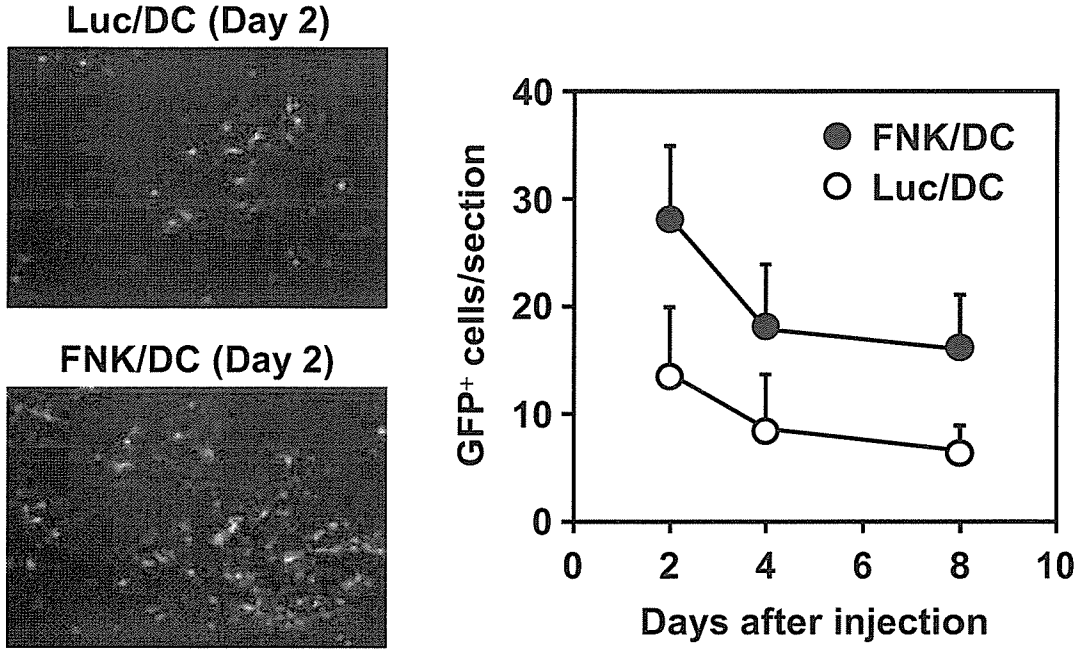


Fig. 92 Enhanced accumulation of FNK/DCs from administration site to regional lymph node. DCs derived from GFP transgenic mice were transfected with AdRGD-FNK or AdRGD-Luc at 25 MOI, and then were cultured for 24 h. These transduced GFP^+ DCs were injected intradermally into right flank of wild type C57BL/6 mice at 2×10^6 cells. The draining inguinal lymph nodes were harvested on days 2, 4, and 6 after injection. Frozen sections (6-μm thickness) of lymph node were prepared, and then the number of GFP^+ DCs was counted under fluorescence microscopy. Each point represents the mean ± SE of results from three mice.

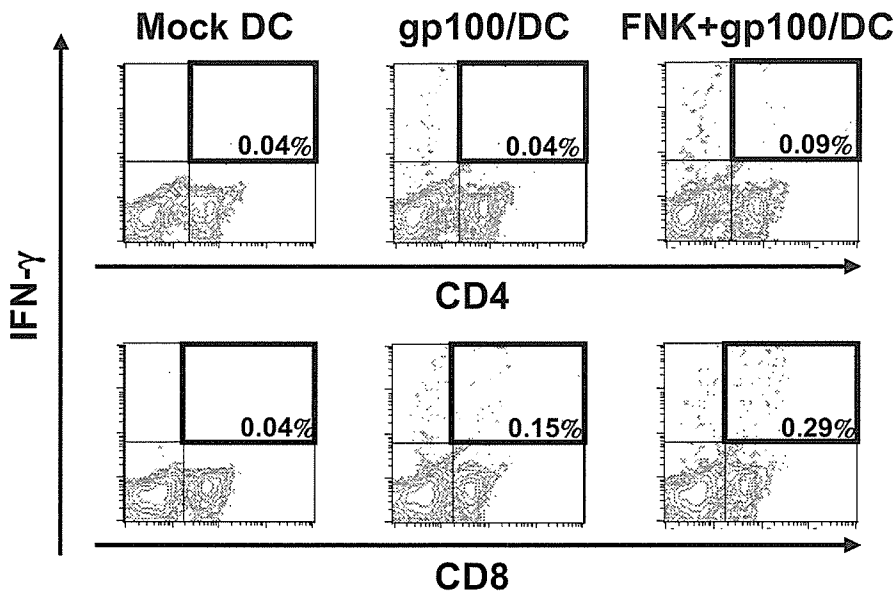
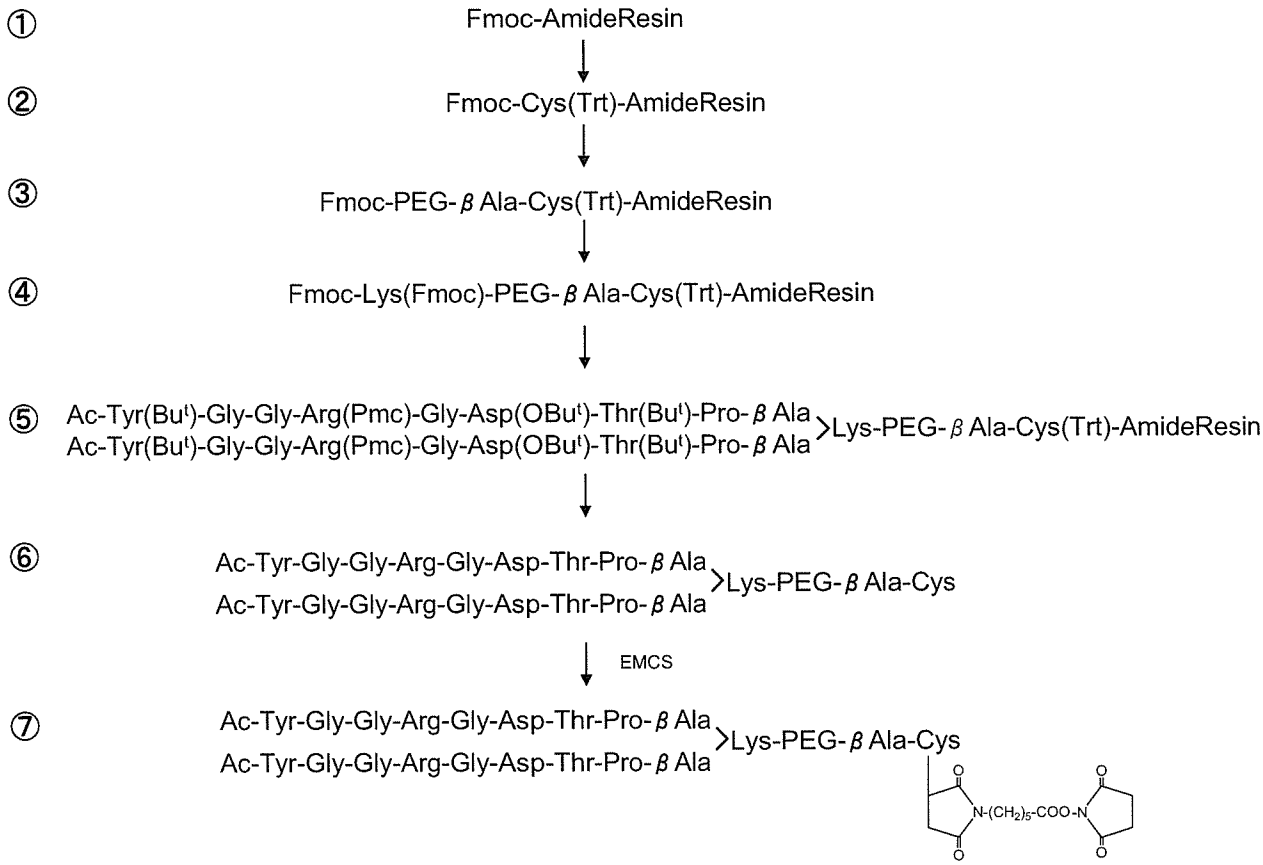
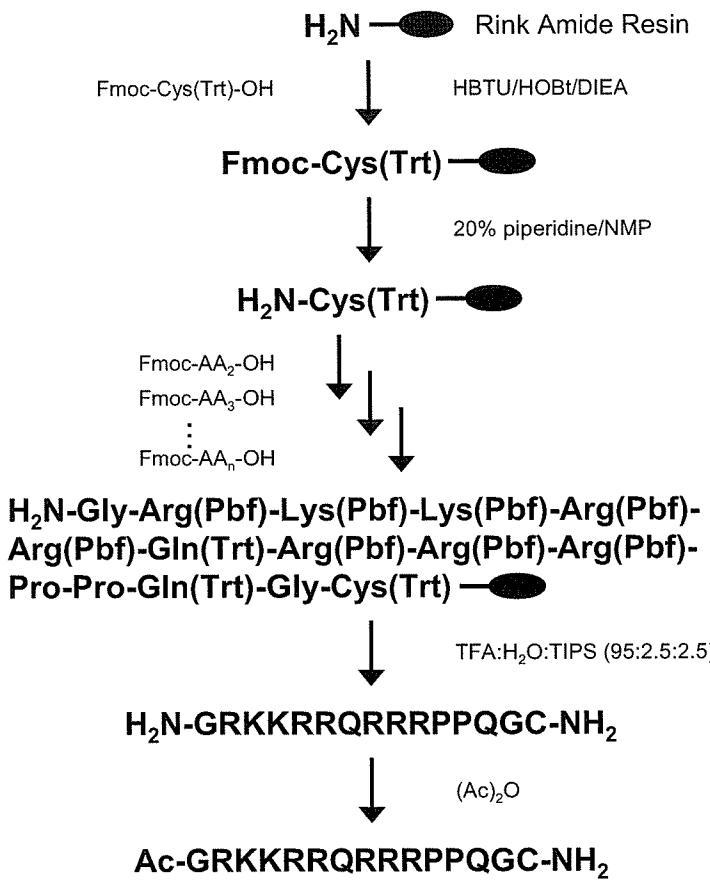


Fig. 93 gp100-specific $CD4^+$ or $CD8^+$ T cell immune response in mice immunized with DCs cotransduced with gp100 and Bcl-xFNK genes. gp100/DCs and FNK+gp100/DCs were prepared using corresponding vectors at 25 MOI, and then culture for 24 h. These transduced cells and mock DCs were vaccinated once intradermally into C57BL/6 mice at 1.5×10^6 cells. At 1 week after immunization, regional lymph node cells were prepared from these mice, and were re-stimulated *in vitro* with mitomycine C- inactivated B16BL6 cells for 24 h. The number of IFN-γ-producing $CD4^+$ or $CD8^+$ T cells was analyzed by intracellular IFN-γ staining followed by flow cytometry.



Scheme 1. Structure scheme for RGD-PEG



Scheme 2. Structure scheme for Tat peptide.

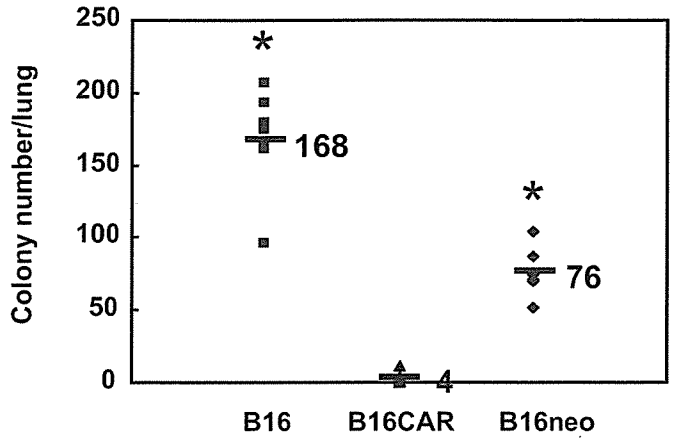


Fig. 94. Effect of CAR expression on lung metastasis. B16, B16CAR or B16neo cells (1.5 x 105) were injected into the tail vein of mice. After 14 days, the mice were sacrificed, their lungs were fixed, and the colonies on the lung surface were counted. The values close to the *horizontal bars* indicate the average numbers of colonies per lung. $P < 0.0001$ ($n = 7$).

