

Fig. 51. Transduction of B16BL6 cells by RGD-PEGylated adenovirus vectors in the presence or absence of adenovirus vectors antiserum. B16BL6 cells (2×10^4 cells) were transduced 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 \pm S.D. (n=3).

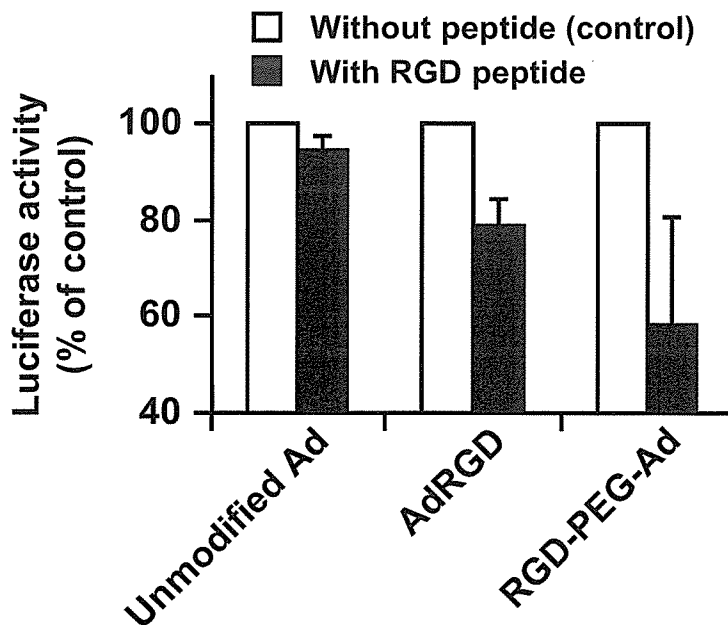


Fig. 52. Inhibitory effect of RGD peptide on gene transduction with RGD-PEG-Ad. B16BL6 cells were transduced with unmodified Ad-Luc, AdRGD-Luc, or RGD-PEG-Ad-Luc at 3000 VP/cell in the presence or absence of RGD peptide (200 μ g/ml). Twenty-four hours later, luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean \pm SE of results from three independent cultures.

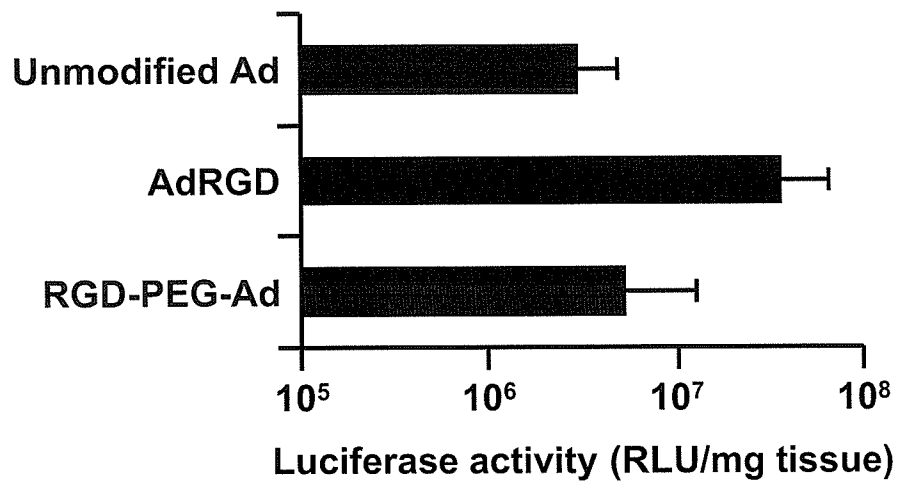


Fig. 53. Gene expression in liver of mice injected i.v. with RGD-PEG-Ad. BALB/c mice were intravenously injected with unmodified Ad-Luc, AdRGD-Luc, or RGD-PEG-Ad-Luc at 1.5×10^{10} VP/mouse. Two days later, livers were harvested and homogenized with buffer. Luciferase activity was then measured using the kit according to the manufacture's instructions. Data represent the mean \pm SE of results from four mice.

