Suppression of Murine Melanoma Growth by Fermented Grain Extracts

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[ABSTRACT]
We evaluated the anti-tumor activity of fermented grain extracts using a mouse tumor model. An experimental diet containing materials from fermented rice germ, wheat germ, hulled rice, soybean and seaweed (fermented materials, FM) was fed to 4-week-old female C57BL6 mice for 14 days prior to and 21 days following the subcutaneous implantation of B16 melanoma (5×10⁵ cells/mouse). FM retarded tumor growth and increased the duration of host survival. We further examined the anti-tumor activity of FM using the B16 metastasis model. An experimental diet containing FM was fed to C57BL6 mice for 14 days prior to and 21 days following B16 tail vein administration (5×10⁴ cells/mouse). The decrease in observed metastasis in the lungs of mice treated with FM was also significant. In order to identify this anti-tumor activity of FM, NK activity in the FM fed mice was evaluated. However, the values were comparable to the control mice. These results suggest that the fermented grain extracts induce anti tumor activity in vivo, although the mechanism of this activity is not yet clear.

1. INTRODUCTION
It has been suggested that certain plant extracts have an ability to inhibit tumor growth¹,². Some fermented grain-germ extracts such as wheat and rice are also good candidates for reducing the risk of cancer³. Katayama et al. reported on the potential of anti-cancer properties of fermented brown rice⁴,⁵. Another group pointed out the usefulness of fermented wheat germ extract as an anti-tumor compound because of its immunostimulatory effect⁶. Recently, Comin-Anduix et al. reported that fermented wheat germ extract (Avemar) led to apoptosis through the activation of poly (ADP-ribose) polymerase⁷. Another group suggested that the inhibitory effect on tumor growth of fermented wheat germ extract is due to metabolic changes in cells⁸.

We have reported on the anti-microbial activity of pine seed shell extract⁹,¹⁰. In this study, we focused on the anti-tumor activities of various kinds of plant extracts including grains, seaweed and pine seed shell as well as their fermented extracts.

2. MATERIALS AND METHODS
2.1 Preparation of fermented materials (FM)
FM ¹
Various kinds of materials such as wheat germ, hulled rice, seaweed, soybean and rice germ were fermented using
Aspergillus oryzae. The fermented extract was boiled for 30 min and then freeze-dried before use.

**FM 2**

Extracted materials from rice germ and soybean, fermented using Bacillus subcillus natto under aerobic conditions, were filtered. Aloe extract, raw coffee bean extract, perilla extract fiber and pine seed shell extract were also added to the filtered extract.

### 2.2 Animals

Mouse melanoma B16 cells were obtained from the Cell Resource Center for Biomedical Research, Tohoku University (Sendai, Japan) and maintained in DMEM (Nacalai tesque) with 5% heat-inactivated fetal bovine serum (FBS). Six-week-old female C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan).

### 2.3 Tumor injection

For subcutaneous tumor implantation, B16 cells (5×10^5 cells/mouse) were maintained in DMEM containing 5% fetal bovine serum. The mice were anesthetized using a pentobarbitone injection. All tumors were allowed to grow to a size not exceeding 17 mm in any dimension in accordance with UKCCCR guidelines.

To evaluate the effects of FMs on experimental metastasis, B16 cells (5×10^4 cells/mouse) were injected into the tail veins. The mice were sacrificed three weeks after inoculation and the lungs were examined to determine the number of tumor nodules.

### 2.4 NK assay

The YAC-1 cell was used as the target cell for NK cytotoxicity. The standard 51Cr-release assay was performed as described elsewhere. Briefly, target cells were incubated with 370 kBq Na_2^51CrO_4 for 60 min at 37°C. After washing, the target cells were seeded into a microtiter plate at a density of 1×10^4/well. As the effector cells, splenic cells were obtained from the tumor-bearing animals and seeded into the microtiter plate at various effector to target ratios. After 4 h of incubation at 37°C in 5% CO_2/95% humidified air, supernatants were collected and the radioactivity was measured on a gamma counter. Specific 51Cr-release was calculated with the standard formula.

### 3. RESULTS AND DISCUSSION

At first, we evaluated the effects of FM1 or FM2 on the growth of B16 tumor xenografts in the mouse model. Experimental diets containing FM1 or FM2 (FM1 0.4%, FM2 1.7%) were fed to 4-week-old female C57BL6 mice for 14 days prior to and 21 days following subcutaneous tumor implantation (5×10^5 cells/mouse). The same diet without FM was fed to mice as the control.

The weight of FM-administrated mice was comparable to that of control mice, suggesting that FM1 and FM2 have no serious side effects on mice (Fig. 1).

Tumor growth was significantly inhibited in the mice treated with FM1, (Fig. 2). In contrast, FM2 did not show any anti-tumor effect in this experiment (Fig. 2). All mice treated with FM1 survived more than 27 days post-inoculation, although FM2-treated mice and control mice were sacrificed by that time according to UKCCCR guidelines.

