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In vivo Anti-tumor Efficacy of Polyethylene Glycol-modified Tumor Necrosis Factor-α against Tumor Necrosis Factor-resistant Tumors

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We previously reported that the optimally PEGylated tumor necrosis factor-α (MPEG-TNF-α), in which 56% of the TNF-α-lysine amino groups were coupled with polyethylene glycol (PEG), had about 100-fold greater anti-tumor effect than native TNF-α. Here, we assessed the usefulness of MPEG-TNF-α as a systemic anti-tumor therapeutic drug, using B16-BL6 melanoma and colon-26 adenocarcinoma, which have been reported to be resistant to TNF-α *in vivo*, as compared with Meth-A fibrosarcoma. MPEG-TNF-α markedly inhibited the growth of both tumors without causing any TNF-α-mediated side-effects, whereas native TNF-α had no anti-tumor effects and caused adverse side-effects. In addition, MPEG-TNF-α drastically inhibited the metastatic colony formation of B16-BL6 melanoma. MPEG-TNF-α may, thus, be a potential systemic anti-tumor therapeutic agent.

Key words: Tumor necrosis factor-α — Polyethylene glycol — PEG-modified TNF-α — TNF-resistant tumors — Anti-metastasis

Tumor necrosis factor-α (TNF-α) exhibits strong cytotoxicity to various kinds of tumor cells, but not to normal cells *in vitro*, and causes hemorrhagic necrosis of certain transplanted solid tumors.[1–3] Thus, TNF-α was considered a promising new drug for cancer therapy. However, continuous-infusion or frequent administration of high doses of TNF-α was required to sustain a sufficient plasma TNF-α level for significant anti-tumor effects, because of its short plasma half-life (2–20 min), and TNF-α was found to have unexpected toxic side-effects in phase I studies.[4–6] The severe toxicity of TNF-α precluded the administration of the dosages required for the anti-tumor activity observed in preclinical studies.[7] As a result, the clinical application of TNF-α as a systemic anti-tumor agent has been limited, though intratumoral administration of TNF-α showed marked anti-tumor effects in phase I studies.[8, 9]

We recently reported that chemical modification of TNF-α with polyethylene glycol (PEG) drastically increased its anti-tumor potency and reduced its adverse side-effects in Meth-A fibrosarcoma and Sarcoma-180 murine solid tumor models.[10–14] In particular, PEG-modified TNF-α (MPEG-TNF-α), in which 56% of the lysine amino groups of TNF-α were coupled with PEG, had 100-fold greater anti-tumor activity than did native TNF-α.[10] Plural intravenous administrations of MPEG-TNF-α alone resulted in the complete regression of Meth-A solid tumors in all treated mice without any side-effects, whereas native TNF-α had only a slight life-prolonging effect even at a toxic dose. We found that the marked increase in the anti-tumor potency of MPEG-TNF-α was the result of enhanced blood-residency and tumor-accumulation.[14] Thus, we suggested that MPEG-TNF-α may be a potential candidate for a systemic anti-tumor drug.

In this study, we evaluated the anti-tumor effects of MPEG-TNF-α on tumors other than Meth-A fibrosarcoma and Sarcoma-180 to clarify its usefulness as a new anti-cancer drug. Anti-tumor effects were assessed using highly metastatic and invasive B16-BL6 melanoma and a highly malignant colon-26 adenocarcinoma, which have been reported to be resistant to TNF-α *in vivo*, compared with Meth-A fibrosarcoma.[15–19]

MATERIALS AND METHODS

Materials Natural human TNF-α was kindly supplied by Hayashibara Biological Laboratories (Okayama). *N*-Succinimidyl succinate monomethoxy PEG [SS-PEG; number-average molecular weight (Mn) = 5,000] was obtained from Sigma (St. Louis, MO). A typical procedure for preparation of MPEG-TNF-α (Mn = 108,000), in which 56% of the lysine amino groups of TNF-α were coupled with SS-PEG, was described elsewhere.[10] The specific activities of native TNF-α and MPEG-TNF-α are 2.18×10^6 and 1.14×10^6 Japan Reference Units (JRU)/mg, respectively. The specific activities of TNF-α are measured in terms of the cytotoxic activity against L-M cells, and expressed in terms of JRU defined previ-

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ously by Yamazaki *et al.*[20] Other reagents and solvents were of analytical grade.