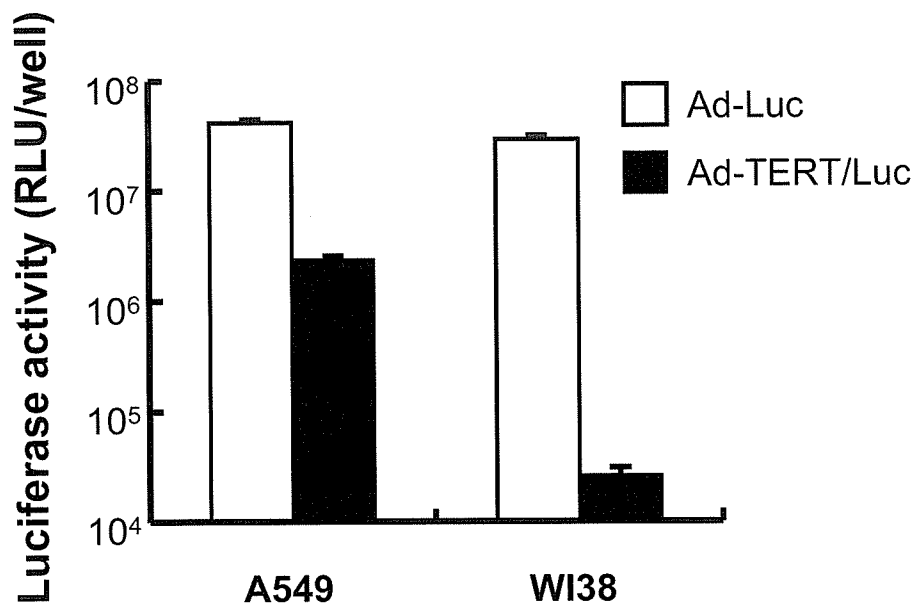


Fig. 54. Transduction efficiency of Ad-Luc and Ad-TERT/Luc into tumor cells or normal cells. A549 tumor cells (2×10^4 cells) and WI38 normal cells (2×10^4 cells) were transduced with 10,000 particles/cell of Ad-Luc or Ad-TERT/Luc. Luciferase expression was measured after 24 hr. Each point represents the mean \pm S.D. (n = 3). RLU, relative light units.

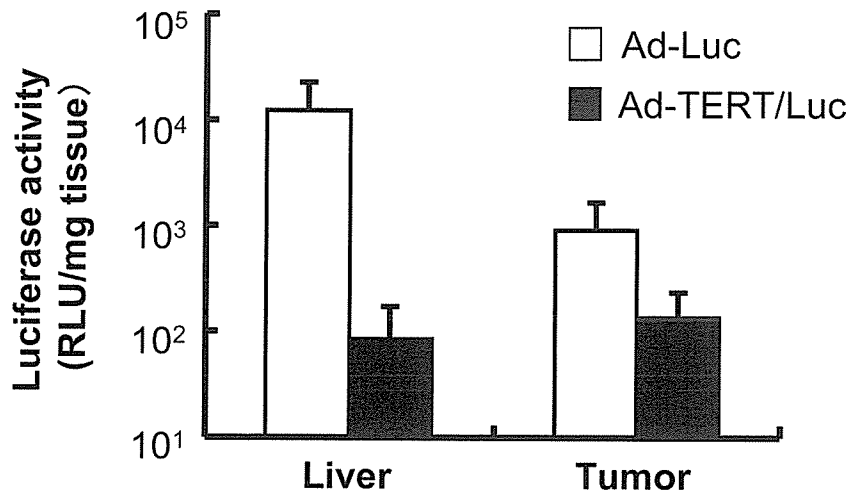


Fig. 55. The gene expression of Ad-Luc and Ad-TERT/Luc in liver and tumor. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of Ad-Luc and Ad-TERT/Luc. After 48 h, liver and tumor tissues were harvested and luciferase activity was measured. Data are presented as means \pm S.D. (n = 6). RLU, relative light units.

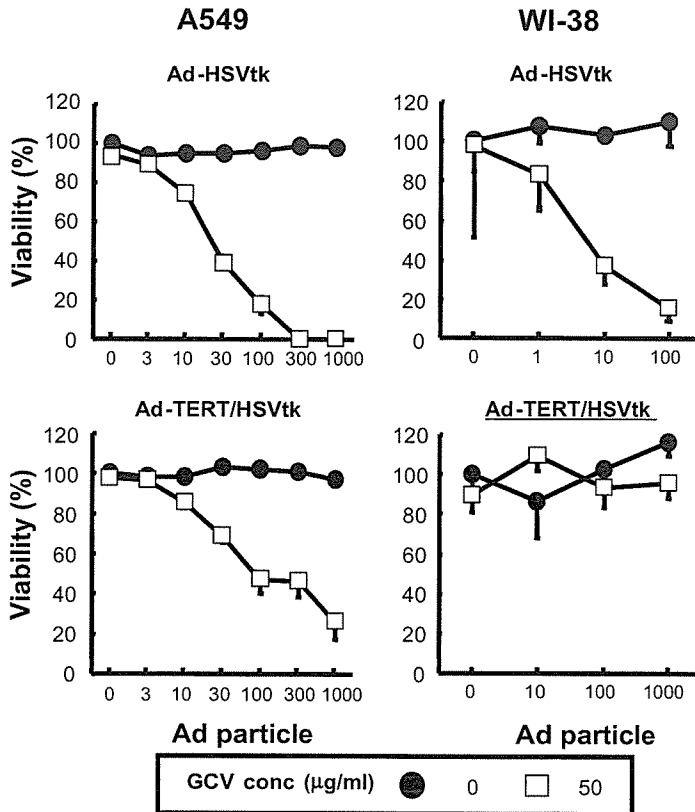


Fig. 56. Selective cytotoxicity of Ad-TERT/HSVtk in cancer cells but not in normal cells. Tumor A549 cells and normal WI38 cells were transduced with Ad-HSVtk and Ad-TERT/HSVtk at indicated virus particle. After 2h, these cells were cultured with media containing GCV. Four days later, cell viability 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 \pm S.D. (n = 6).

