

Herpes simplex virus-thymidine kinase 가 Gancyclovir

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¹,¹,¹,¹,²,²,²

Antitumor Effect of Carcinoma cells Transduced with Herpes simplex virus-thymidine kinase by Gancyclovir and Radiation

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= Abstract =

Background: Many types of cancer become resistant to current chemotherapeutic and radiotherapeutic intervention. To overcome this situation application of gene therapy by the introduction of suicide genes followed by their prodrugs may be promising. A viral enzyme, Herpes simplex thymidine kinase (HSV-tk), which converts gancyclovir from an inactive prodrug to a cytotoxic agent by phosphorylation, are being actively investigated for use in gene therapy for cancer. The purpose of this study was to determine whether combining prodrug-activating gene therapy and irradiation might result in enhanced antitumor effects. **Methods:** The HSV-tk gene was cloned into the retroviral vector, pLXSN and established the clones producing retroviruses carrying the HSV-tk gene. The carcinoma cell line, HCT116 and Huh-7 were transduced with high-titer recombinant retroviruses. These cell lines were treated with gancyclovir before or after irradiation for the defining combinational effect of suicide gene therapy and radiotherapy. **Results:** The titers of cloned PA317 amphotropic retroviruses ranged from 4 to 6 X 10⁶ CFU/ml. After selectional periods, the expression of HSV-tk was confirmed by reverse-transcriptase polymerase chain reaction (RT-PCR). The growth of cells expressing HSV-tk was inhibited as increase of GCV dose after 48 hr and the growth inhibitory effect of GCV was much higher after 72 hr. When the cells transduced with HSV-tk gene were exposed to radiation, the growth inhibitory effect of GCV was significantly increased, as compared with non-transduced parental cells. **Conclusions:** The results suggest that the addition of HSV-tk gene therapy to standard radiation therapy may improve the effectiveness of treatment for solid tumors.

Key Words: HSV-tk, gancyclovir, suicide gene therapy, radiotherapy.

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, GCV bystander (15,16).
(suicide gene) rat gliosarcima HSV-tk 가
(gene therapy)가 (17).
HSV-tk
가 가
가 retrovirus , HSV-tk
가
p53 tumor suppressor gene (1),
drug-sensitive gene (2),
Multidrug resistance (MDR) gene (3)
antisense RNA antisense
DNA (4).
1. HSV - tk
retroviral vector
(pro-drugs) (5).
HSV-tk HSV-1 strain CL/01 5'
primer GCCGCATCTGGTGGCGTGAAACT, 3' primer
CCGTGTTTCAGTTAGCCTC PCR
1%
agarose gel , gel extraction
kit (QIAGEN, Hilden, Germany) HSV-tk
pBluescript SK(+)
vector Bam HI
neomycin (Neo^R)
HSV-tk retroviral vector
pLXRN retroviral
vector (Clontech, Palo Alto, USA) pLTRN
(Fig. 1). retroviral vector
DNA-CaPO₄ transfection (Gibco BRL, Rockville, USA)
NIH-3T3 5 μ
g/mL Gancyclovir (GCV: Roche, Mannheim, Germany), 72 trypan blue (Sigma, St. Louis, USA)
HSV-tk
2.
T HSV-tk (12-14),
glioblastoma HSV-tk , HCT116 (ATCC CCL-247)

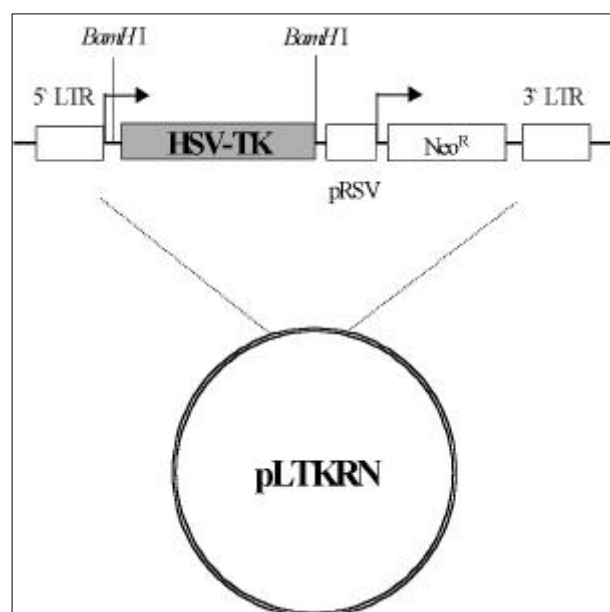


Fig. 1. Map of the recombinant retrovirus vector, pLTKRN. The direction of transcription is indicated by an arrow. Restriction endonuclease sites containing the *Bam*HI cloning site are shown and cloned with HSV-tk gene.