***In vivo* anti-tumor effects against B16-BL6 melanoma** Highly metastatic and invasive B16-BL6 melanoma cells were subcultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS). B16-BL6 melanoma cells (4×10^5 cells/mouse) were implanted intradermally into the abdomen of 5-week-old male C57BL/6 mice (SLC, Hamamatsu) on day 0. Native TNF-α or MPEG-TNF-α was given intravenously on days 7, 10, 14, 17, 21 and 24 (twice a week for 3 weeks; total 6 times). The drug efficacy against B16-BL6 melanoma is expressed as mean tumor volume and life span. Tumor volume was calculated by use of the formula described by Haranaka *et al.*[17]

Inhibitory effects on pulmonary metastasis of B16-BL6 melanoma cells B16-BL6 melanoma cells were maintained as described above. B16-BL6 melanoma cells (4×10^5 cells/mouse) were injected into the tail vein of 5-week-old male C57BL/6 mice on day 0. Native TNF-α or MPEG-TNF-α was given i.v. as a single injection on day 0, 3 or 6. The mice were killed on day 14. The lungs were excised and their wet weight was recorded. They were fixed in Bouin's solution, and the pulmonary metastatic colonies on the surface were counted microscopically.

Therapeutic effects on lung-colony formation of B16-BL6 melanoma cells B16-BL6 melanoma cells were maintained and intravenously injected as described above. Native TNF-α or MPEG-TNF-α was given i.v. on days 3, 6, 9 and 12 (total 4 times). The mice were killed 14 days after tumor inoculation. The lungs were excised and their wet weight was recorded. They were fixed in Bouin's solution, and the pulmonary metastatic colonies on the surface were counted microscopically.

***In vivo* anti-tumor effects against colon-26 adenocarcinoma** Highly malignant colon-26 adenocarcinoma cells were subcultured in RPMI 1640 supplemented with 10% FCS. Colon-26 adenocarcinoma cells (4×10^5 cells/mouse) were implanted intradermally into the abdomen of 5-week-old female BALB/c mice (SLC) on day 0. Native TNF-α or MPEG-TNF-α was given intravenously on days 7, 9, 11, 14, 16 and 18 (every 2 days for 2 weeks; total 6 times). The drug efficacy against colon-26 adenocarcinoma is expressed in terms of the mean tumor volume and life span.

Statistical analysis Statistical evaluations of tumor volume, survival time of mice and the number of lung metastatic colonies were analyzed by using Student's *t* test.

RESULTS

Human natural TNF-α was covalently bioconjugated with PEG via the formation of an amide bond between a lysine amino residue of TNF-α and the succinimidyl succinate group of PEG. The PEGylated TNF-α was purified from unmodified TNF-α (Mn=51,000) and separated into various Mn fractions by gel filtration chromatography. Of the separated PEG-TNF-αs, the MPEG-TNF-α in which 56% of the lysine amino groups of TNF-α were PEGylated had the most potent anti-tumor activity. The Mn of this MPEG-TNF-α was 108,000, and MPEG-TNF-α showed 52.3% of the *in vitro* bioactivity of native TNF-α as measured by cytotoxicity assay against L-M cells.

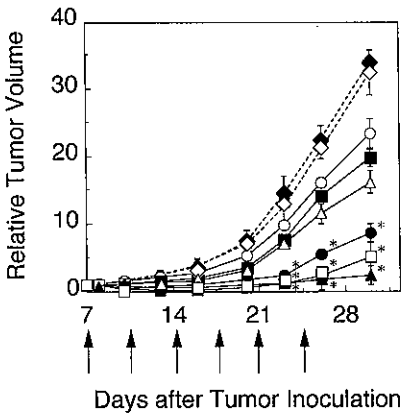

Fig. 1. Anti-tumor effects of native TNF-α and MPEG-TNF-α on B16-BL6 melanoma solid tumor. Native TNF-α and MPEG-TNF-α were given by intravenous injection twice a week for 3 weeks. Mice were used in groups of 5. Each value is the mean±SE. * Significant difference from the saline-control group ($P<0.001$). Control (◇ saline, ◆ PEG). Native TNF-α (○ 2,000 JRU, ■ 5,000 JRU, △ 10,000 JRU). MPEG-TNF-α (● 200 JRU, □ 500 JRU, ▲ 1,000 JRU).