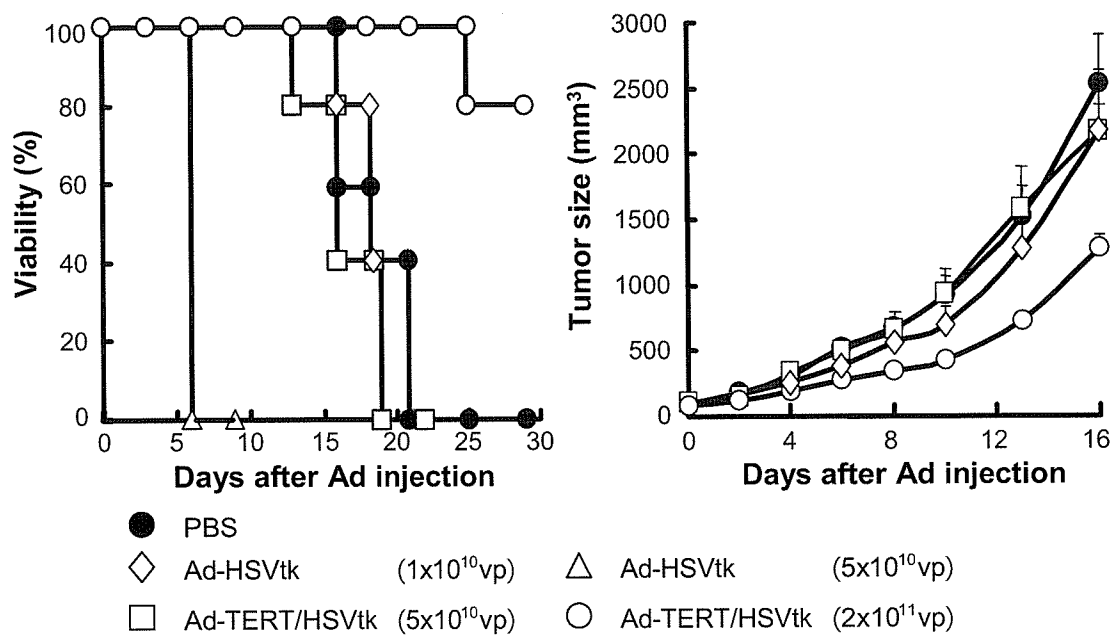


Fig. 57. Therapeutic effect in primary tumor models treated with intravenous injection of Ad-TERT/HSVtk or Ad-HSVtk. Established Meth-A primary tumors-bearing BALB/c mice were i.v. injected with PBS, Ad-HSVtk or Ad-TERT/HSVtk. Then the mice received daily injections of GCV (50 mg/kg) intraperitoneally for 10 days. Tumor size and survival were monitored. Data are presented as means \pm S.E. (n = 5). Mice were euthanized when tumor volume was greater than 4000 mm³.

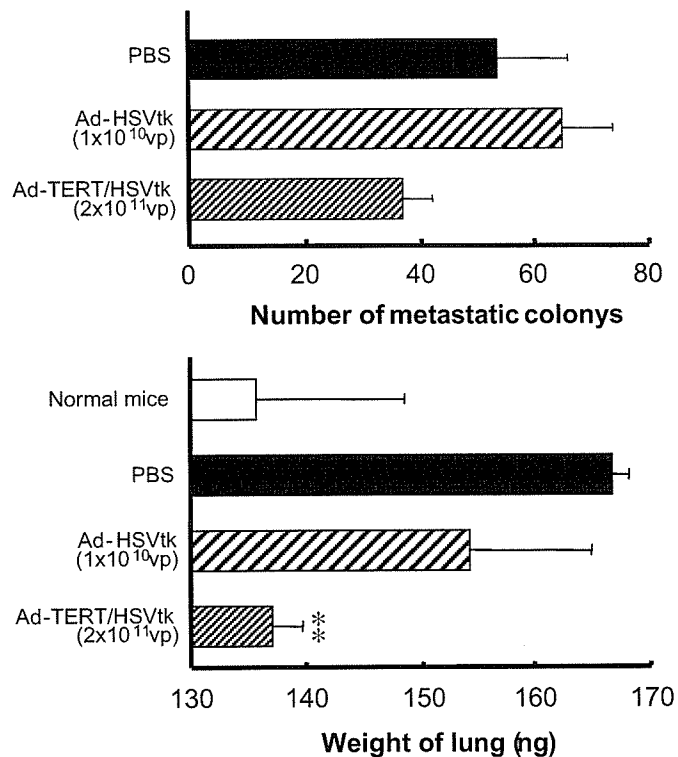


Fig. 58. Therapeutic effect in metastasis models treated with intravenous injection of Ad-TERT/HSVtk or Ad-HSVtk. Established metastasis-bearing BALB/c mice were i.v. injected with PBS, Ad-HSVtk (1×10^{10} VP) and Ad-TERT/HSVtk (2×10^{11} VP) on day 7 after CT26 inoculation. Then these mice were i.p. 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 \pm S.D. (n = 4; **, P < 0.01 compared with value for PBS).