2 mM L-glutamine 10% (Fetal bovine serum; FBS: Gibco BRL, Rockville, USA)
RPMI-1640 (Gibco BRL, Rockville, USA)
5% CO₂/37°C, mouse
fibroblast NIH-3T3, Huh-7,
ecotropic retrovirus, GP&E86
amphotropic retrovirus, PA317 2 mM
L-glutamine 10% DMEM
(Gibco BRL, Rockville, USA).

3. HSV - tk retrovirus virus 가

pLTKRN retroviral vector
DNA-CaPO₄ transfection 60 mm
5 x 10⁵ 16~18 ecotropic
retrovirus GP&E86, 10%
DMEM 1 mg/mL
neomycin (G418) (Invitrogen, Carlsbad, USA)
가, G418 가
60 mm
3 x 10⁵ 16~18 amphotro-
pic retrovirus PA317 8 µg/mL

polybrene (Sigma, St. Louis, USA) 가

retrovirus G418
96-well 1 cell/well

retrovirus
0.45 µm -70
Virus 가 60 mm
2 x 10⁵ NIH-3T3 10
retrovirus 8 µ
g/mL polybrene 4 10%
DMEM 48
NIH-3T3 20:1 split
1 mg/mL neomycin (G418) 가
neomycin colony
, 5 µg/mL GCV
72 HSV-tk

4. HSV - tk

가 retrovirus
HSV-tk 60 mm 4 x
10⁵ 16 ~ 18
8 µg/ml polybrene MOI 10
retrovirus 가 4 10%
DMEM
48 1 mg/mL
neomycin (G418) 가 DMEM 10
HSV-tk

HSV-tk
retrovirus 가
RNA 1st Strand cDNA Synthesis
Kit (Roche, Mannheim, Germany)
(10X reaction buffer 2 µL, 25mM MgCl₂ 4 µL,
Deoxynucleotide Mix 2 µL, RNase inhibitor 1 µL,
Oligo-p(dT)₁₅ primer 2 µL, AMV Reverse Transcriptase
0.8 µL, D.W 1.2 µL, mRNA 7 µL) 25 10, 42
60, 99 5, 4 5 cDNA
HSV-tk primer set
(Polymerase Chain Reaction)
1.5% agarose

5.

¹³⁷Cs

HSV-tk 가

GCV

HSV-tk 가

HSV-tk 가

96-well 1 x 10⁴/well

GCV cell

proliferation kit (XTT: Roche, Mannheim, Germany)

3

1. HSV - tk 가

retrovirus

pLXRN retroviral 2.8 kb HSV-tk

HSV-tk BamHI

BamHI

(Fig. 1).

