



6

- 1 Ad5 .
- 2 YKL - 1 C33A E1A E1B 19kDa .
- 3 YKL - 1 .
- 4 YKL - 1 .
- 5 YKL - 1 .
- 6 YKL - 1 .

55kDa E1B p53 가 ,

가 , 10

가 , 가 가

70% 가 , 가 (in vivo delivery) ,

가 ; ,

, p53 DNA , G1  
 DNA , , (apop  
 tosis) 가 , ( : Kirsch, D.G. et al., J. Clinical Oncology, 3158 - 3168, 1998). p53  
 50% ,  
 p53 mdm2 p53  
 가 ( : Talis, A.L. et al., J. Biol. Chem., 273:6439 - 45, 199  
 8). E1B 가  
 55kDa p53 ,  
 1996 (McCormick) p53 가 , 가  
 ( : Bischoff, J.R. et al., Science, 274:373 - 6, 1996).

36kb DNA , (ITR), (Ps  
 i), E1, E2, E3 E4 ,  
 가 , 2 , E1A E1B E1 ,  
 1) 가 DNA , 2) E1B ( E2, E3, E4) , E1B 55kDa 19kDa  
 E1A 가  
 ( : Telling, C. C. et al., J. Virol., 68:541 - 7, 1994). E1B mRNA  
 p53  
 ( : Goodrum, F.D. et al., Virology, 237:404 - 13, 1997). E1B 55kDa  
 (E1B - attenuated adenovirus: ONXY - 015) p53  
 E1A p53  
 가 , p53  
 (cisplatin) , p53 가  
 ( : Heise, C. et al., Nat. Med., 3:639 - 45, 1997). ,  
 phase - I/II ( : McCormic  
 k, F., The American Society of Gene Therapy(1<sup>st</sup> Annual Meeting), 1998).

가  
 , 가  
 가  
 E1B 55kDa 가 가 ,  
 p53 가 가 ,

, 가 , p53  
가

E1B 가 55kDa  
E1B 55kDa

; , 가

, 가

E1B 55kDa 가  
E1B 55kDa p53 가

Ad5

E1B 55 kDa  
(PCR) , 가 가 E1

E1B 55kDa E1A E1B 19kDa

PCR . PCR

pCA14(Microbix, Canada)

(shuttle vector)

, E1 E3

pTG - CMV(Microbix, Canada)

(E

schierichia coli) BJ5183 (cotransformation)

DNA , E1B 55kDa

가 E1

가 293 (transfection)

'YKL - 1' , 1999 7 1  
(KFCC) 'KFCC - 11099'

E1B 55kDa

가 DNA , PCR

(Western blotting) , E1B 19kDa

가

E1B 55kDa

Ad 5

MOI (Multiplicity of Infection)

0.1 10 37 8 10

가

C33A

5 6

7x10<sup>8</sup>

5x5mm<sup>2</sup>

3x10<sup>8</sup>

1: YKL - 1

1 - 1: YKL - 1

Ad5 (1) E1 E1B 55kDa  
E1A E1B 19kDa  
robix, Canada) DNA PCR . , pXC1(Mic

5' 5' - GCCCTCGAGATGAGACATATTATCTGCCACG - 3' (1) , 3'  
5' - TCTTGGATCCAGATCTATACAGTTAAGC - CACCTATACAAC - 3' (2) ,  
BamHI , 3' E1B 55kDa  
E1B 55kDa . PCR DNA 10ng  
100ng , 94 2 , 94 1 , 55 1 72 1  
35 , 72 10 (extension) .

PCR 2kb DNA , E1A , E1B  
E1B 19kDa E1B 55kDa  
가 , E1B 19kDa ( 3)  
PCR pCA14(Microbix, Canada) BglIII  
'pCA14 - E1A/E1B 19kDa'  
XmnI 가 , E1 E3  
pTG - CMV(Microbix, Canada) ClaI , BJ5183  
DNA ,  
HindIII 가 E1B 55kDa 가  
'pYKL - 1' pYKL - 1 PacI 가 , 293  
(transfection) , 'YKL - 1' , 1999 7 1  
(KFCC) 'KFCC - 11099'