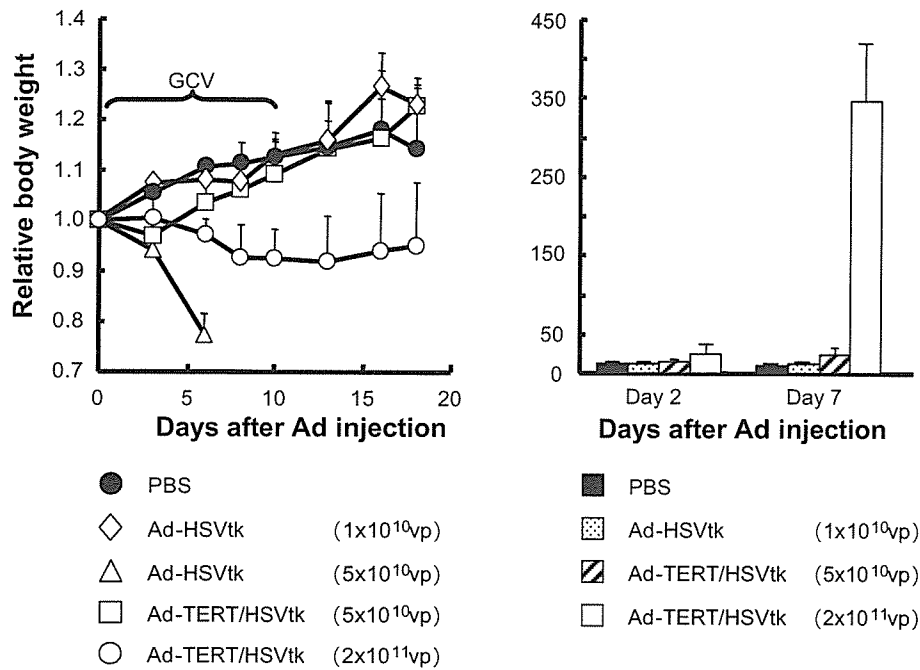


Fig. 59. Side effect of treatment via intravenous injection of Ad-TERT/HSVtk and GCV in primary tumor model. Established Meth-A primary tumors-bearing BALB/c mice were i.v. injected with PBS, Ad-HSVtk or Ad-TERT/HSVtk. Then the mice received daily injections of GCV (50 mg/kg) intraperitoneally for 10 days. Body weights and GPT activity in blood were monitored with time. Data are presented as means \pm S.D. (n = 5).

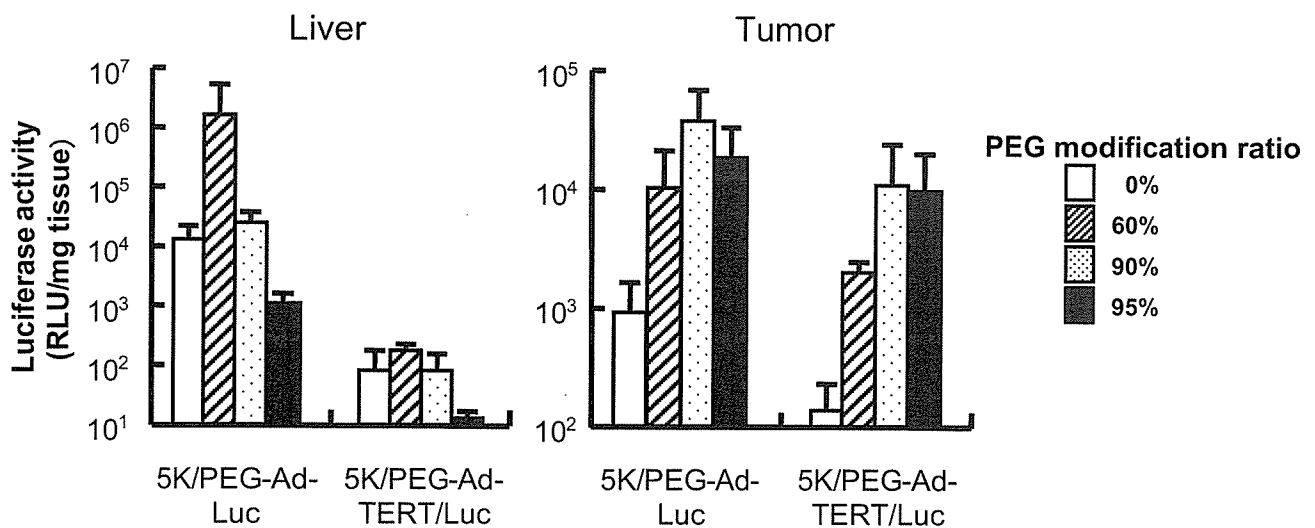


Fig. 60. The gene expression of PEG-Ad-Luc and PEG-Ad-TERT/Luc in liver and tumor. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of PEG-Ad-Luc and PEG-Ad-TERT/Luc with indicated PEG modification ratio. After 48 h, liver and tumor tissues were harvested and luciferase activity was measured. Data are presented as means \pm S.D. (n = 6). RLU, relative light units.