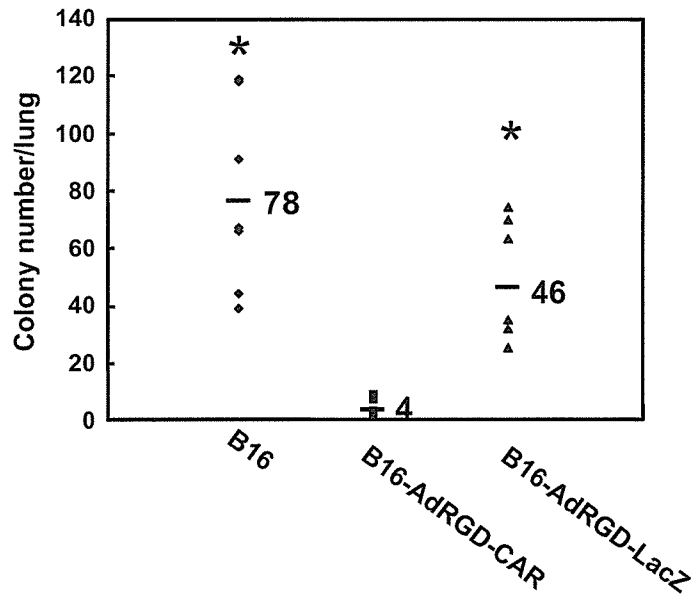


Fig. 95. Effect of transient CAR expression on lung metastasis. B16 cells were transduced with AdRGD-CAR or AdRGD-LacZ at 1000 VP/cell. Two days later, the cells (1.5 x 105) were injected into the tail vein of mice. After 14 days, the mice were sacrificed, their lungs were fixed, and the colonies on the lung surface were counted. The values close to the *horizontal bars* indicate average of colonies per lung. *, $P < 0.0001$ ($n = 7$) compared with B16-AdRGD-CAR cells.

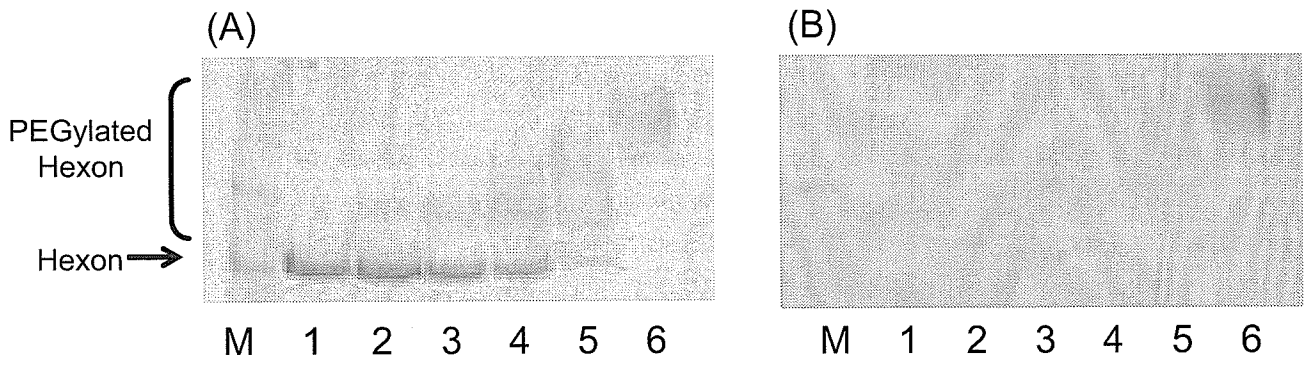


Fig. 96 SDS-PADE analysis of PEGylated adenovirus vectors. Comparison of two SDS-gels (A, B) that were run under identical conditions and loaded as follows : lane M, protein markers ; lane 1, Ad : PEG=1:0 (unmodified-Ad) ; lane 2, 1:25 ; lane 3, 1:100 ; lane 4, 1:400 ; lane 5, 1:1600 ; lane 6, 1:6400. (A) The gel was stained with Coomassie blue. (B) The gel was stained for PEG using barium iodide.

Table 12 Relationship between degree of PEGylated-Hexon and adenovirus vector size.

Ratio (Ad:PEG)*	PEG modification ratio (%)	Vector size (nm)	Serum half-life (min)
1:0	0	113.3±0.76	1.6
1:25	10	120.6±0.64	1.8
1:100	34	123.8±0.98	1.8
1:400	61	128.5±1.25	5.0
1:1600	89	137.6±0.91	12.0
1:6400	100	148.2±1.48	78.6

* ; Amount of PEG to lysine residue of adenovirus vector capsid protein (mol : mol)

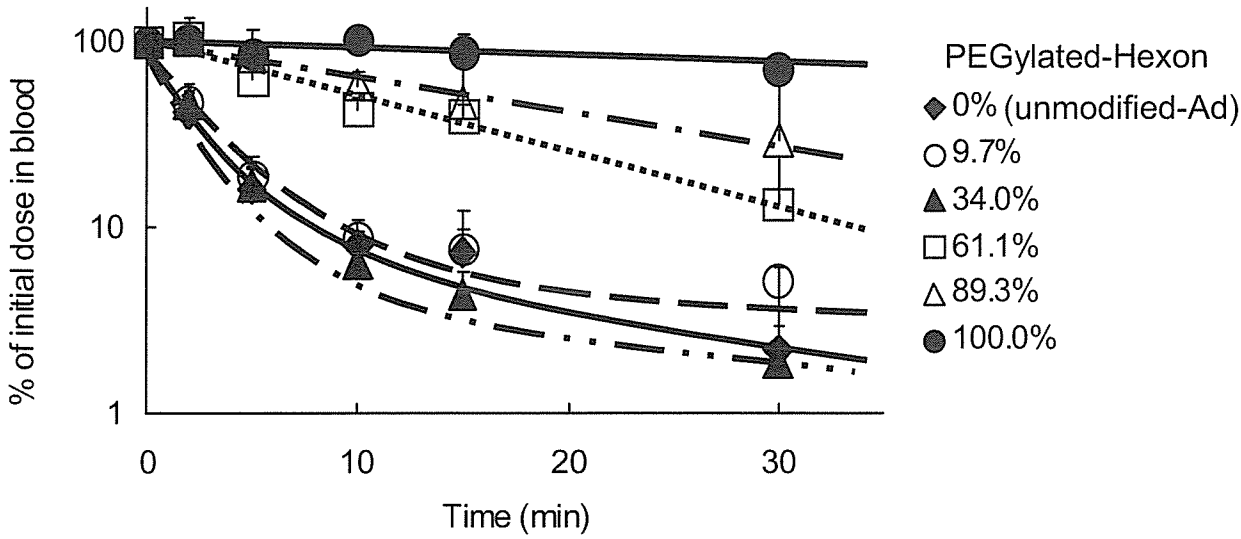


Fig. 97 Pharmacokinetics of PEGylated adenovirus vectors. Normal female BALB/c mice were administrated intravenously with $1x10^{10}$ particles of unmodified-Ad or PEG-Ads. Blood samples were drawn at different times. The concentration of adenovirus vectors in serum was quantitated with southern blot method. A standard curve was made for each PEG-Ads. Each point was represented as mean ±S.D. (n=4).

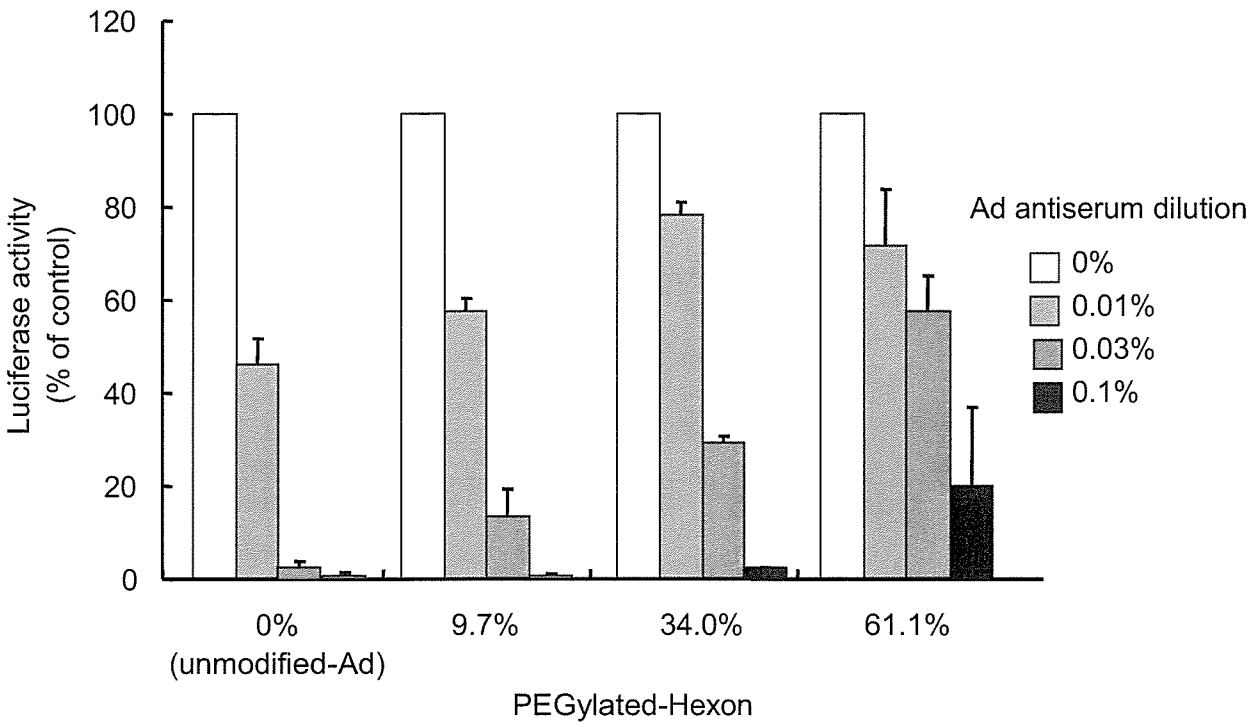


Fig. 98 Transduction of A549 cells by PEGylated adenovirus vectors in the presence or absence of adenovirus vectors antiserum. A549 cells ($1x10^4$ cells) were tranceduced with 1000 particles/cell of unmodified-Ad or PEG-Ads in the presence or absence of Ad antiserum respectively. Luciferase expression was measured after 24 hr. Each point was represented as mean ±S.D. (n=3).