To clarify the usefulness of MPEG-TNF-α as a new anti-tumor agent, we assessed its effects on tumors reported to be resistant to TNF-α *in vivo*, as compared with Meth-A fibrosarcoma. First, highly metastatic and invasive B16-BL6 melanoma was used to evaluate the anti-tumor potency of MPEG-TNF-α. Native TNF-α or MPEG-TNF-α were given intravenously twice a week for 3 weeks. Native TNF-α slightly inhibited tumor growth (Fig. 1), and had a negligible life-prolonging effect even at the maximum dose (10,000 JRU/mouse; Table I). No case of complete regression was observed in the case of native TNF-α treatment (Table I), and all native TNF-α-treated mice died during the experimental period (Table I). Two of the 5 mice administered native TNF-α at a dose of 10,000 JRU/mouse (total 60,000 JRU/mouse) died within 24 h after injection, and the remaining 3 mice developed piloerection, tissue inflammation and a transient decrease in body weight during the experimental period (Fig. 2). At the dose of 10,000

Table I. Anti-tumor Effects of Native TNF-α and MPEG-TNF-α in Terms of Survival Days after B16-BL6 Melanoma Inoculation

Run	Injection dose[a] (JRU/mouse/day)	Survival time[b] (days)		Complete[c] regression
Saline	0	30±1.3	(27, 29, 30, 31, 35)	0/5
PEG	0	32±1.3	(28, 31, 32, 33, 36)	0/5
Native TNF-α	10,000	29±7.5	(8, 15, 37, 40, 46)	0/5
	5,000	38±1.5	(32, 37, 39, 39, 41)	0/5
	2,000	36±1.5	(33, 33, 37, 39, 40)	0/5
MPEG-TNF-α	1,000	70±12.9[d,e]	(37, 54, 59, 100<, 100<)	2/5
	500	46±3.3[d]	(39, 41, 42, 49, 57)	0/5
	200	42±2.2[d]	(36, 38, 41, 45, 48)	0/5

a) Native TNF-α and MPEG-TNF-α were i.v. injected on days 7, 10, 14, 17, 21 and 24.
b) Days after tumor inoculation (mean±SE).
c) Complete regression was defined as no tumor regrowth for more than 100 days.
d) Significant difference from the saline-control group ($P<0.02$).
e) Significant difference from the 10,000 JRU native TNF-α-treated group ($P<0.03$).

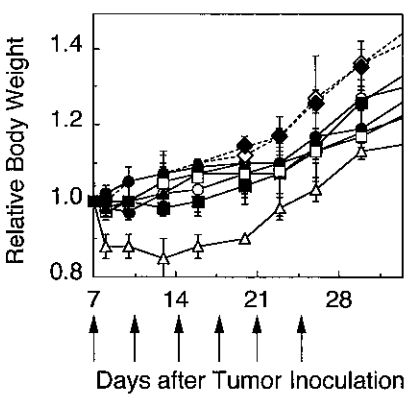

Fig. 2. Body weight of B16-BL6 melanoma solid tumor-bearing mice treated with native TNF-α and MPEG-TNF-α. Mice were used in groups of 5. Each value is the mean±SE. Data were expressed as relative body weight by using the equation: (relative body weight) = (mean body weight at a given time) / (mean body weight on day 7). Control (◇ saline, ◆ PEG). Native TNF-α (○ 2,000 JRU, ■ 5,000 JRU, △ 10,000 JRU). MPEG-TNF-α (● 200 JRU, □ 500 JRU, ▲ 1,000 JRU).

JRU/mouse of native TNF-α, such sudden death and side-effects are always observed, as they were in this study. Thus, 10,000 JRU/mouse of native TNF-α was the maximal achievable therapeutic dose. In contrast, MPEG-TNF-α markedly suppressed tumor proliferation, and completely inhibited tumor growth during the administration period (Fig. 1). Complete cure was obtained in 2 of 5 mice at the dose of 1,000 JRU/mouse of MPEG-TNF-α (total 6,000 JRU/mouse; Table I). During the experimental period, all doses of MPEG-TNF-α were well tolerated and body weight reduction was not observed (Fig. 2).