2

plasmid

BamHI HSV-tk

retroviral vector 2.8 kb HSV-tk

band

retroviral vector NIH-3T3

DNA-CaPO₄ transfection

retroviral vector 가

PA317 retrovirus

retrovirus

NIH-3T3 Neo^R- colony

가 4 6 x 10⁶ CFU/mL

(Table 1), 5 µg/mL GCV 72

HSV-tk

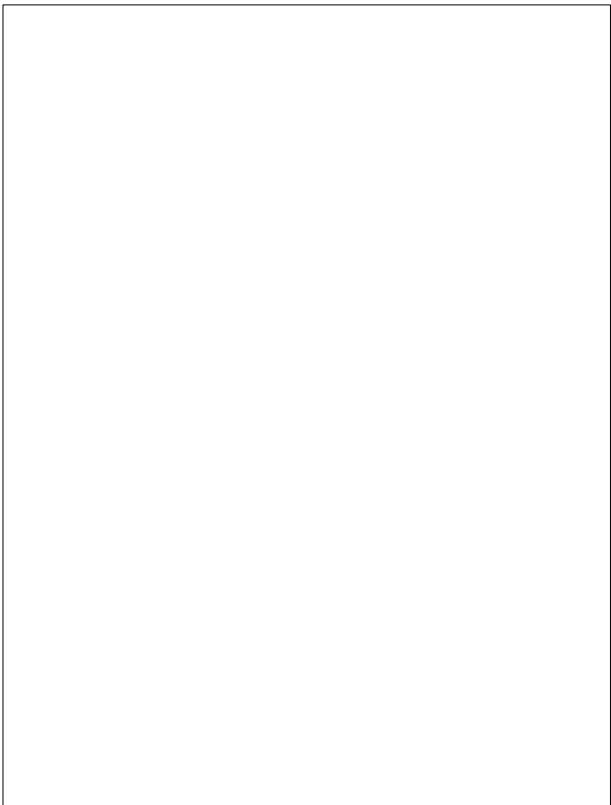


Fig. 2. Electrophoresis of pLTKRN was digested with *Bam*HI. Large DNA band of lane is 6.7 kb of pLXRN vector and small DNA band of lane is 2.8 kb of HSV-tk gene.

Table 1. Titrations of PA317 clones producing amphotropic recombinant retroviruses

Clone	No. of G418R colony*	Titer per milliliter†
1	115	5.75 X 10 ⁶
2	103	5.15 X 10 ⁶
3	128	6.40 X 10 ⁶
4	109	5.45 X 10 ⁶

* : 1:20 split in 100 mm plate 2 days after transduction.

† : Viral titer was determined as the average number of drug-resistance colony cells multiplied by a factor to account for magnification, plate size, and dilution of the infectious stock. In determining the G418-resistance titer, the number of colonies was divided by 4 to account for two cell doublings.

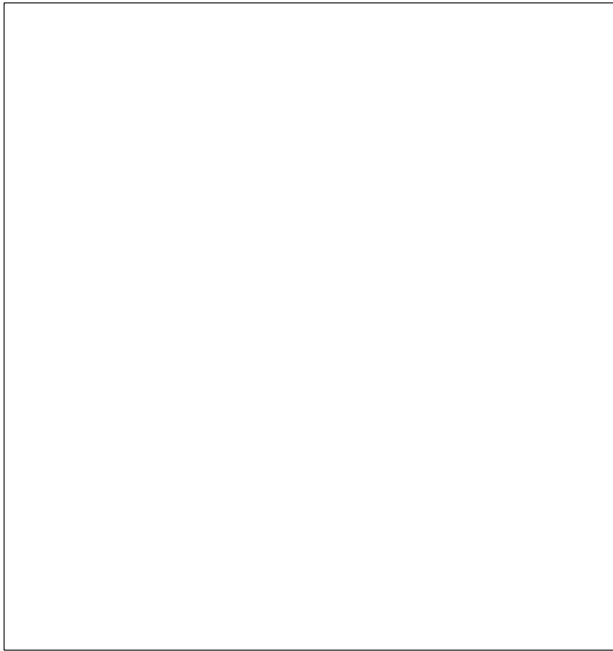


Fig. 3. Expression of HSV-tk gene in HCT/TK and Huh-7/TK. RT-PCR revealed HSV-tk specific 1.4 kb sized mRNA bands.

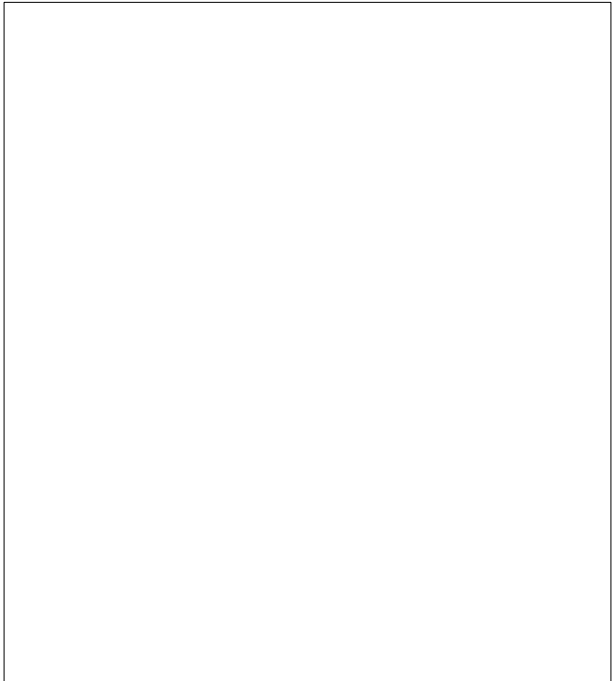


Fig. 4. Effects of Gancyclovir on the growth of human coloectal carcinoma, HCT116 (A) and HSV-tk-transduced HCT116 (HCT/TK; B). The cells were seeded at 1×10^4 cells/well and treated with 0 μg (\blacklozenge), 5 μg (\blacksquare), 10 μg (\blacktriangle), 20 μg (\bullet), and 40 μg (\ast) of GCV.

