



9) cytotoxic T lymphocyte(CTL) class I MHC B7 T CD28 T 가 . DNA CpG dinucleotide cytosine . 5 - aza - 2 ' - deoxycytidine(ADC) DNA CpG dinucleotide cytosine 가 .<sup>10)</sup> ADC melanoma antigen gene(MAGE) melanoma G antigen gene(GAGE) CTL 가 .<sup>11-13)</sup> ADC (immunogenicity) CTL 가 .

cell scraper 가 Trizol 1/10 chloroform 가 12,000 rpm 15 RNA 1.5 mL RNA 100% isopropanol 가 - 20 16 RNA RNA - isopropanol 12,000 rpm RNA pellet ice - cold 70% ethanol 1 mL 가 RNA pellet ethanol RNA pellet DEPC - DW Spectrophotometer RNA (reverse transcriptase - polymerase chain reaction, RT - PCR) . Total RNA 70 10 RNA 5x RT buffer 2 μL, 10 mmol dATP 0.25 μL, 10 mmol dGTP 0.25 μL, 10 mmol dTTP 0.25 μL, 10 mmol dCTP 0.25 μL, MMLV reverse transcriptase(200 U/μL) 0.25 μL, RNase inhibitor(28 U/μL) 0.25 μL, 50 μmol oligo dT primer 0.5 μL, Diethyl pyrocarbonate 4 μL PCR tube RT - mixture . total RNA (1 μg/μL) 2 μL 가 mineral oil 1 10 . PCR machine(Cetus 480,Perkin Elmer, CT, USA) 42 60 1 : 1 PCR . PCR 10x PCR buffer 3 μL, 25 mmol MgCl<sub>2</sub> 1.8 μL, 10 mmol dATP 0.3 μL, 10 mmol dGTP 0.3 μL, 10 mmol dTTP 0.3 μL, 10 mmol dCTP 0.3 μL, 50 μmol sense antisense primer 0.25 μL, Taq polymerase(5 U/μL, Promega, WI, USA) 0.25 μL 25 μL PCR mixture . PCR mixture PCR tube 5 μL mineral oil 1 PCR machine(GeneAmp PCR system 2400, Perkin - Elmer, CT, USA) PCR . 94 5 가 94 30 , 57 45 , 72 45 1cycle 18~35 cycles DNA , 72 5 PCR . 1% agarose gel PCR Gel Doc 2000(Gibco-BRL, NY, USA) DNA band

Phosphate buffered saline(PBS) 3 , 1 mL Trizol

B16F10

RT-PCR primer (Bioneer, Choongbook, Korea), primer

Table 1

Flow cytometry

PBS 1 × 10<sup>7</sup> cells/mL 100 μL 95% 200 μL 가 4 1 . PBS , RNase가 12.5 μg 가 1.12% sodium citrate 250 μL 가 37 30 DNA propidium iodide (50 μg/mL) 250 μL 가 30 (FACScan ; Becton Dickinson, USA) 2% fetal bovine serum(FBS ; Hyclone, USA) (FBS - PBS) 2 FBS - PBS 가 1 × 10<sup>6</sup> cells/mL 1 mL pellet Fluorescein isothiocyanate(FITC)가 (0.5 mg/mL) 10 μL 가 4 30 FBS - PBS 1 mL 가 2 , 500 μL FBS - PBS FITC - anti - mouse CD80(B7 - 1) anti - body , FITC - anti - mouse - H - 2K<sup>b</sup> antibody purified anti - mouse - H - 2D<sup>b</sup> antibody (Phar - Mingen, San Diego, CA, USA)

Table 1. Primers used for RT-PCR

Name	Primer sequence (sense/antisense)
GM-CSF	5'-GGATGTGGCTGCAGAATTACTT-3' / 5'-TCATTTTTGGACTGGTTTTTGGCA-3'
IL-12p40	5'-ACATGTGTCCTCAGAAGCTAACCATC-3' / 5'-ATCCTAGGATCGGACCCTGCAGGG-3'
MAGE-2	5'-ATCGCTCTGCAGTGCCAGTT-3' / 5'-GTCCACCAAGTCATACACCT-3'
MAGE-5	5'-GACATTGTGGACTCAGCACC-3' / 5'-GTCCACCAAGTCATACACCT-3'
G3PDH	5'-GCCACCCAGAAGACTGTGGATGGC-3' / 5'-CATGTAGCCATGAGGTCCACCAC-3'