TNF-α has been reported to promote metastasis of B16 melanoma by some investigators,[21,22] so we assessed the inhibitory effects of MPEG-TNF-α on pulmonary metastasis of B16-BL6 melanoma. Native TNF-α or MPEG-TNF-α were given i.v. as a single injection on day 0, 3 or 6. Native TNF-α and MPEG-TNF-α had no direct cytotoxicity against B16-BL6 melanoma cells (data not shown). The administration of 2,000 and 5,000 JRU/mouse of native TNF-α resulted in a 3- to 3.5-fold increase in the number of metastases over that in the saline control, when administered immediately after i.v. injection of B16-BL6 melanoma cells on day 0 (Fig. 3A). An approximately 3.5-fold increase in the number of lung metastases by MPEG-TNF-α at a dose of 200 JRU/mouse was also observed. However, native TNF-α at a toxic dose of 10,000 JRU/mouse on day 0 did not significantly promote pulmonary metastasis. MPEG-TNF-α at a dose of 500 and 1,000 JRU/mouse also did not enhance the experimental metastasis. A systemic single administration of native TNF-α on day 3 after tumor inoculation showed no effect on metastatic colony formation (Fig. 3B). MPEG-TNF-α did not enhance, but rather reduced pulmonary metastasis. As shown in Fig. 3C, the intravenous administration of native TNF-α 6 days after tumor inoculation did not significantly inhibit lung metastasis. Marked inhibitory effects of MPEG-TNF-α on metastatic colony formation were observed.

We examined the therapeutic effects of MPEG-TNF-α on metastatic colony formation produced by highly metastatic and invasive B16-BL6 melanoma cells by using systemic plural administrations (Fig. 4). Native TNF-α had a slight inhibitory effect on pulmonary colonization of B16-BL6 melanoma, but MPEG-TNF-α nearly completely inhibited colony formation.

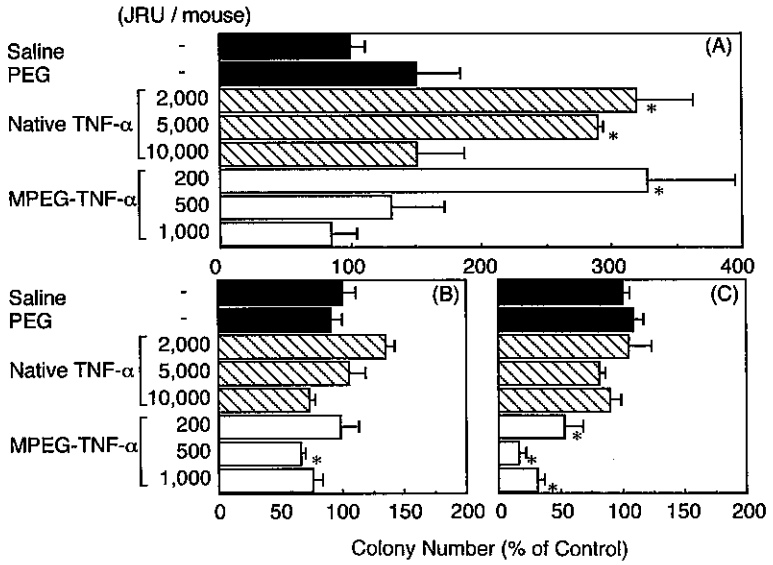

Fig. 3. Inhibitory effect of native TNF-α and MPEG-TNF-α on pulmonary metastasis of B16-BL6 melanoma. Native TNF-α and MPEG-TNF-α were i.v. injected on day 0 (A), 3 (B), or 6 (C). Mice were killed 2 weeks later and colonies on the lung were counted microscopically. The data are mean±SE ($n=5$). * Significant difference from the saline-control group ($P<0.02$).

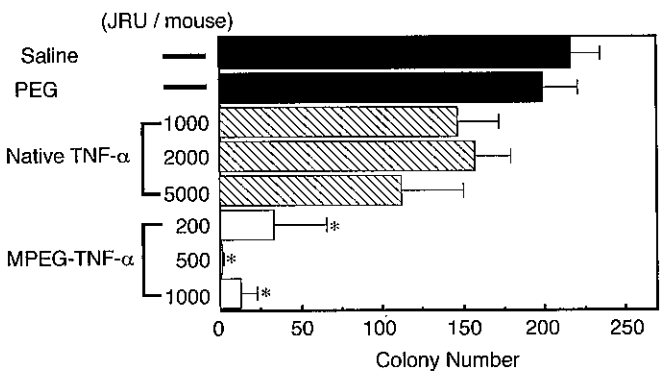

Fig. 4. Anti-tumor effects of native TNF-α and MPEG-TNF-α on experimental lung metastasis. Native TNF-α and MPEG-TNF-α were i.v. injected on days 3, 6, 9 and 12 after B16-BL6 melanoma inoculation. Lung metastasis was counted microscopically on day 14. * Significant difference from the saline-control group ($P<0.002$). (mean±SE, $n=4$)