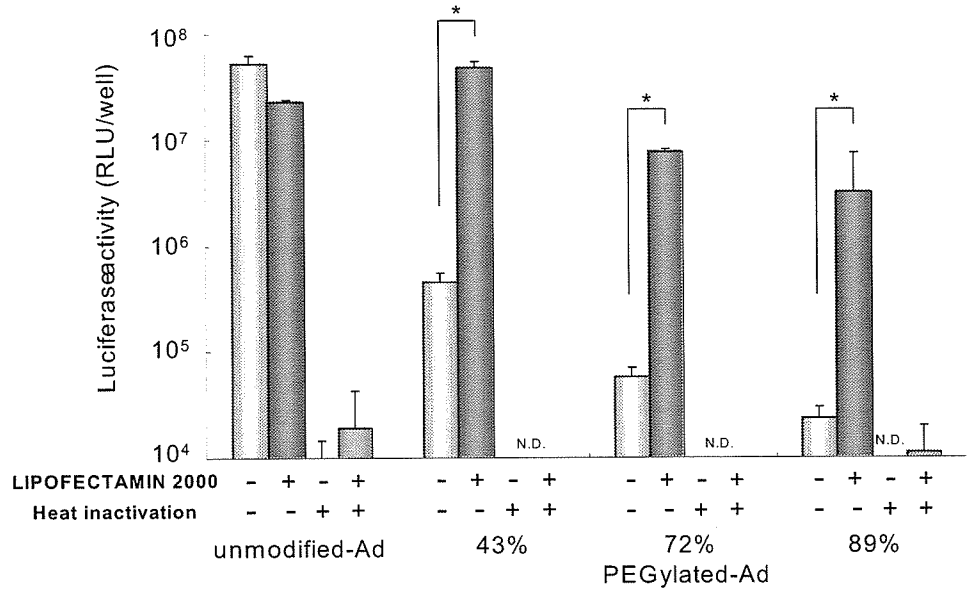


Fig. 99 Transduction efficiency of PEGylated adenovirus vectors into A549 cells in the presence or absence of LIPOFECTAMINE 2000. A549 cells ($2x10^4$ cells) were transduced with 1000 particles/cell of unmodified or PEGylated Ad-Luc in the presence or absence of 20 μg/ml of LIPOFECTAMINE 2000. After 4 hr, the virus solution was replaced with fresh medium, and the cells were incubated for 24 hr. Luciferase expression was measured. Each point represents the mean ± S.D. (n=3). * $P<0.05$ (*t-test*).

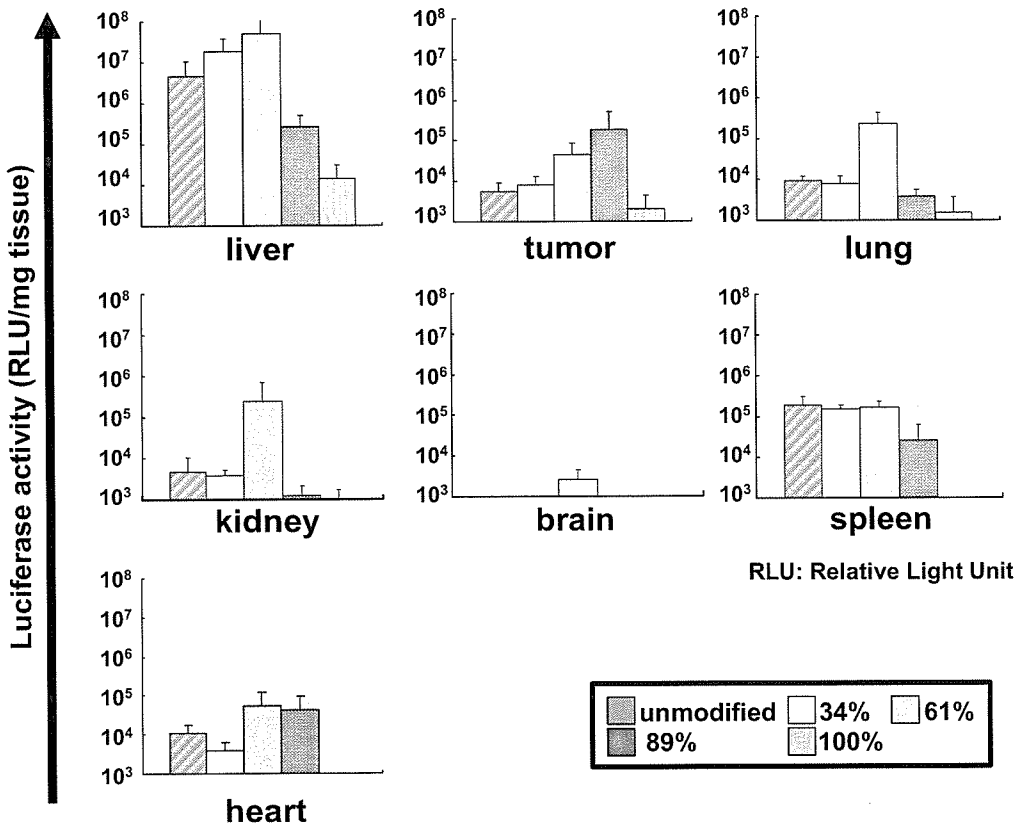


Fig. 100 *In vivo* gene expression pattern of PEG-Ad after i.v. administration into mice. 2×10^6 Meth-A fibrosarcoma tumor cells were inoculated intradermally and 10^{10} particles of unmodified or PEGylated Ad-Luc were injected intravenously after approximately one week. After 2 days, organs were harvested and homogenized with buffer. Luciferase activity was then measured using the kit according to the manufacture's instructions.(n=4).

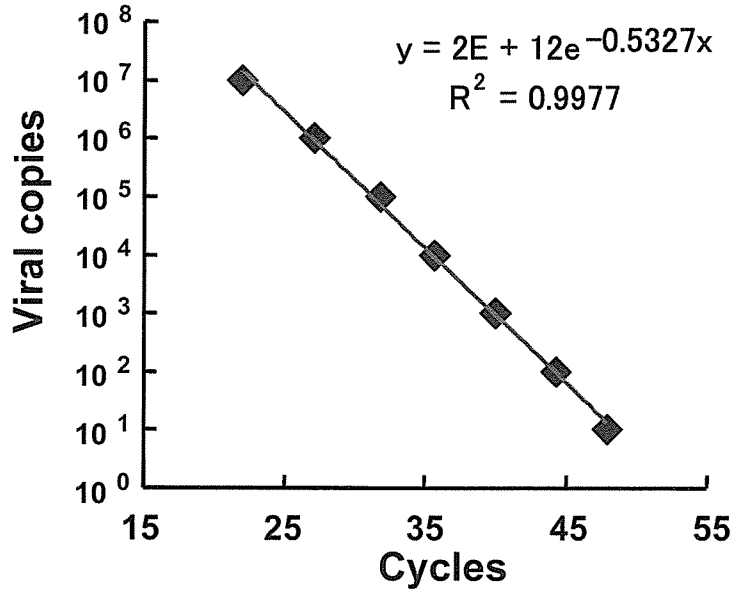


Fig. 101 Standard curve of TaqMan Real-time PCR

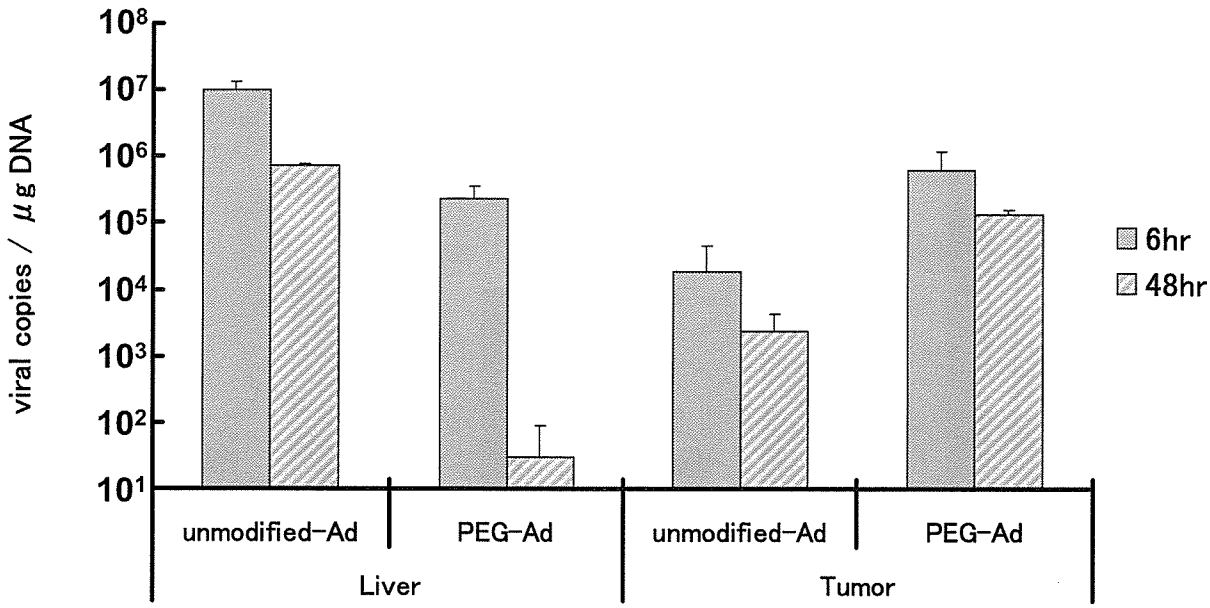


Fig. 102 Accumulation of Ad particles in tumor, and reduction in liver induced by PEGylation. Real-time PCR was carried out for detecting viral particles existence in tumor and liver 6, 48 h after systemically administration of 1 x 10^{11} VP of both unmodified-Ad and PEGylated Ad (89% of modification ratio).

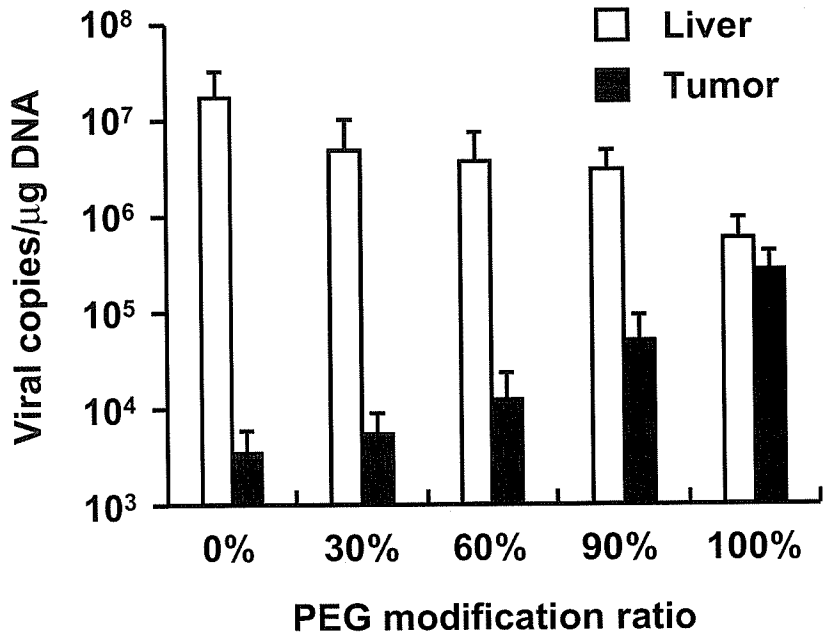


Fig. 103 Biodistribution of PEG-Ad vector in Meth-A tumor-bearing mice. BALB/c mice were intradermally inoculated with Meth-A cells into the flank at 2×10^6 cells/mouse. One week later, these mice were intravenously injected with unmodified Ad-Luc or PEG-Ad-Luc with various modification ratios at 10^{11} VP/mouse. At 6 h after vector injection, tumor and liver were harvested, and then real-time PCR was carried out for detecting viral geneme in DNA isolated from them. Data represent the mean ± SD of results from four mice.