B16F10 whole tumor cell (B16F10 ) ADC 48 B16F10 - ADC B16F10 0.02% trypsin EDTA PBS 2 PBS 2 × 10<sup>6</sup> cells/mL 10 mL (10,000 radiation, IBL437, CIS bio international, France). PBS 1 PBS 5 × 10<sup>6</sup> cells/mL . C57BL/6 200 μL (1 × 10<sup>6</sup> cells/mouse) B16F10 PBS 200 μL tumor 10 2~3 digimatic micrometer(Mitutoyo, Japan) ADC 가 B16F10 ADC DNA B16F10 ADC가 B16F10 B16F10 ADC 0, 0.2, 1 5 μmol 가 24, 48 72 . ADC

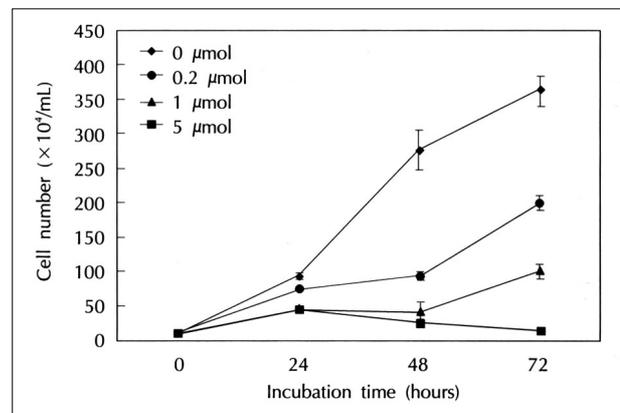
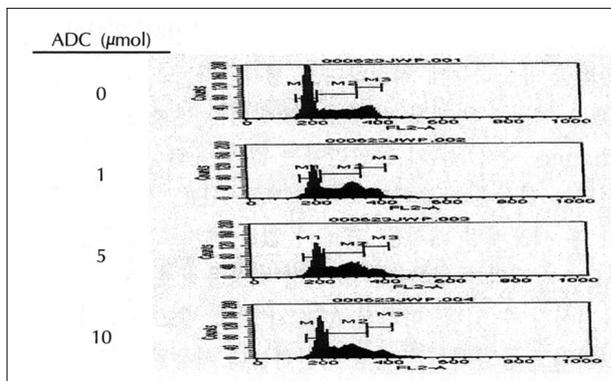


Fig. 1. Effect of ADC on the proliferation of B16F10 cell. B16F10 cells were cultured in medium containing ADC for 24, 48 and 72 hours. Cells were detached by trypsin treatment from plate, and stained with trypan blue. The number of viable cells was counted in hemocytometer.

ADC 가 B16F10  
 , ADC 가 가  
 가 (Fig. 1).  
 ADC 가 B16F10  
 , B16F10 ADC 0  $\mu$ mol,  
 1  $\mu$ mol, 5  $\mu$ mol, 10  $\mu$ mol 24  
 ADC B16F10  
 . ADC  
 S 가 가 (Fig. 2).

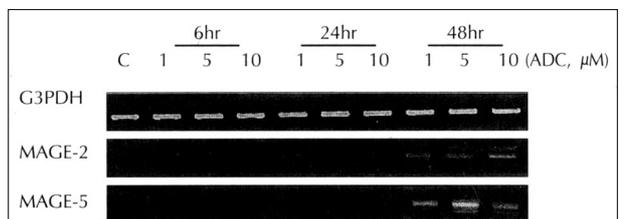
ADC 가 B16F10  
 ADC B16F10 cytotoxic T  
 MAGE - 2 MAGE - 5  
 ADC  
 . ADC  
 MAGE - 2 MAGE - 5 6  
 , 1  $\mu$ mol  
 ADC 24 MAGE - 2 MAGE - 5  
 , 48  
 (Fig. 3).

ADC 가 B16F10 MHC B7  
 ADC B16F10  
 , B16F10 1  $\mu$ mol  
 ADC 48 anti - MHC antibody anti -  
 B7 antibody MHC B7  
 가  
 ADC MHC B7

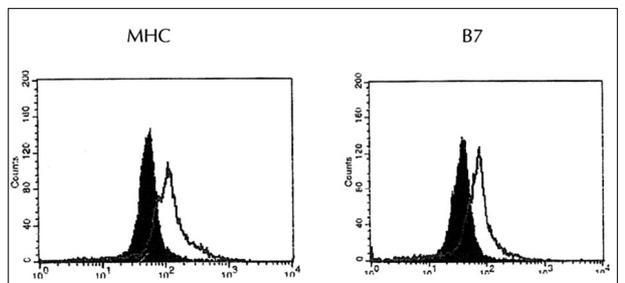


**Fig. 2.** Effect of ADC on the cell cycle of B16F10 cell. B16F10 cells were cultured in medium containing ADC for 24 hours. Cells were detached by trypsin treatment from plate, and stained with propidium iodide, and cell cycle was analyzed by FACS scan. M1, M2 and M3 represent G1, S and G2/M phase, respectively.