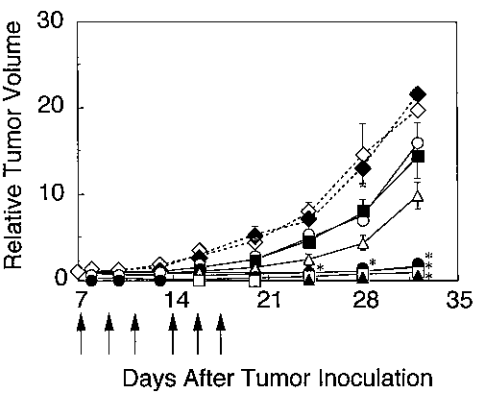

Fig. 5. Anti-tumor effects of native TNF-α and MPEG-TNF-α on colon-26 adenocarcinoma solid tumor. Native TNF-α and MPEG-TNF-α were given by intravenous injection every 2 days for 2 weeks. Mice were used in groups of 5. Each value is the mean±SE. * Significant difference from the saline-control group ($P<0.001$). Control (◇ saline, ◆ PEG). Native TNF-α (○ 2,000 JRU, ■ 5,000 JRU, △ 10,000 JRU). MPEG-TNF-α (● 200 JRU, □ 500 JRU, ▲ 1,000 JRU).

The anti-tumor effects of MPEG-TNF-α was also evaluated using the highly malignant colon-26 adenocarcinoma, which is resistant to TNF-α *in vivo* (Figs. 5 and 6, Table II). Native TNF-α had a negligible life-prolonging effect, even at the dose of 10,000 JRU/mouse that caused death from shock due to side-effects in 60% (3/5) of the treated mice. In contrast, MPEG-TNF-α significantly prolonged the survival time, and complete cure was observed in 1 of the 5 mice at the dose of 1,000 JRU/mouse. In colon-26 adenocarcinoma, tumor proliferation was completely inhibited during the administration period of MPEG-TNF-α. The colon-26 adenocarcinoma-bearing mice showed a profound body weight loss by 14

Table II. Anti-tumor Effects of MPEG-TNF-α in Terms of Survival Days after Colon-26 Adenocarcinoma Inoculation

Run	Injection dose[a] (JRU/mouse/day)	Survival time[b] (days)		Complete[c] regression
Saline	0	28±1.6	(24, 26, 28, 29, 34)	0/5
PEG	0	28±1.4	(25, 26, 27, 28, 33)	0/5
Native TNF-α	10,000	23±7.1	(8, 11, 17, 39, 42)	0/5
	5,000	33±2.0	(27, 29, 34, 36, 37)	0/5
	2,000	31±1.8	(28, 29, 34, 36, 37)	0/5
MPEG-TNF-α	1,000	58±11.2[d,e]	(37, 43, 49, 59, 100<)	1/5
	500	44±4.2[d,e]	(29, 42, 45, 49, 54)	0/5
	200	42±3.3[d,e]	(31, 39, 43, 48, 49)	0/5

a) Native TNF-α and MPEG-TNF-α were i.v. injected on days 7, 9, 11, 14, 16 and 18.
b) Days after tumor inoculation (mean±SE).
c) Complete regression was defined as no tumor regrowth for more than 100 days.
d) Significant difference from the saline-control group ($P<0.04$).
e) Significant difference from the 10,000 JRU native TNF-α-treated group ($P<0.05$).