2.

HSV - tk

가

retrovirus

HCT116

Huh-7

HSV-tk

RNA

HSV-tk

primer

PCR

HSV-tk

가

HSV-tk

retrovirus

1.4 kb

HSV-tk

mRNA

(Fig. 3).

3. HSV - tk

GCV

(HCT116)

HSV-tk

가

(HCT/TK)

GCV

36

HSV-tk

가

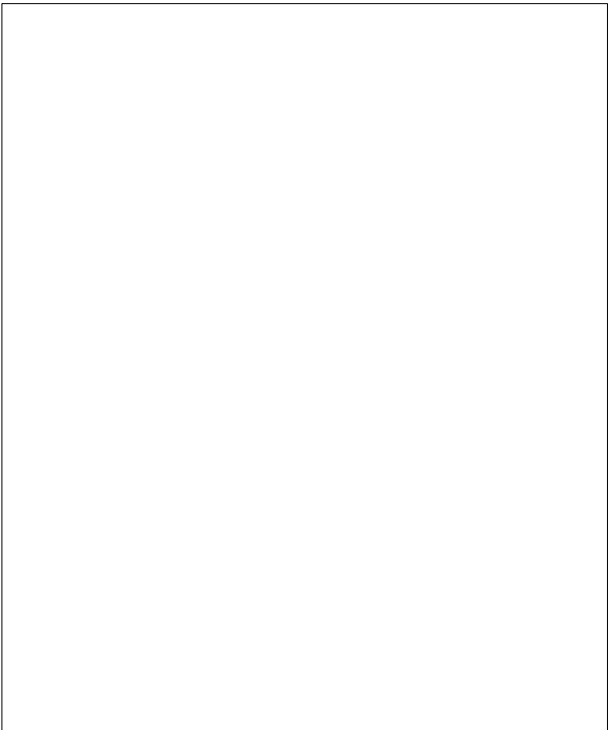


Fig. 5. Effects of Gancyclovir on the growth of human coloectal carcinoma, HCY116 (A) and human hepatocellula carcinoma, Huh-7 (B). Mock-infected (\blacklozenge) and HSV-tk-transduced cells (\blacksquare) were seeded at 1×10^4 cells/well and treated with indicated GCV for 72hr.

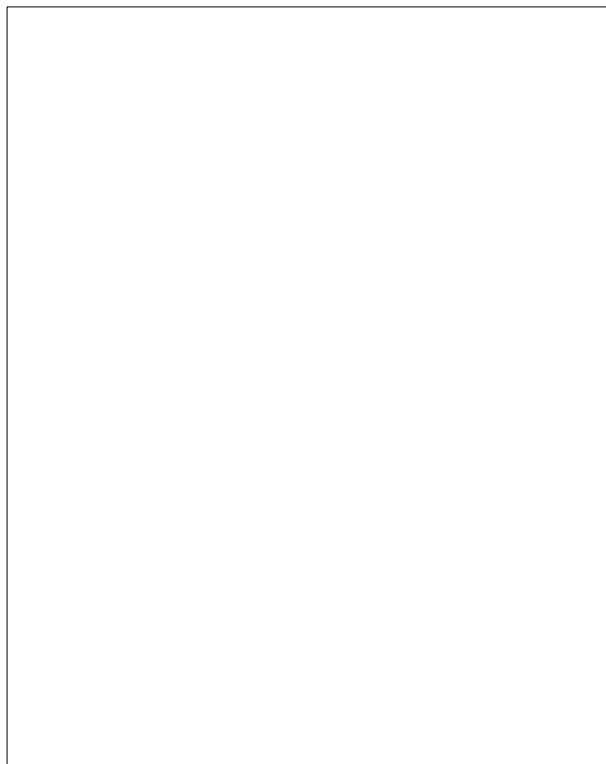


Fig. 6. Effects of irradiation on the growth of human colorectal carcinoma, HCT116 (A) and human hepatocellular carcinoma, Huh-7 (B). Mock-infected (◆) and HSV-tk-transduced cells (■) were irradiated with ^{137}Cs source as indicated dose and seeded at 1×10^4 cells/well.