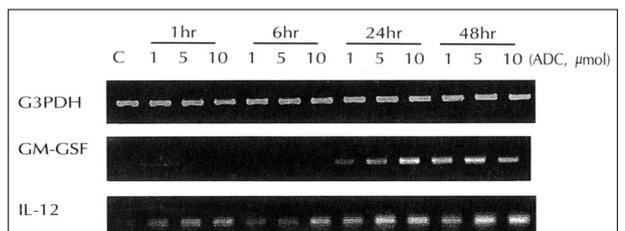
B16F10 가 가  
 (Fig. 4).  
 ADC 가 B16F10  
 가 GM - CSF  
 IL - 12 B16F10  
 total  
 ADC  
 RNA RT - PCR . GM - CSF  
 ADC 1  $\mu$ mol 24



**Fig. 3.** Effect of ADC on the MHC and B7 gene expression of B16F10 cell. B16F10 cells were cultured in medium containing 1  $\mu$ mol of ADC for 48 hours, and stained with FITC-conjugated anti-MHC or anti-B7 antibody for FACS analysis. Black color space represent control group, and white color space represent ADC-treated group. G3PDH : Glyceraldehyde-3-phosphate dehydrogenase.



**Fig. 4.** Effect of ADC on the MHC and B7 expression of B16F10 cell. B16F10 cells were cultured in medium containing 1  $\mu$ mol of ADC for 48 hours, and stained with FITC-conjugated anti-MHC (H-2k<sup>b</sup>) or anti-B7 (CD80) antibody for FACS analysis. Black color space represent control group, and white color space represent ADC-treated group.



**Fig. 5.** Effect of ADC on the GM-CSF and IL-12 gene expression of B16F10 cell. B16F10 cells were cultured in medium containing ADC for 1, 6, 24 and 48 hours, and total RNA was isolated. The gene expression of GM-CSF and IL-12 was measured by RT-PCR. G3PDH : Glyceraldehyde-3-phosphate dehydrogenase.

B16F10

GM-CSF 48 2~3 PBS, ADC B16F10  
IL-12 ADC 1 B16F10-ADC  
가 48 3~7

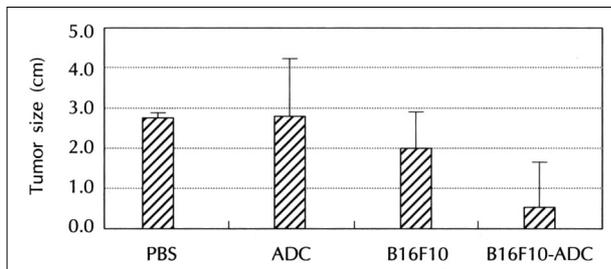
(Fig. 5).

(Table 2).

ADC가 B16F10 가  
ADC B16F10 가  
, B16F10 1 μmol  
ADC 48 MAGE 가  
MHC, B7 가 B16F10  
(B16F10-ADC)

PBS, ADC(1 μmol/mouse), B16F10  
B16F10-ADC  
, 1 B16F10  
ADC DNA 가  
MAGE GAGE NY-ESO-1  
11-13) ADC  
가  
ADC  
가  
ADC B16F10  
ADC  
ADC 가 가  
, S  
ADC 1 μmol  
가 trypan blue  
가 B16F10

B16F10 ( )가 2.6 cm ± 0.2, 2.7 cm ± 1.6, 2.0 cm ± 0.9  
B16F10-ADC 0.5 cm ± 1.1  
B16F10-ADC  
(Fig. 6).



**Fig. 6.** Effect of vaccination with B16F10-ADC on the tumor growth. Mice immunized with PBS, ADC, B16F10 or B16F10-ADC were challenged with live B16F10 cell. Twenty days after tumor challenge, the size of tumor mass of each group was measured. PBS : Phosphate buffered saline, ADC : 5-aza'-2-deoxycytidine

**Table 2.** Delay of mice death by immunization with B16F10-ADC

Day	Number of survived mice			
	PBS	ADC	B16F10	B16F10-ADC
20	5	5	5	5
25	3	4	2	5
28	0	0	2	5
31	0	0	0	3
34	0	0	0	0

PBS : Phosphate buffered saline  
ADC : 5-aza'-2-deoxycytidine

ADC DNA 가  
CTL  
MAGE GAGE NY-ESO-1  
11-13) ADC  
ADC  
ADC B16F10  
ADC  
ADC 가 가  
, S  
ADC 1 μmol  
가 trypan blue  
가 B16F10  
ADC DNA  
epigenetic change, DNA  
가  
ADC 48~72  
ADC가 ADC  
ADC가  
class I MHC  
(tumor - associated antigen) 15) CI-  
class I MHC 가 CTL  
, B7  
CTL MHC -



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