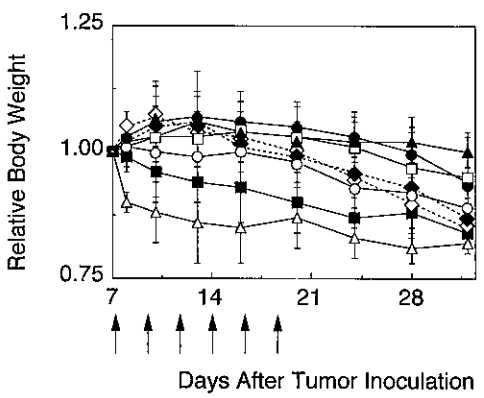

Fig. 6. Body weight of colon-26 adenocarcinoma solid tumor-bearing mice treated with native TNF-α and MPEG-TNF-α. Mice were used in groups of 5. Each value is the mean±SE. Control (◇ saline, ◆ PEG). Native TNF-α (○ 2,000 JRU, ■ 5,000 JRU, △ 10,000 JRU). MPEG-TNF-α (● 200 JRU, □ 500 JRU, ▲ 1,000 JRU).

days after inoculation, probably due to the cachexia induced by colon-26 adenocarcinoma. MPEG-TNF-α effectively suppressed this loss of body weight.

DISCUSSION

When TNF-α is used as a systemic anti-tumor drug, its dose must be restricted to only 1/5 to 1/25 of the amount necessary to obtain sufficient anti-tumor activity, due to its adverse side-effects.[23] Thus, TNF-α is clinically administered only into the tumor or the artery leading to the cancer in current cancer chemotherapy.[8,9] The anti-tumor effects of TNF-α result not only from its direct cytotoxic action against various tumor cells, but also from activation of anti-tumor effector immune cells and specific damage to the tumor vessels.[1,3] Additionally, in the process of bleeding necrosis in the tumor vessels, the vascular permeability of the tumor vessels is selectively increased, promoting transport from blood to the tumor tissue.[24] Therefore, improvement in blood stasis may enhance all the anti-tumor action mechanisms of TNF-α, increasing its bioavailability. The increase in blood-residency would lead to a decrease in the distribution of TNF-α in the liver and spleen, which are the major sites of the unfavorable side-effects.[25,26] More recently, we attempted to improve the blood stasis by PEGylation, to bring about a selective increase in the anti-tumor activity of TNF-α and an effective decrease in its other biological activities.[10,13] As mentioned in the introduction, we found that optimal PEGylation of TNF-α selectively increased its anti-tumor potency *in vivo*, and the marked increase in the anti-tumor potency of PEGylated TNF-αs resulted from the enhanced blood-residency and tumor-accumulation.[11,12,14] In this study, we evaluated its effects on TNF-resistant solid tumors other than Meth-A fibrosarcoma and Sarcoma-180, to assess the usefulness of MPEG-TNF-α as a new anti-tumor agent.

In the present study, we examined the anti-tumor effects of MPEG-TNF-α against highly metastatic and highly invasive B16-BL6 melanoma, which shows extremely strong resistance to TNF-α *in vivo*.[18] As shown in Figs. 1 and 2, Table I, little prolongation of the survival was observed by the administration of native TNF-α even at the highest possible dose (10,000 JRU/mouse). In contrast, not only was tumor growth mark-

edly inhibited, but also complete cure was achieved in 2 of 5 mice without side-effects by the administration of MPEG-TNF-α at 1,000 JRU/mouse. We next assessed the anti-tumor effects of MPEG-TNF-α against colon-26 adenocarcinoma, which shows resistance to TNF-α *in vivo* (Figs. 5 and 6, Table II).[17] Little prolongation of survival was observed with native TNF-α even at 10,000 JRU/mouse, at which dose about half the animals died due to toxic shock. MPEG-TNF-α, however, prolonged the survival period, and complete cure was achieved in 1 of 5 mice administered MPEG-TNF-α at only 1,000 JRU/mouse. Although the complete cure rate is apparently very low with the two TNF-α-resistant tumors, it is of note that intravenous administration of MPEG-TNF-α alone was effective against tumors which are unaffected by systemic administration of TNF-α. In colon-26 adenocarcinoma and B16-BL6 melanoma, tumor proliferation was completely inhibited by MPEG-TNF-α during the administration period. Therefore, complete cure of all mice may be possible by additional administration of MPEG-TNF-α, as was observed in the case of Meth-A fibrosarcoma.