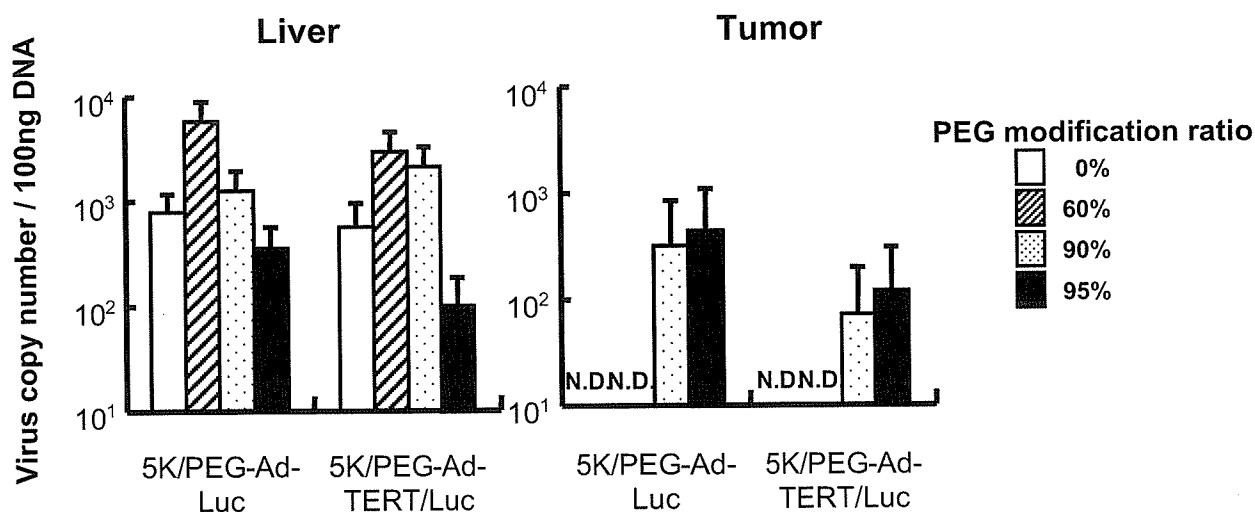


Fig. 61. Tissue distribution of PEG-Ad-Luc and PEG-Ad-TERT/Luc. Once the tumor diameter was approximately 8 mm, Meth-A tumor-bearing mice were injected intravenously with 10^{10} particles of unmodified Ad or each PEG-Ads with indicated PEG modification ratio. After 48 h, the tumor and liver tissues 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 \pm S.D. (n = 5-6).

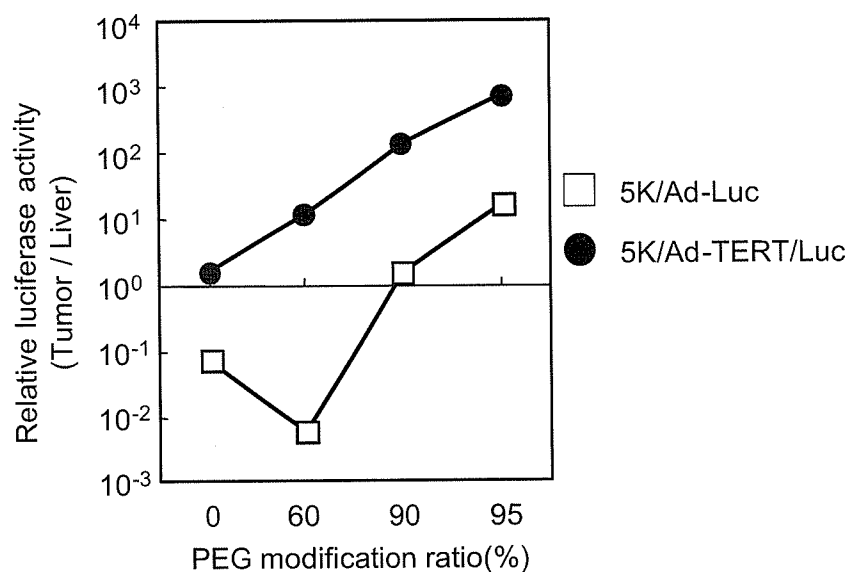


Fig. 62. Ratio of Luciferase activity (Tumor / Liver). Ratio of luciferase activity was calculated as: Luciferase activity in tumor (RLU / mg tumor) / luciferase activity in liver (RLU / mg liver) at each modification ratio.

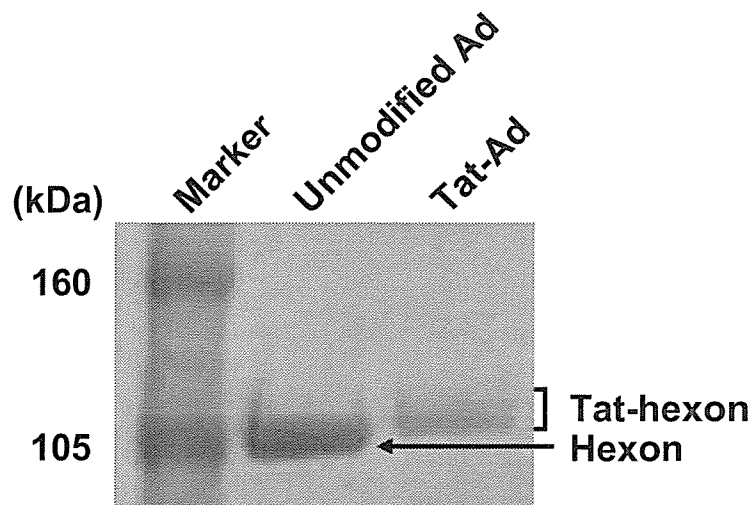


Fig. 63. SDS-PAGE analysis of Tat-Ad.

Table 5. The surface charge of Tat-Ad.