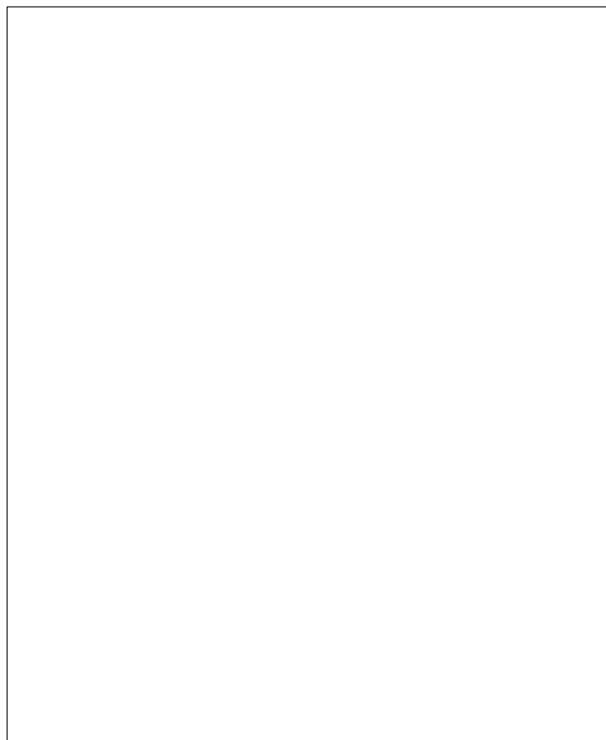


Fig. 7. Effects of Gancyclovir on radiation response of human colorectal carcinoma, HCT116 (A) and human hepatocellular carcinoma, Huh-7 (B). Mock-infected (◆) and HSV-tk-transduced cells (■) were irradiated (20 Gy) and seeded at 1×10^4 cells/well and treated with indicated GCV.

48 GCV 가
가 , 72 HSV-tk
가 . HSV-
tk 가 GCV 40 μg
GCV
(Fig. 4). GCV
HCT116 Huh-7
HSV-tk GCV
(0.75 μg) GCV (40 μg) GCV
72
HSV-tk 가
GCV
HSV-tk 가 1.25 μg
GCV ,
GCV 가 가 가
(Fig. 5).

4. GCV
72
, 20 Gy 가
(Fig. 6).
HSV-tk
20 Gy
GCV GCV
. HSV-tk 가
GCV 가
HCT116 5 μg GCV ,
GCV HSV-tk GCV 20 μg
5 μg GCV , Huh-7

Herpes simplex virus-thymidine kinase	가	Gancyclovir
가	(Fig. 7).	junction metabolic cooperation (19), HSV-tk/GCV apoptosis endocytosis (20), (21).
Retroviral vector		GCV 72
genomic DNA		HSV-tk/GCV 가
. Retrovirus 가		GCV가 HSV-tk
vector, , retrovirus virus		GCV
가 . long		. 40 μ g GCV
terminal repeat	가	GCV
rous sarcoma virus promoter neomycin		HSV-tk/GCV 가
phosphotransferase	가	(Fig. 5).
pLXRN retroviral vector		rat gliosarcima
. polyclonal retrovirus		HSV-tk
	가	가 가 (17)
가 retrovirus		HSV-tk/GCV
limited dilution ~		.
10 ⁶ CFU/mL 가 retrovirus		80 Gy
(Table). Culver (18) mouse brain		GCV
HSV-tk retrovirus		
	retrovirus	가
GCV 가		.
가 retrovirus HSV-tk/GCV		
가		
HCT116		
Huh-7	HSV-tk/GCV	
,	HSV-tk 가	
	가	
retrovirus 10		
HSV-tk		
,	PCR HSV-tk	
(Fig. 3).	HSV-tk GCV	
	apoptosis	
	,	
가		
가		
'bystander , 가		
(5). bystander gap		

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Antitumor Effect with HSV-Tk and Radiation