sub>50</sub> values of 11, 50, and 50 µg/ml, respectively. Tumor growth in F9 teratocarcinoma-bearing BALB/c mice, orally administered with 80 and 200 mg/kg/day of SSPs, was inhibited 10.3% and 52.4%, respectively, and survival days increased by 11.9% and 22.1%, respectively, compared to the control group. The results of antitumor activities exerted by SSP *in vitro* and *in vivo* suggest the feasibility of using SSP as an antitumor agent.

**Key words:** Korean soy sauce, peptide, antiproliferation, antitumor, teratocarcinoma

Peptides, isolated and identified from various biological sources such as amphibians and prokaryotes, possess antimicrobial and anticancer activities [1, 10, 11, 12]. In addition, some artificially synthesized peptides show anticarcinogenic activity. For example, MDP-Lys, a synthetic muramyl dipeptide derivative, enhances the antitumor activity of inactivated tumor vaccine [14], and RGD peptides show anticancer activity by inducing apoptosis through direct activation of caspase-3 [2]. Peptides in food have also been reported to show various physiological activities including antihypertension, antithrombosis, opioid, phagocytosis, and anticarcinogenesis [8, 9, 13]. Furthermore, a nonapeptide derived from soy protein hydrolysates inhibited the proliferation of cancer cell lines by arresting at the G2/M phase [6].

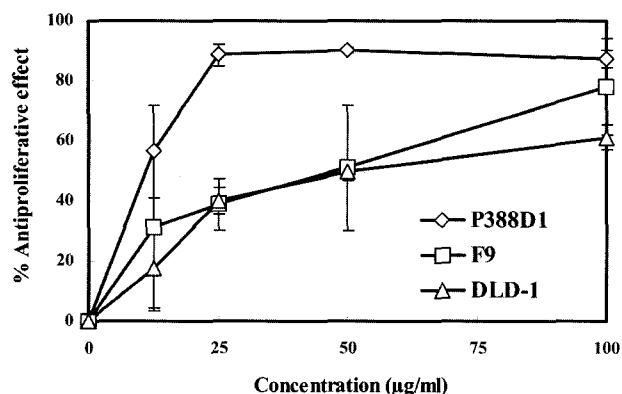
Traditional Korean soy sauce is made of soybean balls, which are cooked and then fermented by a mixture of

molds (i.e., Meju), and is a peptide-rich all-purpose seasoning with a salty taste and sharp flavor. The cytotoxic effects of peptide fractions derived from Korean traditional soy sauce were studied *in vitro* using several tumor cell lines [7]. However, until now, no studies on the *in vivo* antitumor activity of a peptide fraction from traditional Korean soy sauce have been carried out. In this study, a peptide fraction exerting antiproliferative effects on several cancer cell lines was separated and *in vivo* antitumor activity of the fractionated peptide fraction (SSP) was then investigated using a teratocarcinoma-bearing mouse model.

Korean traditional soy sauce was obtained from a local convent (Daegu, South Korea). Separation and fractionation of antitumor peptide fraction from the soy sauce were sequentially conducted by ultrafiltration (mol. cutoff <2000, DOW, Chemical Company, Copenhagen, Denmark), butanol extraction, silica gel column chromatography (Merck, Hamburg, Germany), and Sep-pak C<sub>18</sub> chromatography (Waters, Corp, Milford, MA, U.S.A.). Before proceeding to antiproliferative experiments with the finally obtained peptide fraction, thin layer chromatography with ninhydrin reaction (with ninhydrin: acetone: acetate=0.2:100:3, w/v/v) was performed on an aluminium plate coated with 0.1 mm of silica gel (Merck) to verify the presence of peptides in the peptide fraction. Cells were plated at a density of 1×10<sup>4</sup> per well in 96-well plates. Peptide fractions were filtered through a 0.2 µm filter, and put into the wells after being adjusted to an appropriate concentration with the culture medium. Cells were incubated with or without peptide fractions at 37°C in 5% CO<sub>2</sub>-containing atmosphere for 48 h for P388D1 and for 72 h for other cell lines. Twelve hours before the cell harvest, <sup>3</sup>H-thymidine (1 µCi per well) was added into each well. The cells were then harvested on a glass fiber filter using a cell harvesting apparatus (Cambridge Scientific, Cambridge, MA, U.S.A.), loaded with 3 ml of scintillation cocktail, and counted in a liquid scintillation counter (Wallac Co., Turku, Finland). More detailed description of the <sup>3</sup>H-thymidine uptake assay can be found elsewhere [5].

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**Fig. 1.** *In vitro* antiproliferative effect of peptide fraction from traditional Korean soy sauce on P388D1 mouse lymphoma, F9 mouse teratocarcinoma, and DLD-1 human colon cancer cells.

*In vivo* antitumor activity was investigated by the previously described method [4]. Six-week-old male BALB/c mice were kept in plastic cages in a temperature-controlled room with food and water given *ad libitum*. F9 cells were incubated in DMEM medium (GibcoBRL, Grand Island, NY, U.S.A.) containing 10% fetal bovine serum (FBS, GibcoBRL). They were washed with phosphate buffered saline (PBS), suspended in sterile saline, adjusted to an appropriate concentration for injection, and inoculated into mice.