(Microbix, Canada) , 가 , pSpE1SP1A/ - gal pXC1  
'ad - E1/ - gal' 'ad - XJ'

1 - 2: DNA PCR

DNA , YKL - 1 E1B 55kDa (KFCC - 11099) C33A MOI(Multiplicity of Infection) 10  
(Qiagen Genomic Isolation Kit, Qiagen, U.S.A.)  
DNA , E1A 5' E1B  
55kDa 3' PCR  
YKL - 1 E1B 55 kDa 가 YKL - 1 E1B 55 kDa  
가

1 - 3: DNA

YKL - 1(KFCC - 11099) , E1A E1B  
19 kDa  
(Western blotting) C33A YKL - 1 MOI 10 , 2  
(lysis buffer, 50mM HEPES, 0.15M NaCl, 0.5% NP - 40, protease inhibitor: P  
MSF, TLCK, TPCK) , SDS - PAGE . PVDF (membrane)  
E1A E1A(sc - 430), E1B 19kDa  
E1B 19kDa(DP17) - actin , HRP(Horse Radian Peroxi  
dase) 2 , ECL(Enhanced Chemi - Luminescence:RPN2108, Amersha  
m) X - ( : 2).  
2 , 1 E1A E1B 19kDa 293 ; 2 C33A; 3  
YKL - 1 C33A  
YKL - 1 C33A E1A E1B 19kDa

2: YKL - 1

2 - 1: YKL - 1

## YKL - 1

ATCC(American Type Culture Collection) FHs 738Lu, FHs 173We, SK - Hep1, HepG2, Hep3B, C33A, Hela H460, YKL - 1, ad - XJ ad -  $\Delta$  E1/ - gal MOI 10, 1 0.1 , 8 10 , 1% (crystal violet, 50% ) , 173We 738Lu YKL - 1, ad - XJ ad -  $\Delta$  E1/ - gal , MOI가 10, 1, 0.1 , YKL - 1 ad - XJ 1/1000 . YKL - 1 . 3 , 1 ad -  $\Delta$  E1/ - g al ; 2 YKL - 1 ; , 3 ad - XJ .

YKL - 1 C33A, Hep - 3B, SK - Hep1, HepG2, Hela H4 60 . YKL - 1, ad - XJ ad -  $\Delta$  E1/ - ga l , MOI가 10, 1, 0.1 , YKL - 1 , p53 가 C33A Hep - 3B ( : 4). 4 , 1 ad -  $\Delta$  E1/ - gal ; 2 YKL - 1 ; , 3 ad - XJ .

YKL - 1 p53 가

## 2 - 2: YKL - 1

YKL - 1 , p53 가  $1 \times 10^7$  C33A 5 6 ,  $5 \times 5 \text{mm}^2$  ,  $5 \times 10^8 / 50 \mu\ell$  YKL - 1 YKL - 1 , YKL - 1 ( : 5). 5 1.0 , ( ) YKL - 1 , ( ) YKL - 1 .

ematoxylin/eosin) / (h , YKL - 1 가 YKL - 1 , (b) YK ( : 6). 6 , (a) , 40 , 200 , (b) YKL - 1 가 , YKL - 1 (immunohistochemistry) , YKL - 1 , E1B 55kDa 가 YKL - 1 가 .

가 , , 가 , .

Figure 1. p53 and E1B expression in nasopharyngeal carcinoma cell lines. The expression of p53 and E1B in nasopharyngeal carcinoma cell lines was detected by Western blotting. The results showed that p53 and E1B were expressed in all cell lines. The molecular weight of p53 was 55kDa. The molecular weight of E1B was 17kDa.

(57)

1.

2.

가 (Adenovirus) Ad 5 E1B p53 55kDa  
(Adenovirus) YKL - 1 (KFCC - 11099).

3.

2 YKL - 1 (KFCC - 11099)

4.

5.