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

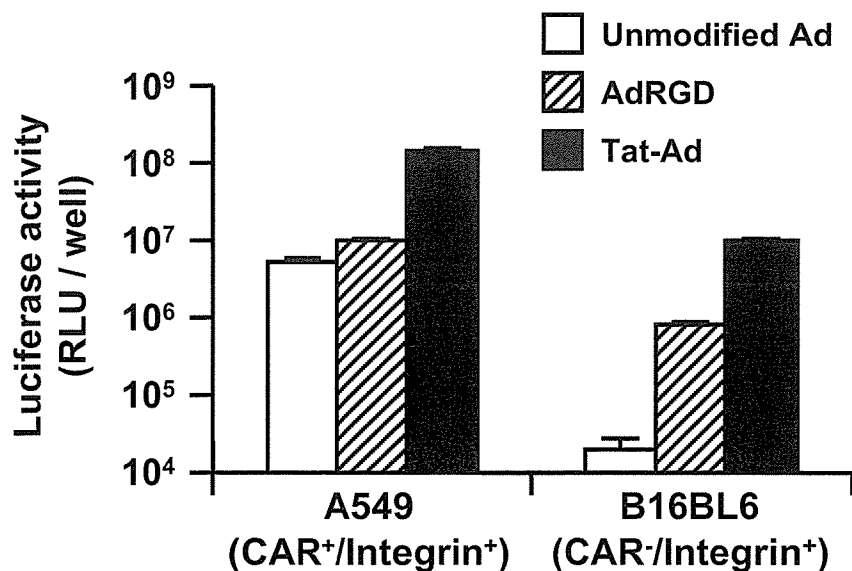


Fig. 64. Transduction efficiency of Tat-Ad in A549 and B16BL6 cells. A549 and B16BL6 cells were transduced with unmodified Ad-Luc, AdRGD-Luc, or Tat-Ad-Luc at 10000 VP/cell. After 24 h-cultivation, luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean \pm SD of results from triplicate culture.

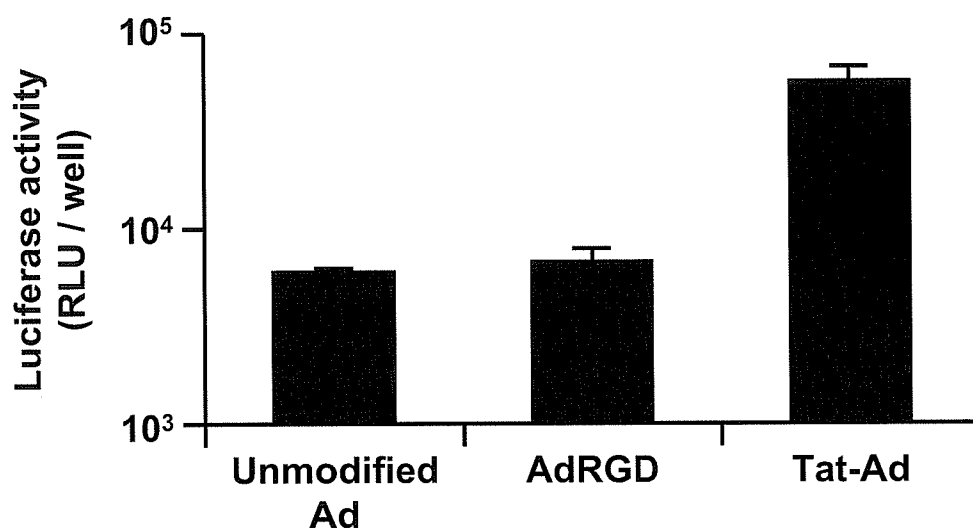


Fig. 65. Transduction efficiency of Tat-Ad in KG-1a cells. KG-1a cells were transduced with unmodified Ad-Luc, AdRGD-Luc, or Tat-Ad-Luc at 10000 VP/cell. After 24 h-cultivation, luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean \pm SD of results from triplicate culture.