At the beginning of the experiment, a total of 48 mice were divided into three groups. To investigate the tumor growth inhibition effect, six mice in each group were sacrificed 25 days after tumor cell inoculation, and the remaining ten mice were used to determine the survival rate for 45 days after the tumor inoculation. The tumor growth inhibition rate was calculated as follows [3]:

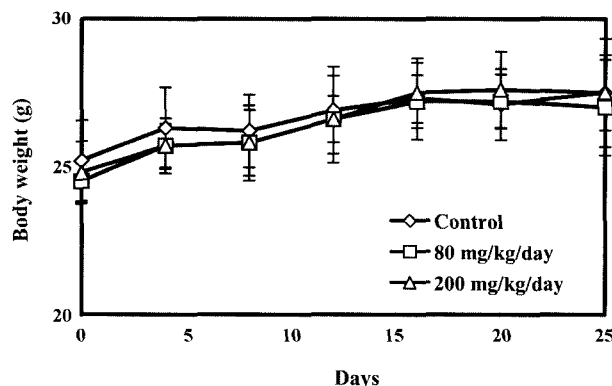
$$\text{Inhibition rate (\%)} = \frac{(C - T)}{C} \times 100$$

**Table 1.** Antiproliferative activity of peptide fraction from traditional Korean soy sauce, SSP, against cancer cell lines.

Name	Origin	IC <sub>50</sub> (µg/ml)
P388D1	A mouse lymphoma cell line	11
F9	A mouse teratocarcinoma cell line	50
DLD-1	A human colon cancer cell line	50
SNU-C2A	A human colorectal cancer cell line	- <sup>a</sup>
SNU-1	A human stomach cancer cell line	-
A549	A human lung cancer cell line	-
K562	A human (male) blood cancer cell line	-
HepG-2	A human liver cancer cell line	-
HL60	A human (female) blood cancer cell line	-
MCF-7	A human breast cancer cell line	-

The reported values are the means of triplicates.

<sup>a</sup>-; no antiproliferative effect at the concentration of peptide fraction ranging from 25 to 100 µg/ml.



**Fig. 2.** Mean body weight of teratocarcinoma-bearing mice treated with peptide fraction from traditional Korean soy sauce (SSP). Control group was administered with PBS, and treated groups with 80 and 200 mg/kg/day of the peptide fraction, respectively.

where C and T are the means of tumor weights in the control and treated groups, respectively. F9 teratocarcinoma cells were intraperitoneally inoculated into each mouse with  $2 \times 10^6$  cells/body. Peptide fractions (SSPs) (80 and 200 mg/kg/day) and PBS (control) were administered everyday for a week using a stainless steel feeding needle, and thereafter once every three days for six days. Student's *t*-test was used to analyze the significance of differences between the control and SSP-treated groups.

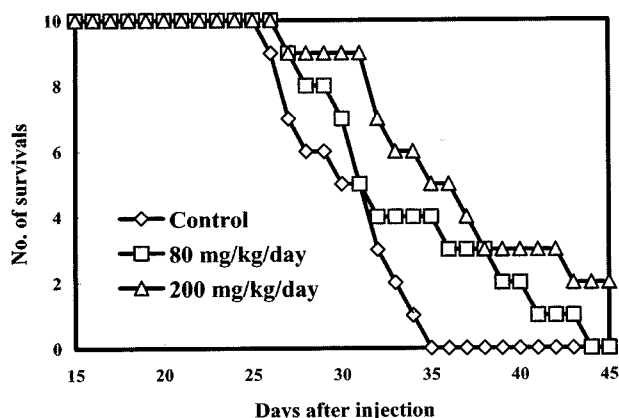
The peptide fraction (SSP) was tested for its antiproliferative activity against ten cancer cell lines. SSP inhibited the growths of P388D1 mouse lymphoma, F9 mouse teratocarcinoma, and DLD-1 human colon cancer cells in a dose-dependent manner (Fig. 1), showing IC<sub>50</sub> values of 11, 50, and 50 µg/ml, respectively. However, SSP did not suppress the growth of other human cancer cell lines (Table 1). Since F9 cell line was a solid tumor and its proliferation was effectively inhibited by SSP *in vitro*, F9 cell line was selected for *in vivo* antitumor activity test.

No significant differences in body weight changes were observed among the experimental groups (Fig. 2). Tumor growth of the SSP-treated groups at a dose of 80 and 200 mg/kg/day was inhibited by 10.3% and 52.4%, respectively (Table 2). The growth inhibitory effect was

**Table 2.** Inhibitory effect of a peptide fraction from traditional Korean soy sauce, SSP, on the tumor growth of F9 teratocarcinoma-bearing BALB/c mice.

Sample	Dose (mg/kg/day)	Number of mice	Tumor weight (mg)	Inhibition rate (%)
Control	-	6	1754.4±1236.5	-
Peptide fraction	80	6	1574.3±925.3	10.3
Peptide fraction	200	6	834.9±751.6	52.4*

\**p*<0.05 compared to the control group.



**Fig. 3.** Survival days of Balb/c mice inoculated with F9 cells. Control group was administered with PBS, and treated groups with 80 and 200 mg/kg/day, respectively, of the peptide fraction (SSP).

significant with 200 mg/kg/day ( $p < 0.05$ ). In addition, the mean survival days increased upon treatment with 80 and 200 mg/kg/day SSP as high as 11.9 and 22.1% in survival days compared to the control, respectively. These results indicate that SSP contains active peptides possessing antitumor activity. Similarly, Buckley *et al.* [2] reported that synthetic peptides containing the Arg-Gly-Asp (RGD) motif induce apoptosis through direct activation of caspase-3. Further investigation is needed to identify the antitumor peptide from the Korean traditional soy sauce and to elucidate the underlying mechanism responsible for the anticarcinogenic properties of the peptide.

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