3 ,

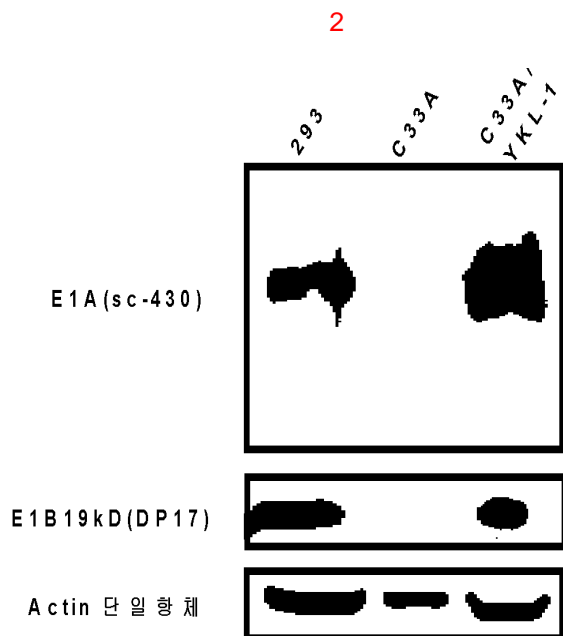
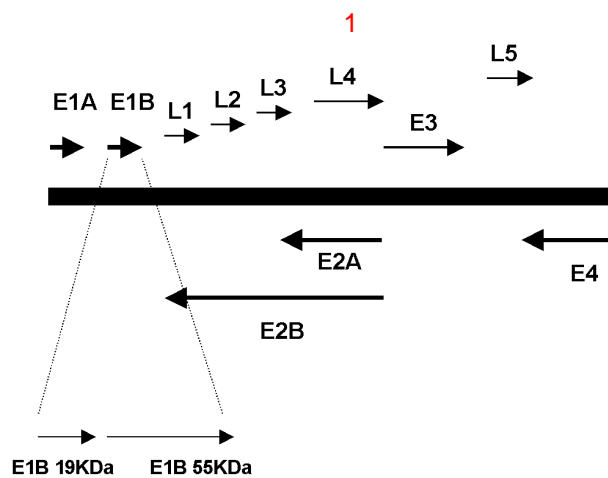
5x5mm<sup>2</sup> 5x10<sup>8</sup> YKL - 1 (KFCC - 11099) MOI 0.1 10

6.

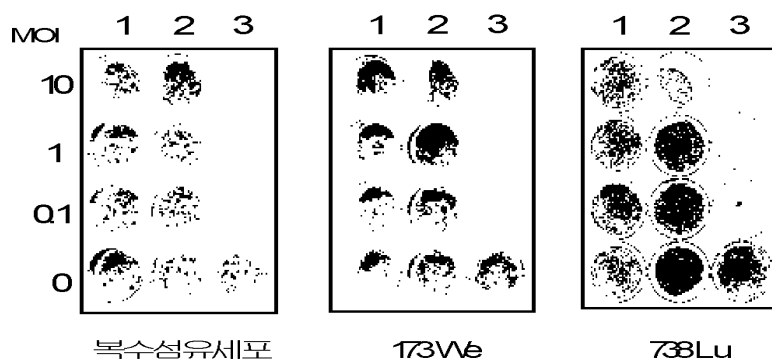
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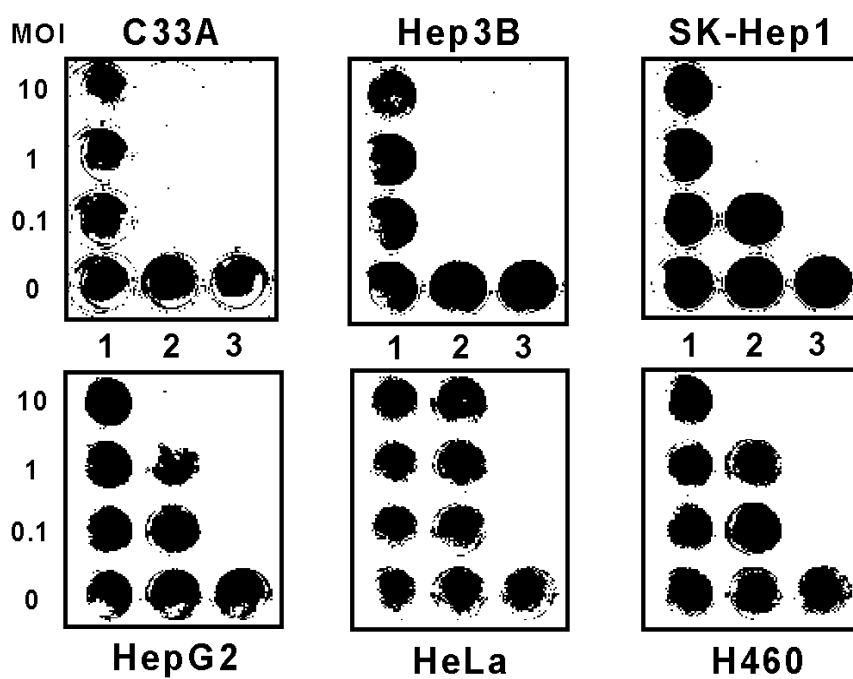
37 , 8 10