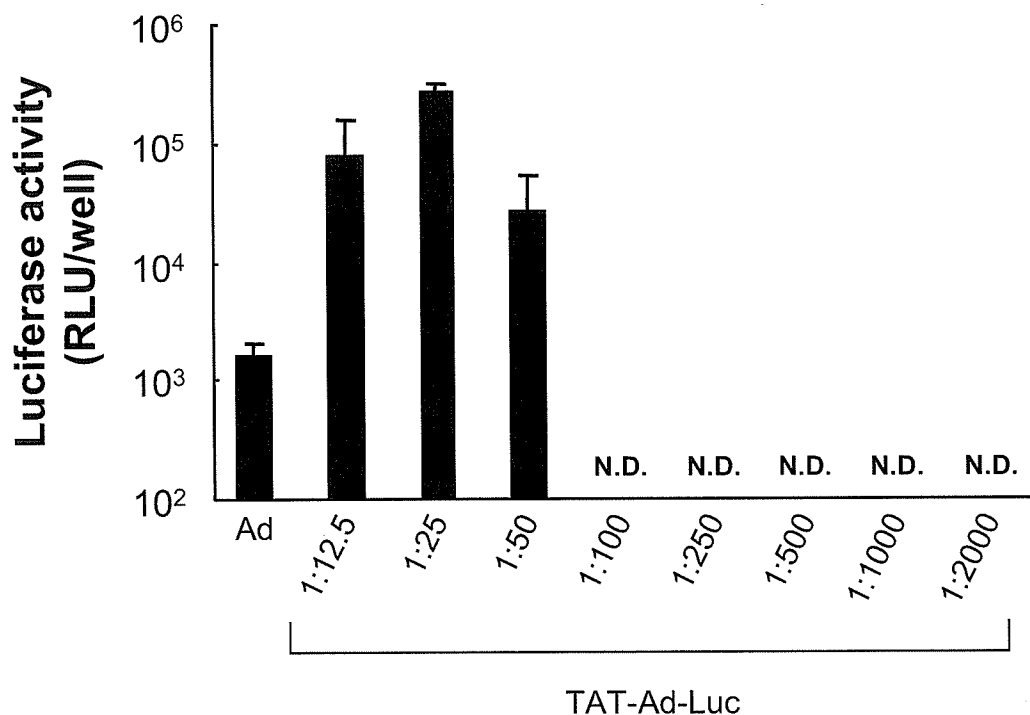


Fig. 66. Transduction efficiency of Tat-Ad with various modification ratio. B16BL6 cells (1×10^4 cells) were transduced with 10,000 particles/cell of Ad or each Tat-Ads encoding the luciferase gene. Luciferase expression was measured after 24 hr. Each bar represents the mean \pm S.D. (n = 4). (Tat-Ad, virus lysine residue:Tat peptide= 1:12.5 (mole:mole), 1:25, 1:50, 1:100, 1:250, 1:500, 1:1000, 1:2000).

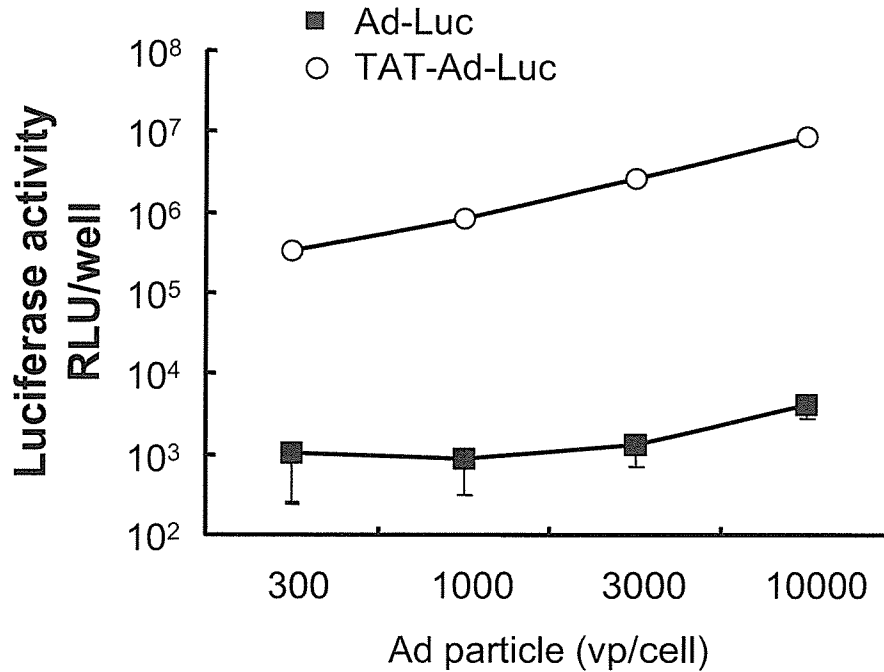


Fig. 67. Transduction efficiency of Tat-Ad into B16BL6 cells. B16BL6 cells (1×10^4 cells) were transduced with 300, 1000, 3,000 or 10,000 particles/cell of Ad/CMV-Luc or each Tat-Ad-Luc encoding the luciferase gene. Luciferase expression was measured after 24 hr. Each bar represents the mean \pm S.D. (n = 4).