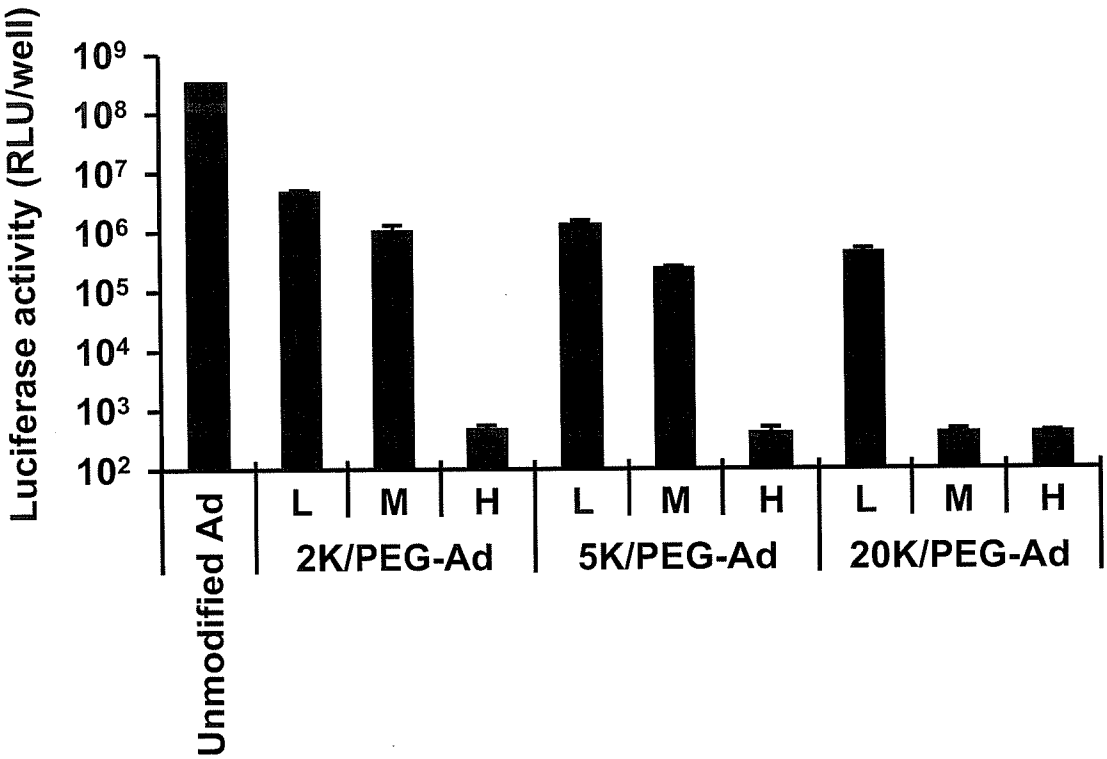


Fig. 104 Transduction efficiency of PEG-Ads modified with various PEG. A549 cells were transduced with unmodified Ad-Luc, 2K/PEG-Ad-Luc, 5K/PEG-Ad-Luc, or 20K/PEG-Ad-Luc at 10000 VP/cell. The modification ratio of PEG-Ad are indicated as follows: L, 30-40%; M, 50-60%; H, 80-90%. After 24 h-cultivation, luciferase activity was measured. Data represents the mean ± SD of results from triplicate culture.

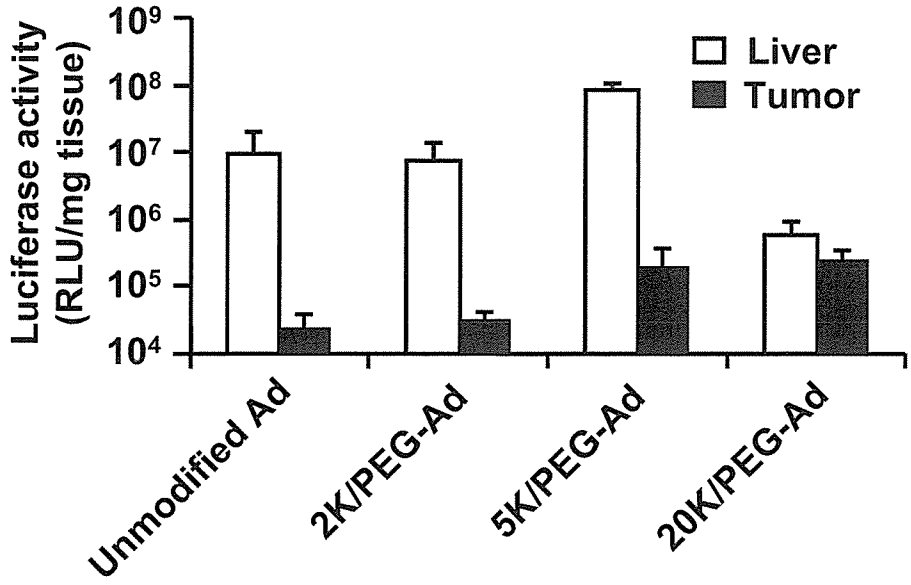


Fig. 105 *In vivo* gene expression of PEG-Ads modified with various PEG after i.v. administration into Meth-A tumor-bearing mice. Meth-A tumor-bearing BALB/c mice were intravenously injected with unmodified Ad-Luc, 2K/PEG-Ad-Luc. 5K/PEG-Ad-Luc, or 20K/PEG-Ad-Luc at 10^{10} VP/mouse. Modification ratio of each PEG-Ad was 30-40%. Two days later, liver and tumor were harvested and homogenized with buffer. Luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean ± SD or results from five mice.

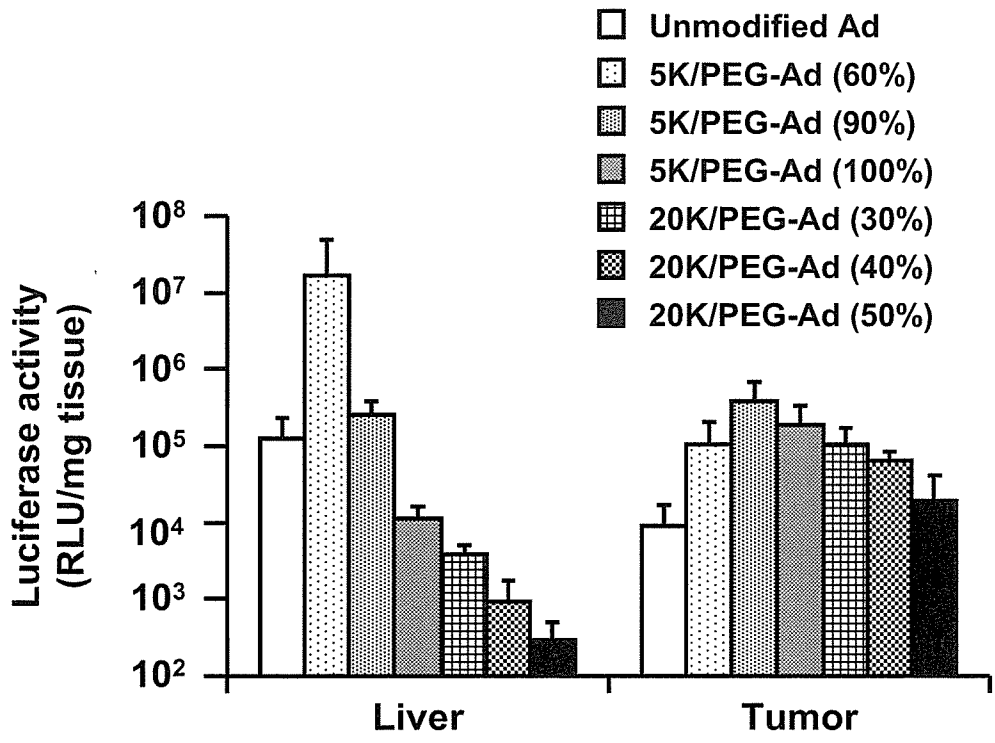


Fig. 106 Gene expression pattern in Meth-A tumor-bearing mice injected i.v. with various PEG-Ads. Meth-A tumor-bearing mice were i.v. injected with unmodified or various PEGylated Ad-Luc at 10^{10} VP. Two days later, liver and tumor were harvested and homogenized with buffer. Luciferase activity was then measured using the kit according to the manufacture's instructions. Data represent the mean ± SE of results from five mice.

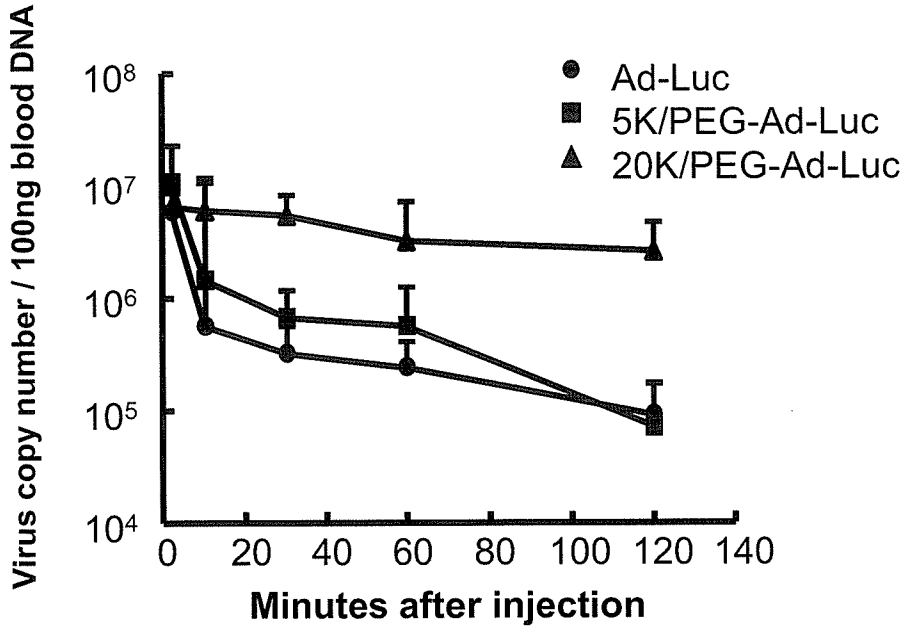


Fig. 107 Pharmacokinetics of PEG-Ads after i.v. injection. BALB/c mice were injected intravenously with unmodified Ad-Luc, 5K/PEG-Ad-Luc, or 20K/PEG-Ad-Luc at 10^{10} VP/mouse. The concentration of Ad in the blood at the indicated time points was determined by real-time quantitative PCR. Data are presented as means ± S.D. (n = 6).

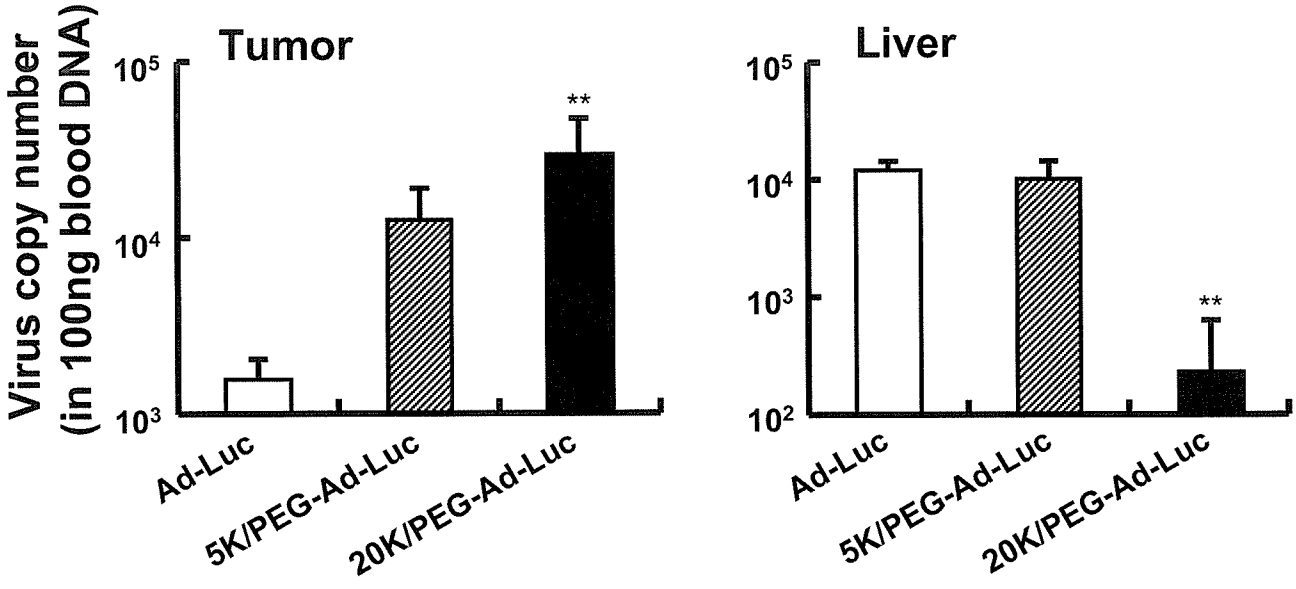


Fig. 108 Tissue distribution of PEG-Ads after i.v. injection. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of unmodified Ad-Luc or PEG-Ad-Luc. Six hours after the injection, the tumor and liver tissues were harvested and DNA extracted. The number of viral genomes in each sample was measured by real-time quantitative PCR. Data are presented as means ± S.D. (n = 4-6; **,$P < 0.01$ compared with value for unmodified Ad).