In the next experiment, we tested the anti-tumor effect of FM1 using the experimental lung metastasis model. FM1- or FM2-containing experimental diets were fed to C57BL6 mice for 14 days and then B16 tumor cells were administered to the tail vein (5×10^4 cells/mouse). The same diet without FM was fed to mice as the control.

The mice were sacrificed 21 days after inoculation and the lungs were examined to determine the number of tumor nodules. The number of metastases observed in the lungs of FM1-treated mice was significantly lower than in FM2-treated and control mice (Fig. 3). These results suggest that FM1 has anti-tumor activities in the solid tumor model as well as the lung metastasis model in mice.

It has been reported that fermented wheat germ extract (Ave- mar) markedly inhibits tumor metastasis formation after chemotherapy and surgery in clinically advanced colorectal cancers. The extract was also reported to significantly pro-

![Fig. 1. Body weight of mice treated with FMs.](image)
long the time-to-progression in high-risk melanoma patients in a randomized clinical study\textsuperscript{14}. Therefore, FM1 may also possess a similar anti-cancer activity to the wheat germ extract.

In order to elucidate the mechanisms of anti-tumor activity, NK activity in the tumor-bearing mice was evaluated. The NK activity of FM fed-mice was not significantly elevated and was comparable to that of the control mice (data not shown), suggesting that the NK activity might not contribute to the anti-tumor activity.

We also tried to evaluate the direct anti-tumor activity of FM\textsubscript{1} \textit{in vitro}. FM1 or FM2 suspended with PBS (final concentration, 1 mg/ml) was added to B16 and the cell growth was evaluated using an MTT assay. Cell growth 24 h after treatment was suppressed significantly (P<0.05) (Fig. 4). A similar
effect was also observed when adding FM2 to the cells (Fig. 4). Therefore, this inhibitory effect on tumor growth in vitro might not correlate with the anti-tumor activity in vivo.

Finally, we hypothesized a synergistic effect between FM1 and FM2 on tumor growth suppression. These two compounds have already been used as functional foods in Japan, Korea, Taiwan and Malaysia. An experimental diet containing FM1 and FM2 was prepared and fed to mice for 14 days, followed by the tumor challenge described previously. A faint synergistic effect on tumor growth inhibition by these two compounds was found in this experiment (data not shown), although it was not statistically significant (p=0.07). Therefore, the anti-tumor activity seems to belong to FM1 alone.

In this study, we demonstrated the anti-tumor activity of fermented grain extract using a mouse model. The extract inhibited the growth as well as metastasis of the B16 solid tumor in vivo. NK activity might not be correlated with this activity. Another immunostimulatory effect may be involved in the anti-tumor activity of this compound or FM1 may inhibit tumor growth directly. It has been reported that the fermented wheat germ extract Avemar inhibits de novo nucleic acid synthesis\(^7\). Indeed, FM1 suppressed tumor growth in vitro as described previously. Further investigation is required to clarify the mechanism of FM1 derived anti-tumor activity.

4. CONCLUSION

Anti-tumor activity of fermented grain extracts (FM) was evaluated using the mouse tumor model. An experimental diet containing materials from fermented rice germ, wheat germ, hulled rice, soybean and seaweed was fed to mice and then the B16 melanoma was implanted. As a result, FM retarded tumor growth and increased the duration of host survival. In addition, the decrease in observed metastasis in the lungs of mice treated with FM was also significant. These results suggest that the fermented grain extracts induce anti tumor activity in vivo.

**GRANT**

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**REFERENCES**


要 旨

発酵抽出エキスによるマウス腫瘍細胞の増殖抑制効果

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穀物の発酵抽出エキスの抗腫瘍活性についてマウスモデルを用いて検討した。米胚芽、小麦胚芽、玄米、大豆などを発酵させ、それに海草ミネラルを加え、さらに発酵させた抽出物を混合させた餌をマウスに14日間与えた後、マウスB16メラノーマ細胞を皮下に接種し、経過を観察した。その結果、発酵抽出エキスを与えたマウスにおいて有為な腫瘍細胞の増殖抑制効果が見られた。さらに肺転移モデルとしてB16細胞を尾静脈より接種し、3週間後に肺における結節数を計測したところ、発酵抽出エキスを与えたマウスにおいて有為な転移数の減少が確認された。腫瘍抑制効果のメカニズムを明らかにする目的でNK活性を対照マウスと比較したが、有為な違いは見られなかった。以上の結果は、その機構は不明なものので、上記の穀物発酵抽出エキスがマウスモデルにおいて抗腫瘍活性を有することを示唆する。

キーワード：穀物発酵抽出エキス、抗腫瘍活性、肺転移阻害、B16メラノーマ