It is of interest to discuss why MPEG-TNF-α showed marked anti-tumor effects against TNF-resistant solid tumors. We have found through our past investigations that vascular endothelial cells possess and retain properties intrinsic to the tissue owing to the effects of humoral factors and extracellular matrix of surrounding cells, and this tissue-specificity can be reproduced *in vitro* by cell cultures of endothelium with conditioned medium of tissue cells and by co-cultures between endothelial cells and various tissue-cells.[27,28] Therefore, we evaluated the sensitivity of vascular endothelial cells to TNF-α in a tumor environment by culturing them in conditioned media of various tumor cells.[29] When vascular endothelial cells were cultured under normal conditions (DMEM medium containing 15% FCS) or conditioned medium of normal vascular endothelial cells, no damage was observed in vascular endothelial cells even at a TNF-α concentration of 1,000 JRU/ml. However, marked cytotoxicity was observed in vascular endothelial cells cultured in conditioned medium of Meth-A fibrosarcoma even at a TNF-α concentration of only 10 JRU/ml. A similar increase in the sensitivity of vascular endothelial cells to TNF-α was also observed in conditioned media of other tumors, such as B16-BL6 melanoma and colon-26 adenocarcinoma. We speculate that this increase in the sensitivity of endothelial cells to TNF-α may be caused by up-regulation of TNF-receptor. Therefore, we thought that TNF-α may exhibit anti-tumor effects against almost all solid tumors. So, frequent administration of native TNF-α at an excessively high dose could induce dramatic anti-tumor activity against almost all solid tumors, if native TNF-α shows no adverse toxic side-effects. In fact, native TNF-α at a toxic dose of 10,000 JRU/mouse induced marked hemorrhagic necrosis in both solid tumors *in vivo* (data not shown). MPEG-TNF-α can reduce the dose to 1/100 or less as compared with native TNF-α, and side-effects of TNF-α, such as weight loss, sudden death, platelet reduction, piloerection, and tissue inflammation, were not observed in the MPEG-TNF-α group even at a dose that would be toxic in the case of native TNF-α (data not shown). Thus, an increase in the dose of MPEG-TNF-α may lead to marked anti-tumor effects against TNF-resistant tumors. However, to clarify the mechanisms of the anti-tumor activity of MPEG-TNF-α against TNF-resistant tumors, more detailed studies, e.g., on host anti-tumor immune-response and vascular permeability of tumor tissue, are necessary.

It is important to note that MPEG-TNF-α at a dose of more than 500 JRU/mouse did not enhance, but rather reduced pulmonary metastasis of B16-BL6 melanoma (Fig. 3) and nearly completely inhibited lung-colony formation (Fig. 4). TNF-α has been reported to promote metastasis of B16 melanoma by some investigators.[21,22] Indeed, native TNF-α at a dose of 2,000 or 5,000 JRU/mouse drastically increased pulmonary metastasis of B16-BL6 melanoma, when intravenously administered on day 0 (Fig. 3). Okahara *et al.* reported that administration of 50–5,000 JRU/mouse of TNF-α increased the number of metastatic lung colonies of B16-BL6 melanoma.[21] Flow cytometric analysis demonstrated a high expression of very late activation antigen 4 (VLA-4) on the surface of B16-BL6 melanoma cells. Immunoperoxidase staining demonstrated that a ligand for VLA-4 (VCAM-1) was expressed on lung vascular endothelium 4 h after administration of TNF-α. Thus, the enhancement of metastasis by TNF-α treatment is probably caused by the interaction between VLA-4 on tumor cells and VCAM-1 on activated endothelial cells. However, we observed that native TNF-α at a toxic dose of 10,000 JRU/mouse did not enhance the metastasis. We speculate that an extremely high dose of native TNF-α may not promote tumor metastasis. Saito *et al.* reported similar results.[30] MPEG-TNF-α at a dose of more than 500 JRU/mouse did not enhance metastasis. In addition, MPEG-TNF-α nearly completely inhibited colony formation of B16-BL6 melanoma. Thus, MPEG-TNF-α may be a useful derivative as a potential anti-tumor therapeutic agent. It is unclear, however, why MPEG-TNF-α showed anti-metastatic effects. Studies of this phenomenon are under way.

To clarify the usefulness of MPEG-TNF-α as a systemic anti-tumor drug, we evaluated its anti-tumor effects on TNF-resistant tumors other than Meth-A fibrosarcoma. MPEG-TNF-α markedly inhibited the growth

of the two tumors studied and inhibited metastasis without causing any TNF-α-type side-effects upon systemic administration, whereas native TNF-α had only slight anti-tumor effects and caused adverse side-effects. Until now, cancer therapy with TNF-α has been limited to intratumoral administration against specified tumors. MPEG-TNF-α may be a potential systemic anti-tumor therapeutic agent.

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