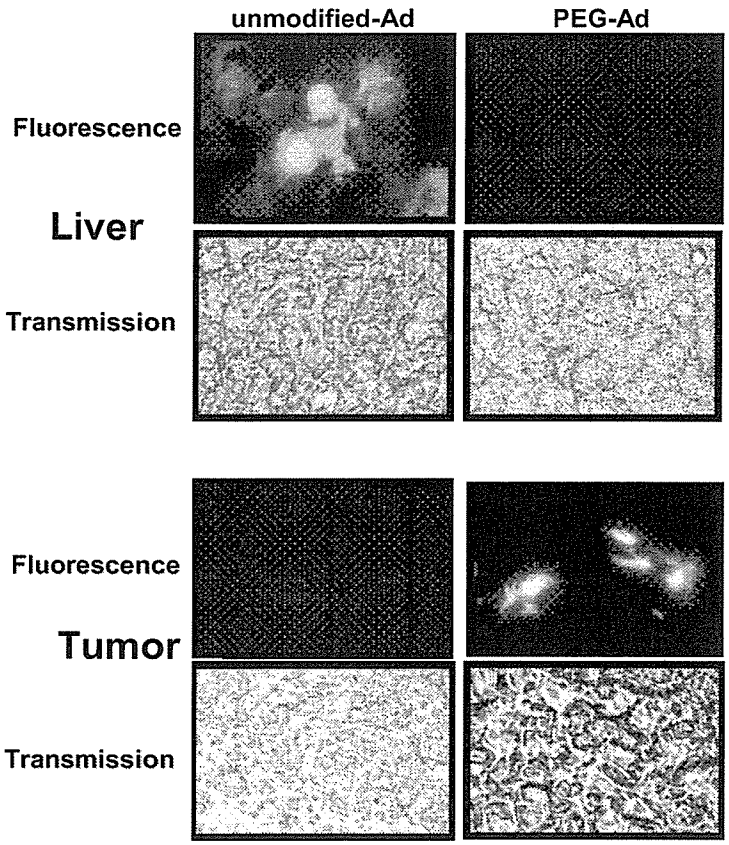


Fig. 109 EGFP gene expression of unmodified-Ad and PEG-Ad. Tumor-bearing mice were administrated intravenously with 1.5×10^{11} particles of unmodified-Ad or PEG-Ad expressing EGFP. After 48 hr, livers and tumors were removed, processed for frozen sectioning. EGFP was observed under a fluorescence microscopy at x 400 magnification.

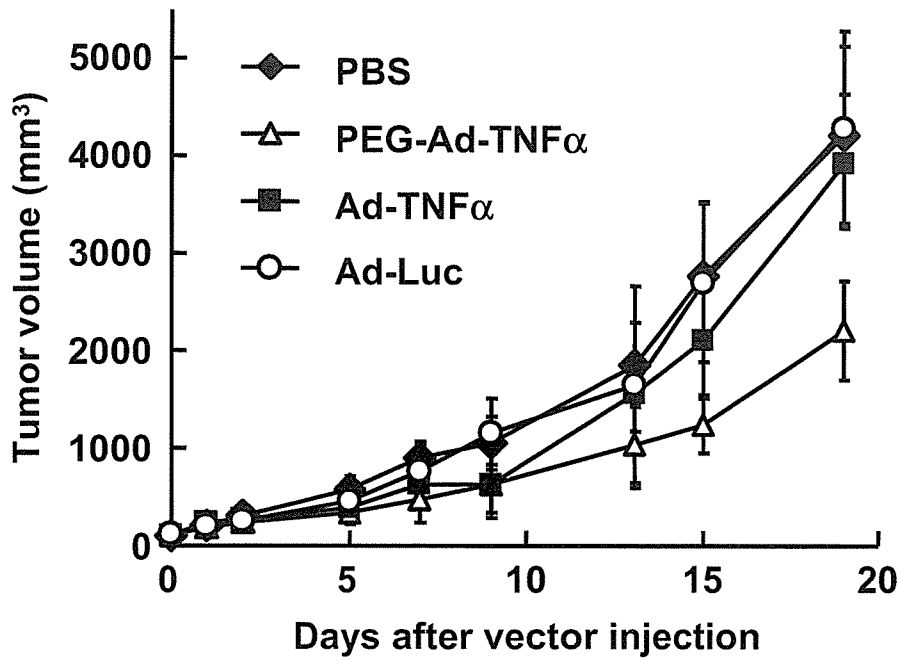


Fig. 110 Anti-tumor efficacy of systemic injection of PEG-Ad-TNFα. Meth-A tumor-bearing BALB/c mice were intravenously injected with Ad-Luc, Ad-TNFα, or PEG-Ad-TNFα (89% modification ratio) at 10^{10} VP/mouse. Tumor volume was measured two or three times per week. Each point represents the mean ± SE from 6-8 mice.

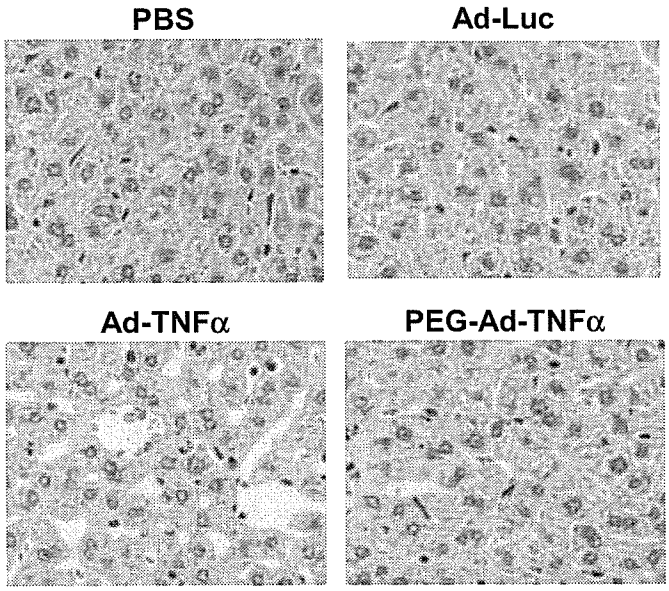


Fig. 111 Histopathological examination of liver after i.v. injection of PEG-Ad-TNFα. Meth-A tumor-bearing BALB/c mice were intravenously injected with Ad-Luc, Ad-TNFα, or PEG-Ad-TNFα (89% modification ratio) at 10^{10} VP/mouse. After 48 h, livers were harvested, placed in neutral 10% formalin, and embedded in paraffin. Sections (5-μm) were prepared for hematoxylin and eosin staining and histopathological examination. Original magnification is ×300.

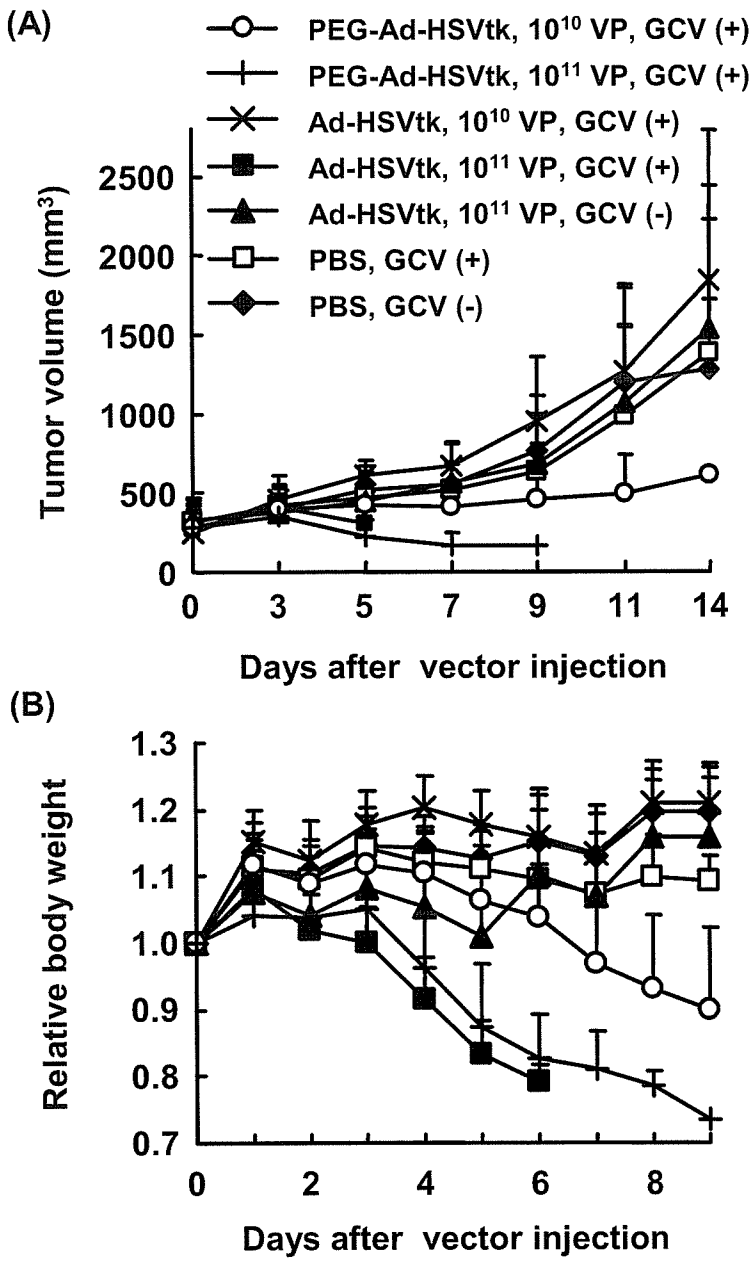


Fig. 112 Tumor growth (A) and body weight change (B) in Meth-A tumor-bearing mice treated with HSVtk/GCV system. Meth-A tumor-bearing BALB/c mice were intravenously injected with Ad-Luc, Ad-HSVtk, or PEG-Ad-HSVtk (90% modification ratio) at 10^{10} or 10^{11} VP/mouse. These mice were treated once daily with intraperitroneal injection of GCV (50 mg/kg/day) for 10 days. The tumor volume (A) and body weight (B) were monitored. Each point represents the mean ± SD of results from six mice.