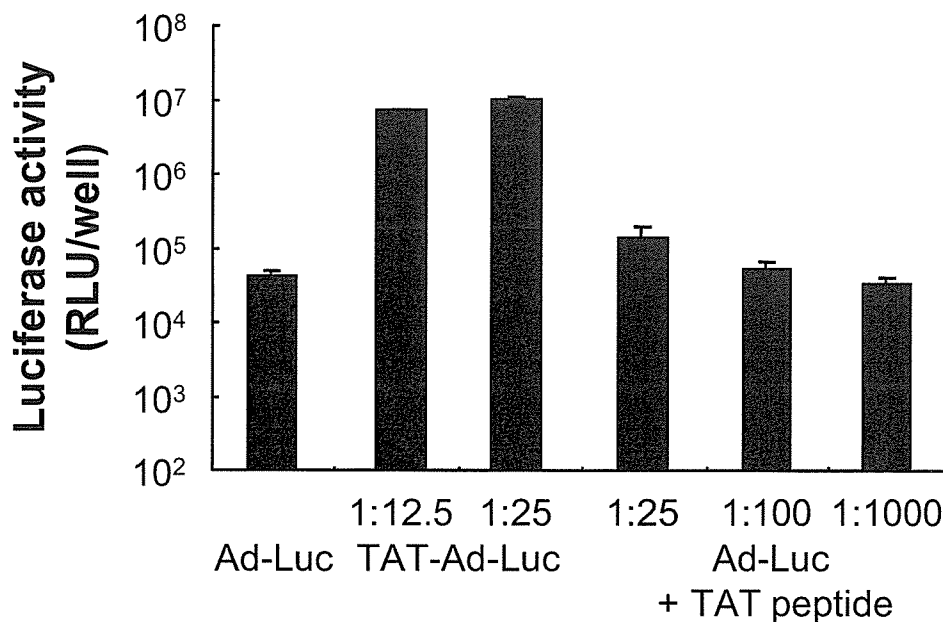


Fig. 68. Transduction efficiency of Ad, TAT-Ad or Ad with TAT peptide. B16BL6 cells (1×10^4 cells) were transduced with 10,000 particles/cell of Ad-Luc, TAT-Ad-Luc or Ad-Luc with TAT peptide. Luciferase expression was measured after 24 hr. Each point represents the mean \pm S.D. (n = 4). (TAT-Ad; virus lysine residue:TAT peptide= 1:12.5 (mole:mole), 1:25), (TAT peptide; virus lysine residue:TAT peptide= 1:25, 1:100, 1:1000).

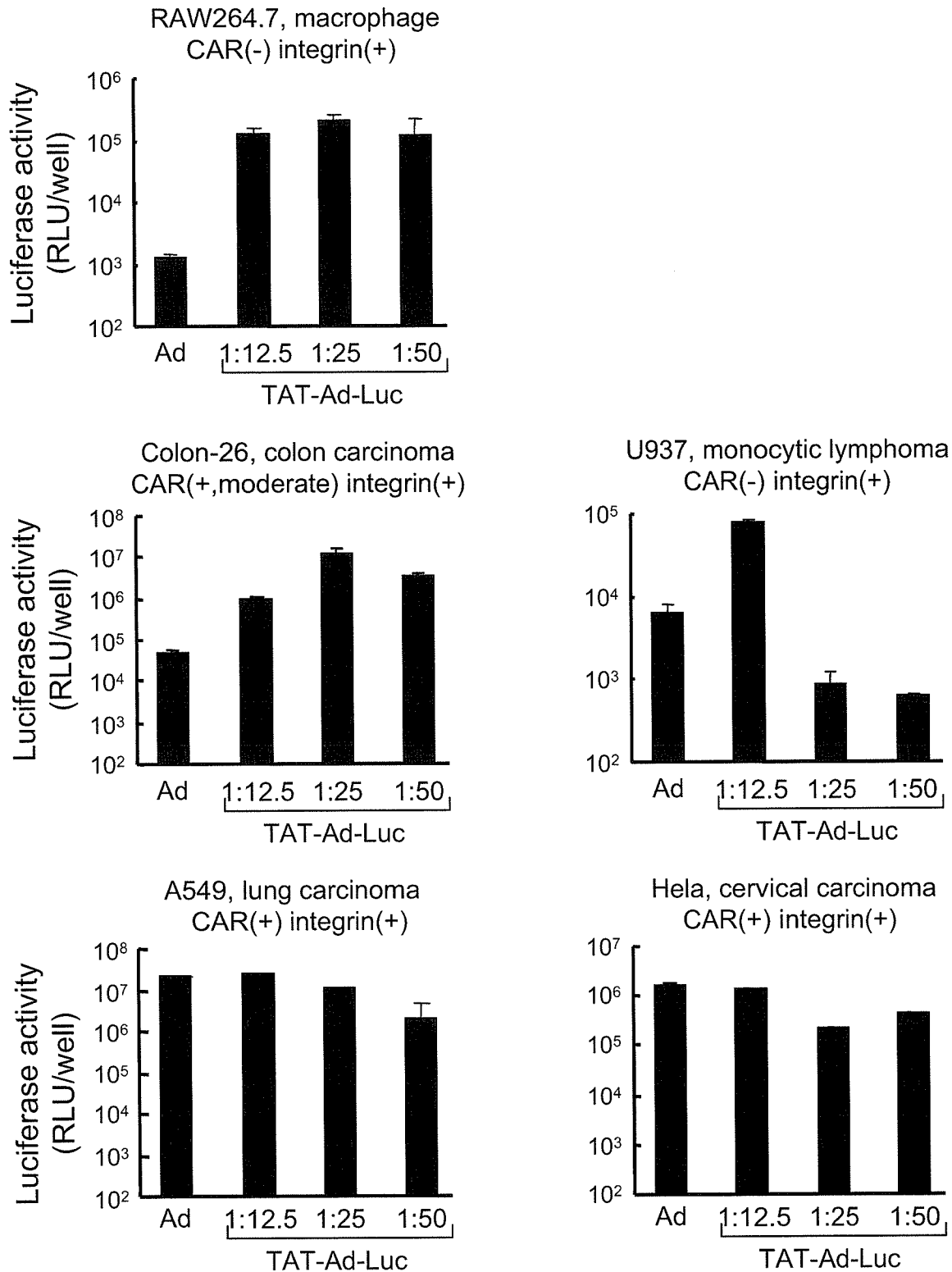


Fig. 69. Transduction efficiency of Tat-Ad into various cells. RAW264.7 cells, CT26 cells, U937 cells, A549 cells and HeLa cells (1×10^4 cells) were transduced with 10,000 particles/cell of Ad-Luc or each Tat-Ad-Luc encoding the luciferase gene. Luciferase expression was measured after 24 hr. Each bar represents the mean \pm S.D. (n = 4).

研究成果の刊行に関する一覧表

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