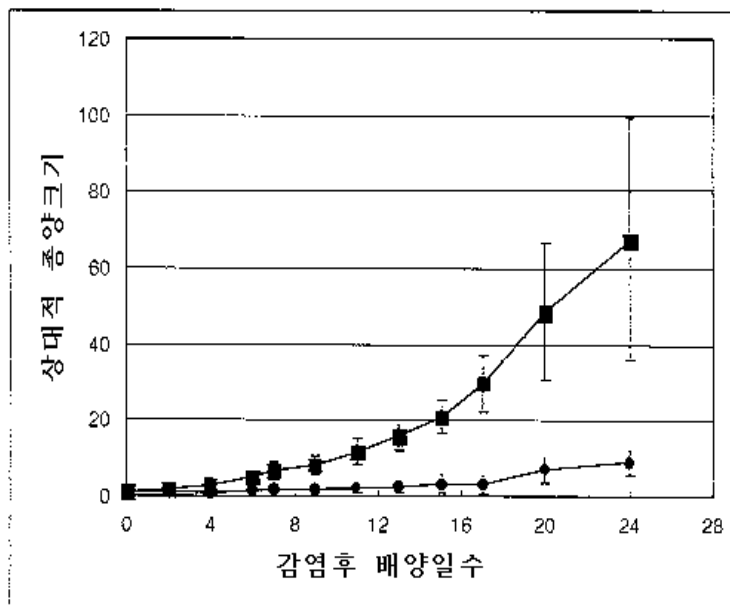
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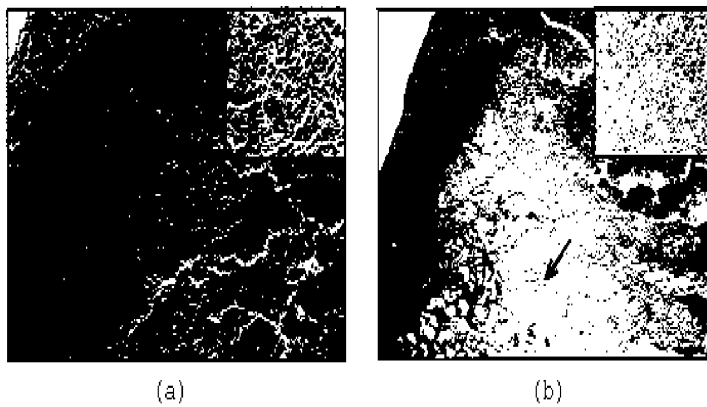
4



5



6



<110> YONSEI UNIVERSITY <120> Tumor-Specific Recombinant Adenovirus and Method  
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3

1 <210> 2 <211> 40 <212> DNA <213> Artificial Sequence <220>  
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cctataca

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ac                               40 <210>      3 <211>      1711 <212>      DNA <213>      Aden
ovirus type 5 <400>      3 atgagacata ttatctgcc a cgaggtgtt attaccgaag aaatggccgc
cagtcttttg      60
gaccagctga tcgaagaggt actggctgat aatcttcac ctcctagcca ttttgaacca      120 cctacccttc
acgaactgta tgatttaga
c gtgacggccc ccgaagatcc caacgaggag      180 gcggtttcgc agatttttcc cgactctgta atgttggcgg
tgaggaagg gattg
actta      240 ctacttttc cgccggcgcc cggttctccg gagccgcctc acctttcccg gcagcccgag
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tcattatcac      480 c
ggaggaata cgggggaccc agatattatg tgttcgcttt gctatatgag gacctgtggc      540 atgtttgtct
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1711

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