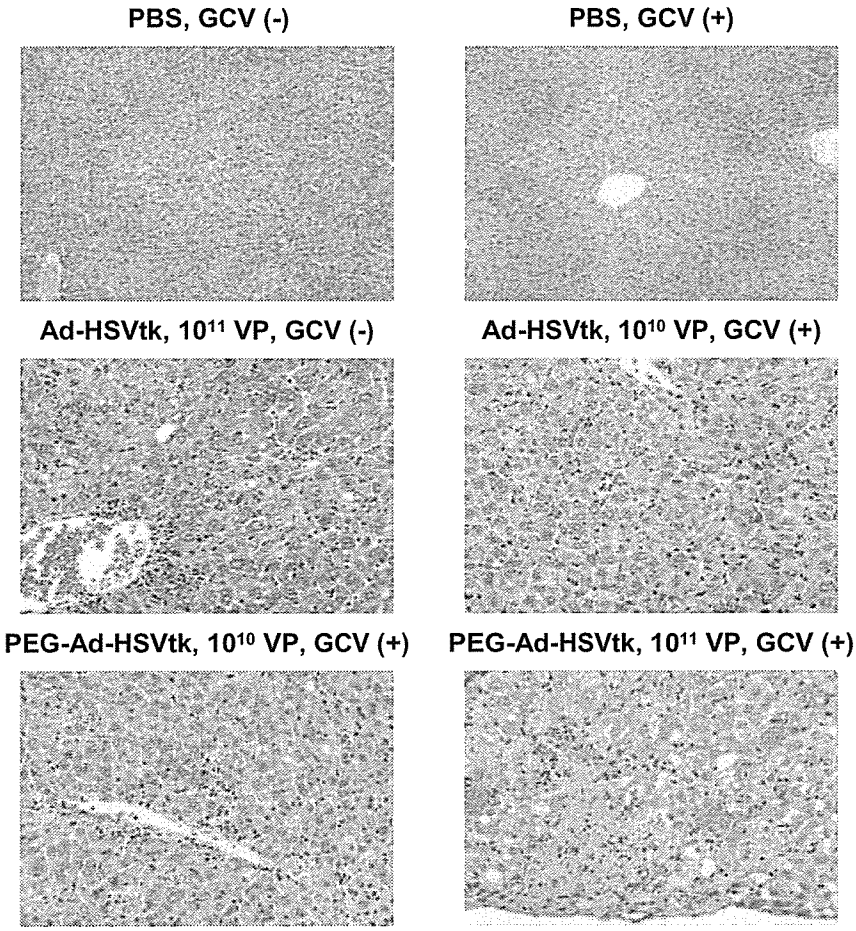


Fig. 113 Histopathological examination of liver after HSVtk/GCV treatment. Meth-A tumor-bearing BALB/c mice were intravenously injected with Ad-HSVtk or PEG-Ad-HSVtk (90% modification ratio) at 10^{10} or 10^{11} VP/mouse. These mice were treated once daily with intraperitroneal injection of GCV (50 mg/kg/day). On day 7 after vector injection, livers were harvested, placed in neutral 10% formalin, and embedded in paraffin. Sections (5-μm) were prepared for hematoxylin and eosin staining and histopathological examination. Original magnification is ×300.

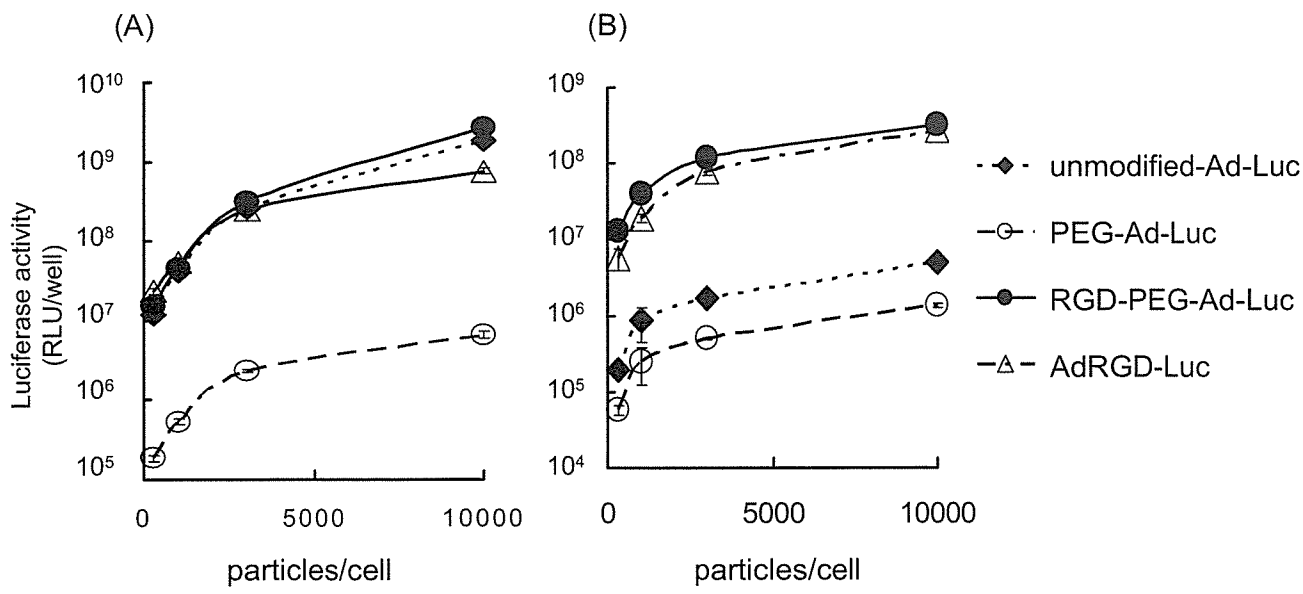


Fig. 114 Transduction of A549 cells and B16BL6 cells by RGD-PEGylated adenovirus vectors. (A) A549 cells and (B) B16BL6 cells ($2x10^4$ cells) were transfected with 300, 1000, 3000 or 10000 particles/cell of Ad, PEG-Ad-Luc, RGD-PEG-Ad-Luc or AdRGD-Luc respectively. Luciferase expression was measured after 24 hr. Each point was represented as mean ±S.D.

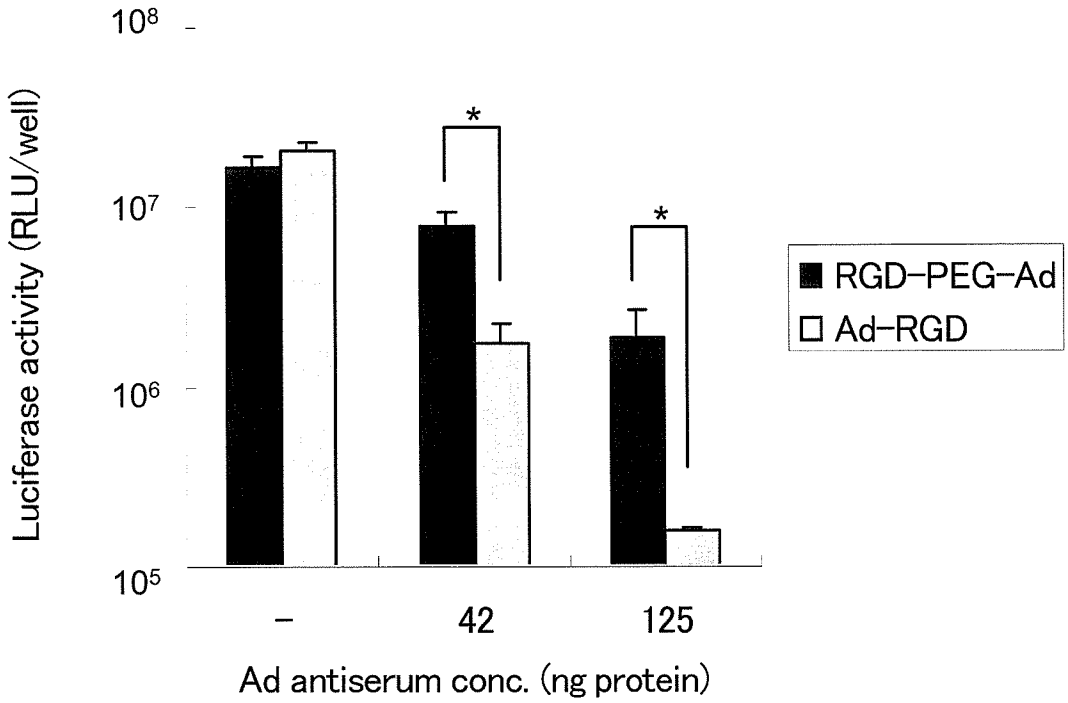


Fig. 115 Transduction of B16BL6 cells by RGD-PEGylated adenovirus vectors in the presence or absence of adenovirus vectors antiserum. B16BL6 cells ($2x10^4$ cells) were tranceduced with 1000 particles/cell of RGD-PEG-Ad or AdRGD in the presence or absence of Ad antiserum respectively. Luciferase expression was measured after 24 hr. Each point was represented as mean ±S.D. (n=3).

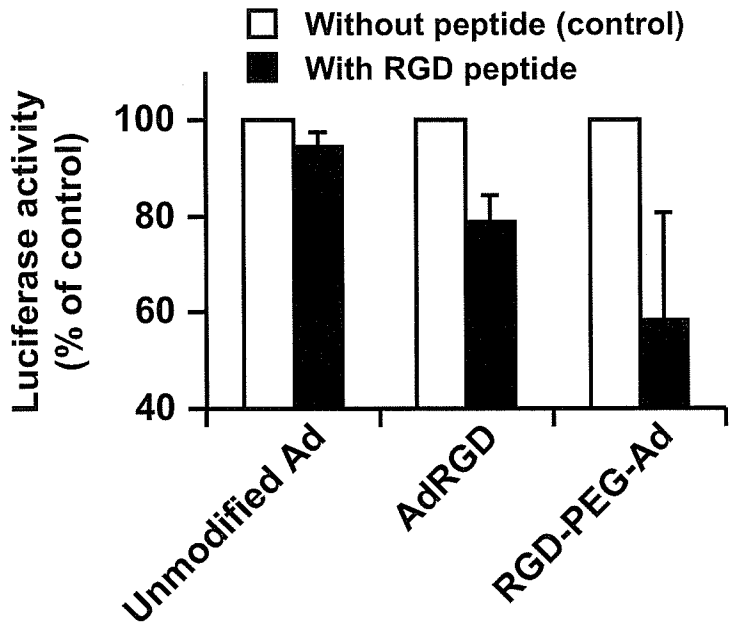


Fig. 116 Inhibitory effect of RGD peptide on gene transduction with RGD-PEG-Ad. B16BL6 cells were transduced with unmodified Ad-Luc, AdRGD-Luc, or RGD-PEG-Ad-Luc at 3000 VP/cell in the presence or absence of RGD peptide (200 μg/ml). Twenty-four hours later, luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean ± SE of results from three independent cultures.

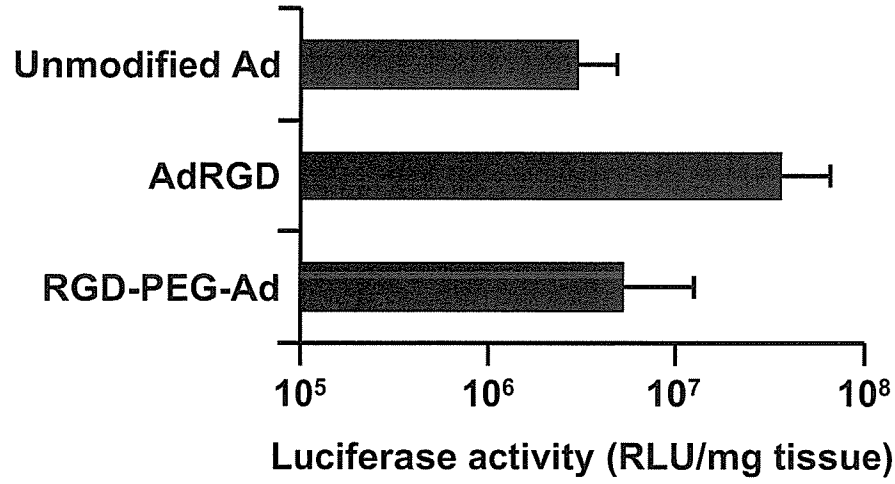


Fig. 117 Gene expression in liver of mice injected i.v. with RGD-PEG-Ad. BALB/c mice were intravenously injected with unmodified Ad-Luc, AdRGD-Luc, or RGD-PEG-Ad-Luc at 1.5×10^{10} VP/mouse. Two days later, livers were harvested and homogenized with buffer. Luciferase activity was then measured using the kit according to the manufacture's instructions. Data represent the mean ± SE of results from four mice.

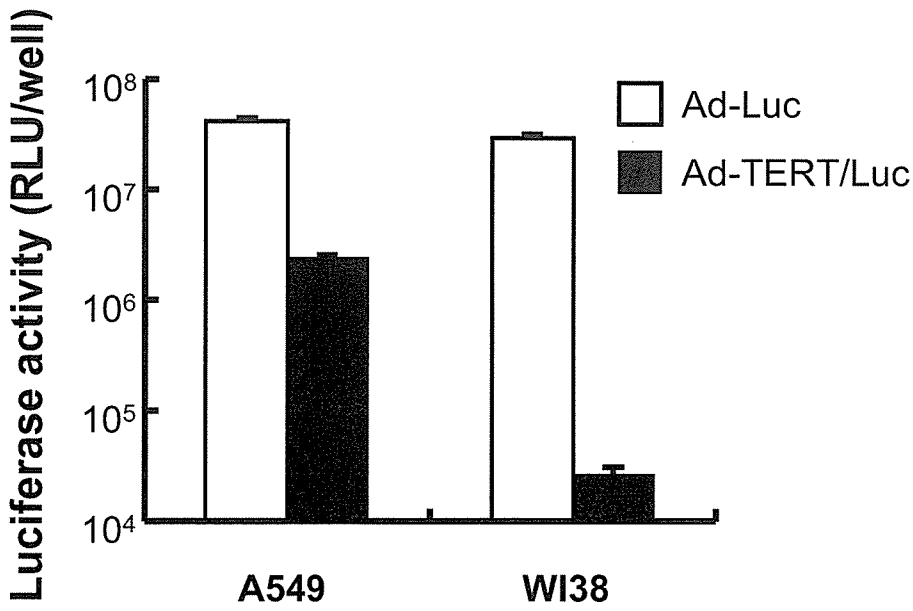


Fig. 118 Transduction efficiency of Ad-Luc and Ad-TERT/Luc into tumor cells or normal cells. A549 tumor cells (2×10^4 cells) and WI38 normal cells (2×10^4 cells) were transduced with 10,000 particles/cell of Ad-Luc or Ad-TERT/Luc. Luciferase expression was measured after 24 hr. Each point represents the mean ± S.D. (n = 3). RLU, relative light units.

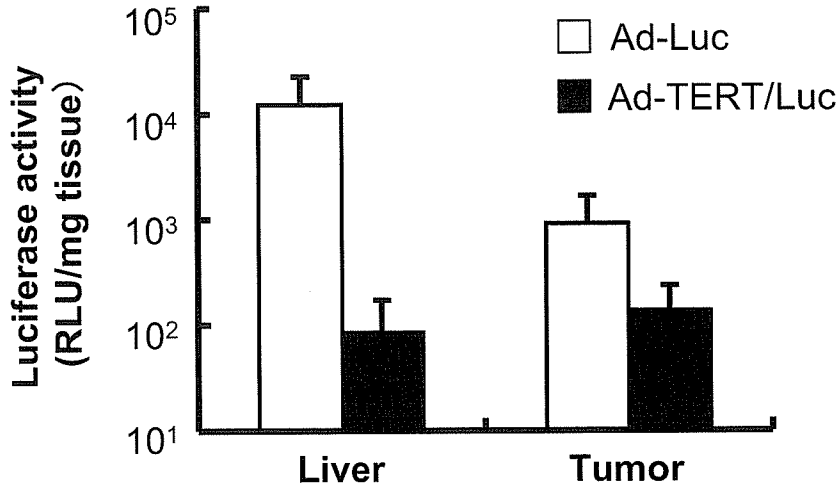


Fig. 119 The gene expression of Ad-Luc and Ad-TERT/Luc in liver and tumor. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of Ad-Luc and Ad-TERT/Luc. After 48 h, liver and tumor tissues were harvested and luciferase activity was measured. Data are presented as means ± S.D. (n = 6). RLU, relative light units.

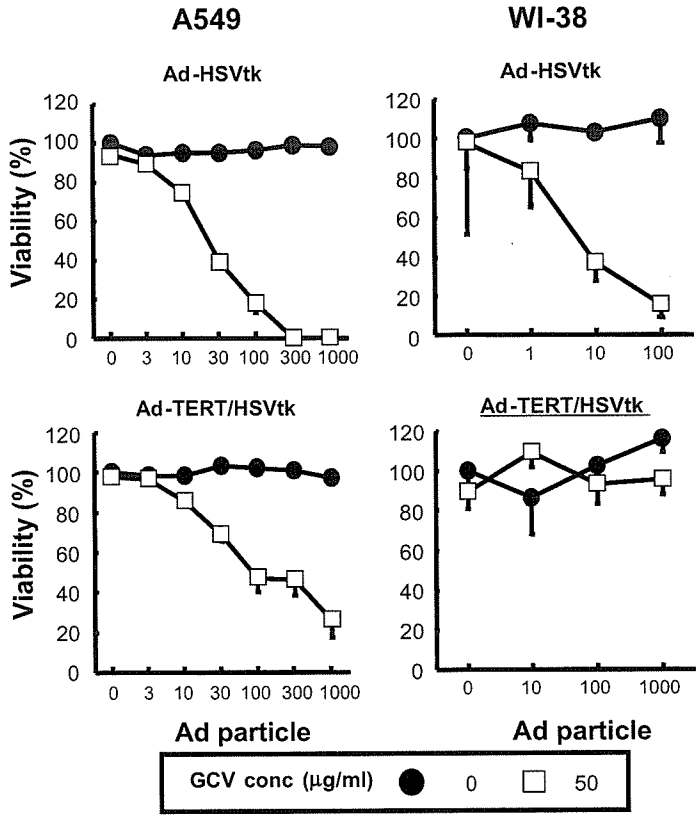


Fig. 120 Selective cytotoxicity of Ad-TERT/HSVtk in cancer cells but not in normal cells. Tumor A549 cells and normal WI38 cells were transduced with Ad-HSVtk and Ad-TERT/HSVtk at indicated virus particle. After 2h, these cells were cultured with media containing GCV. Four days later, cell viability was measured by WST-1 assay. Data represent as a percent of viability of cells that were treated without Ad nor GCV. Data are presented as means ± S.D. (n = 6).

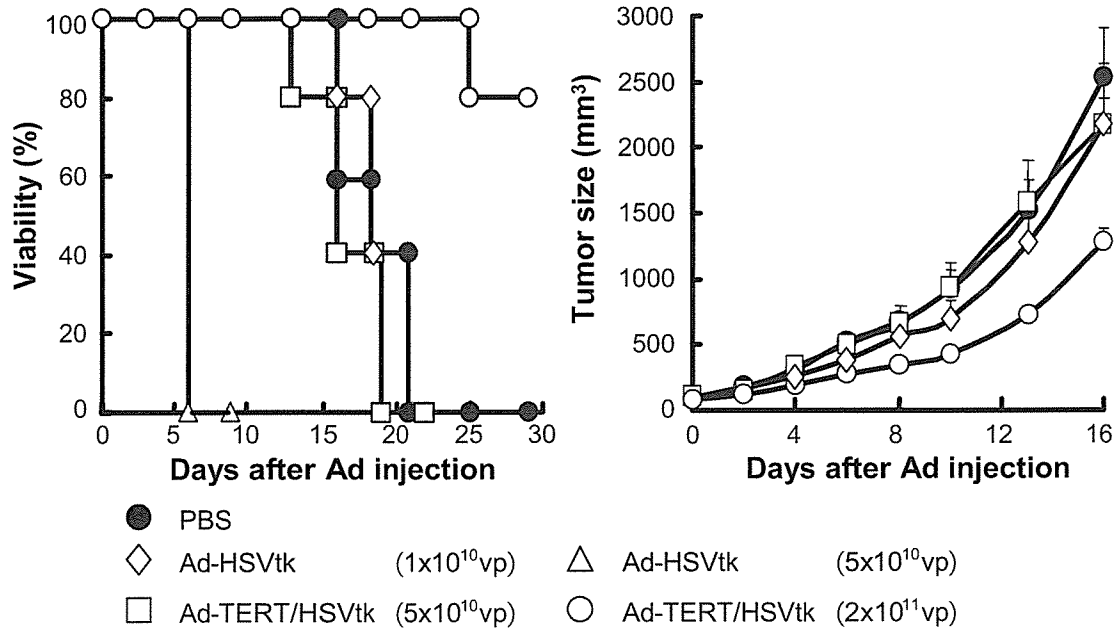


Fig. 121 Therapeutic effect in primary tumor models treated with intravenous injection of Ad-TERT/HSVtk or Ad-HSVtk. Established Meth-A primary tumors-bearing BALB/c mice were i.v. injected with PBS, Ad-HSVtk or Ad-TERT/HSVtk. Then the mice received daily injections of GCV (50 mg/kg) intraperitoneally for 10 days. Tumor size and survival were monitored. Data are presented as means ± S.E. (n = 5). Mice were euthanized when tumor volume was greater than 4000 mm^3.

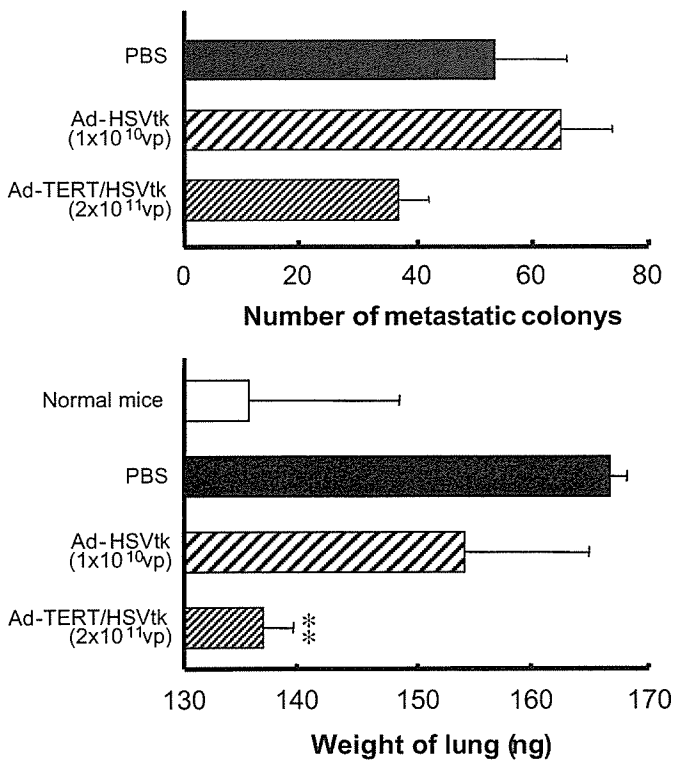


Fig. 122 Therapeutic effect in metastasis models treated with intravenous injection of Ad-TERT/HSVtk or Ad-HSVtk. Established metastasis-bearing BALB/c mice were i.v. injected with PBS, Ad-HSVtk (1×10^{10} VP) and Ad-TERT/HSVtk (2×10^{11} VP) on day 7 after CT26 inoculation. Then these mice were i.p. injected with GCV once daily for 7 days. Lungs were harvest on day 14 after CT26 inoculation. The number of metastasis colonies and lung weights were measured. Data represent the mean ± S.D. (n = 4; **,P < 0.01 compared with value for PBS).

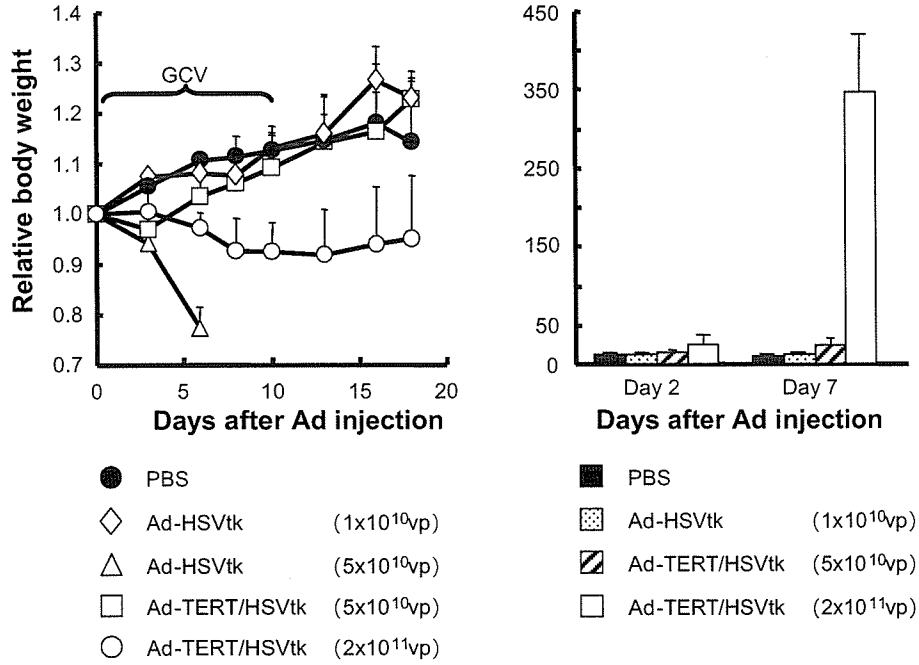


Fig. 123 Side effect of treatment via intravenous injection of Ad-TERT/HSVtk and GCV in primary tumor model. Establised Meth-A primary tumors-bearing BALB/c mice were i.v. injected with PBS, Ad-HSVtk or Ad-TERT/HSVtk. Then the mice received daily injections of GCV (50 mg/kg) intraperitoneally for 10 days. Body weights and GPT activity in blood were monitored with time. Data are presented as means ± S.D. (n = 5).

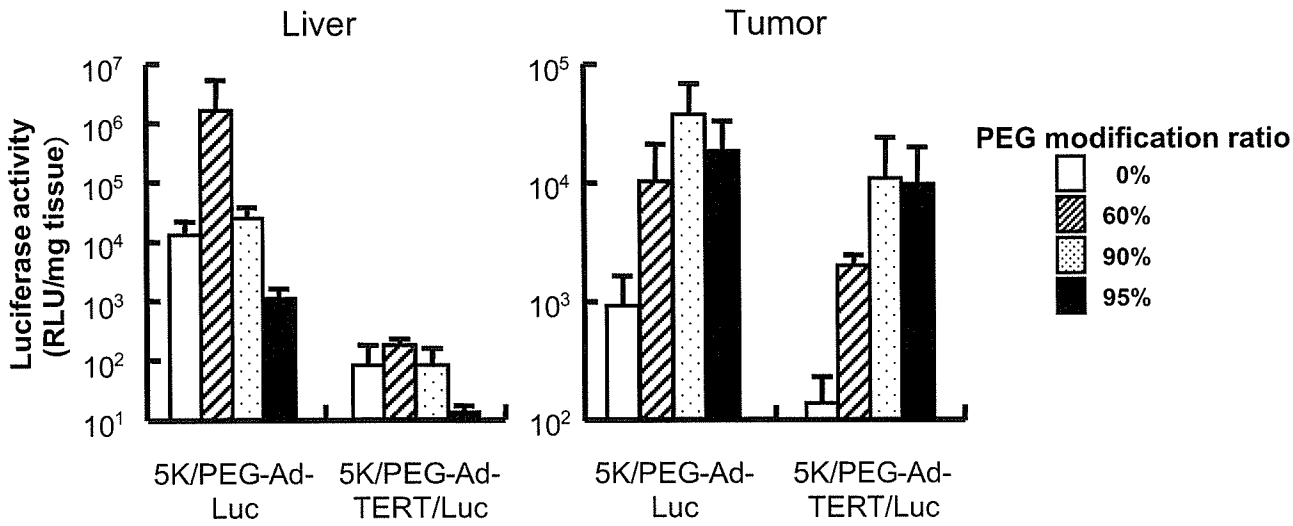


Fig. 124 The gene expression of PEG-Ad-Luc and PEG-Ad-TERT/Luc in liver and tumor. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of PEG-Ad-Luc and PEG-Ad-TERT/Luc with indicated PEG modification ratio. After 48 h, liver and tumor tissues were harvested and luciferase activity was measured. Data are presented as means ± S.D. (n = 6). RLU, relative light units.

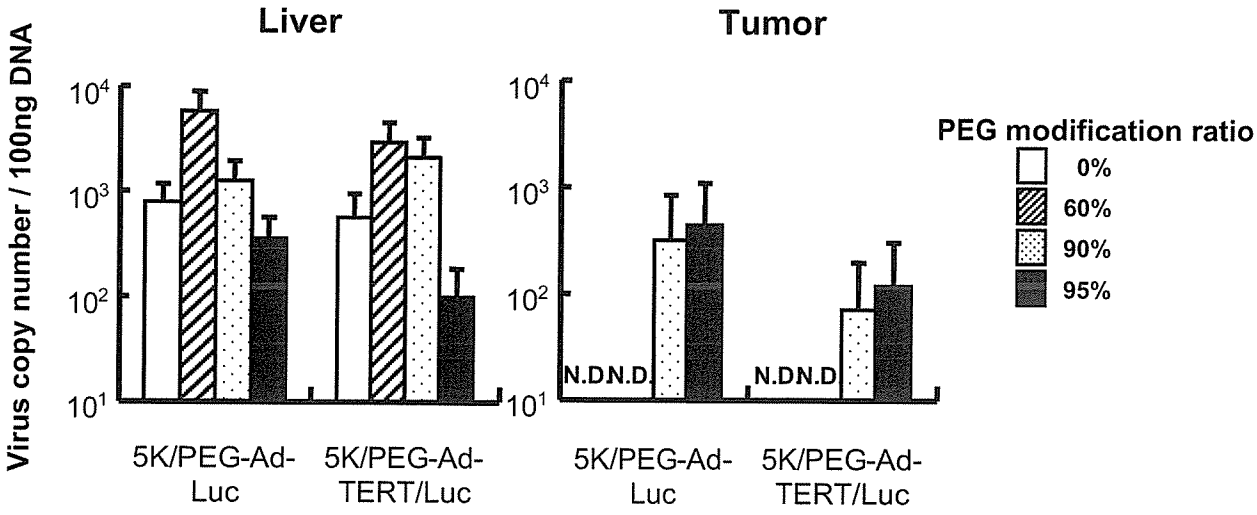


Fig. 125 Tissue distribution of PEG-Ad-Luc and PEG-Ad-TERT/Luc. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of unmodified Ad or each PEG-Ads with indicated PEG modification ratio. After 48 h, the tumor and liver tissues were harvested and DNA extracted. The number of viral genomes in each sample was measured by real-time quantitative PCR. Data are presented as means ± S.D. (n = 5-6).

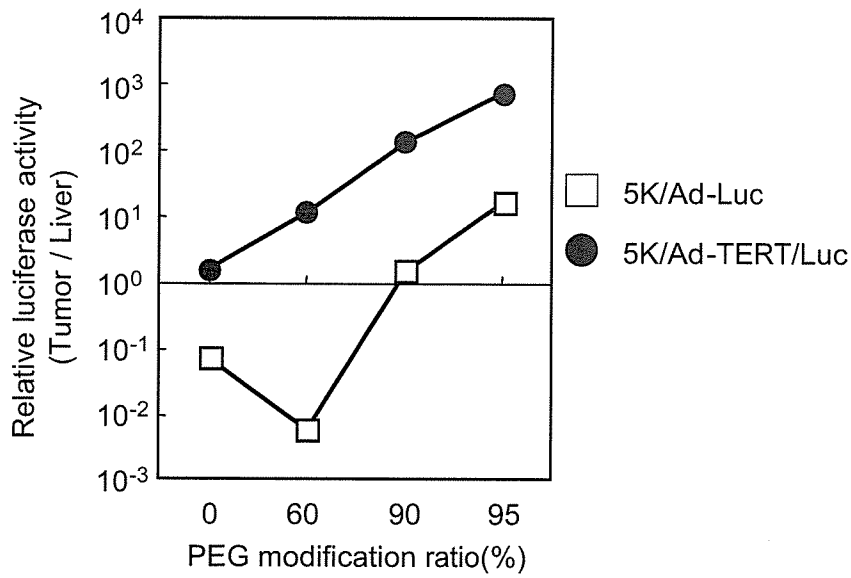


Fig. 126 Ratio of Luciferase activity (Tumor / Liver). Ratio of luciferase activity was calculated as: Luciferase activity in tumor (RLU / mg tumor) / luciferase activity in liver (RLU / mg liver) at each modification ratio.

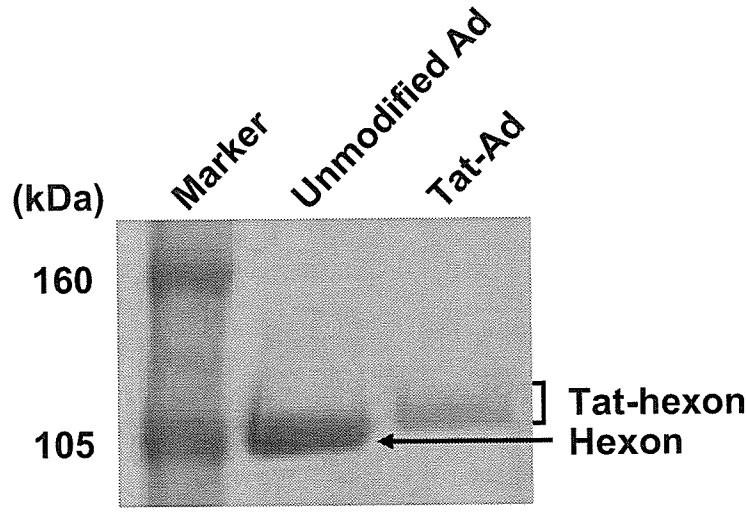


Fig. 127 SDS-PAGE analysis of Tat-Ad.

Table 13 The surface charge of Tat-Ad.

Vector	Surface charge (mV)
Unmodified Ad	-18.7
Tat-Ad	+2.3

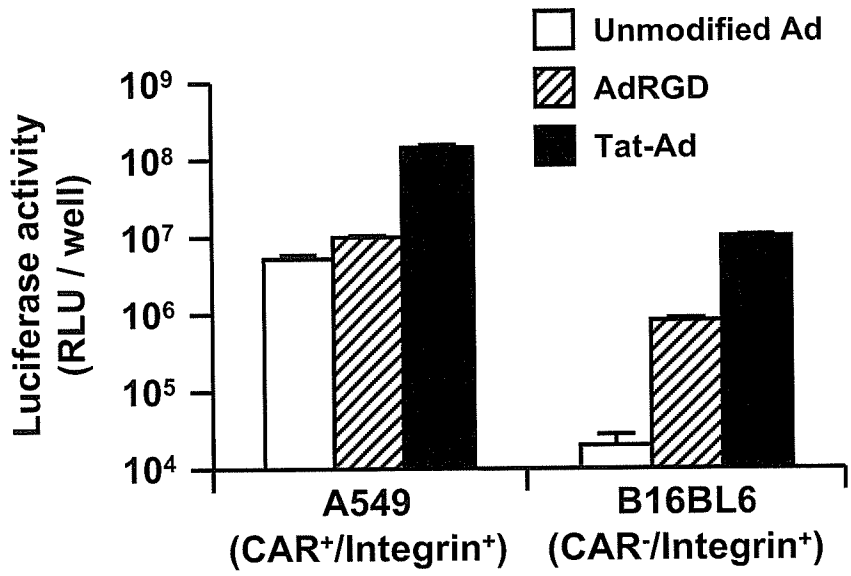


Fig. 128 Transduction efficiency of Tat-Ad in A549 and B16BL6 cells. A549 and B16BL6 cells were transduced with unmodified Ad-Luc, AdRGD-Luc, or Tat-Ad-Luc at 10000 VP/cell. After 24 h-cultivation, luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean ± SD of results from triplicate culture.