

博 士 學 位 論 文

Cisplatin 내성 난소암 세포주로부터 2차
내성 아세포주 수립과 성격규명



濟州大學校 大學院
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박 사 학 위 논 문

Cisplatin 내성 난소암 세포주로부터 2차
내성 아세포주 수립과 성격규명



제주대학교 대학원
의 학 과

안 춘 산

2006년 6월

Cisplatin 내성 난소암 세포주로부터 2차 내성
아세포주 수립과 성격규명

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안 춘 산

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2006년 6월

Establishment and characterization of Secondary
drug- resistance cell line originating
from cisplatin resistant SNU-8

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(Supervised by Professor Sung-Yob Kim, M. D., Ph. D)

A Dissertation Submitted in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY
in Medicine

Date Approved:



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June, 2006

ABSTRACT

Resistant and recurrent ovarian carcinoma patients who had the first chemotherapy with cisplatin have showed low reactivity to the second chemotherapy and high recurrence. The aims of our study are to establish secondary chemotherapy drug-resistant cell line (SNU-8/Fac, SNU-8/Pac). In addition, we tried to investigate mechanism of multi-drug resistance (MDR), and to analyze drug sensitivity in 5-Fluorouracil (5-FU)-resistant cell line (SNU-8/Fac) when we use a MDR1 suppresser Cyclophosphorine A in the present study. Drug sensitivity was measured by MTT assay and multi-drug resistance was analyzed by RT-PCR and western blotting analysis.

In this study, we established secondary drug-resistant cell line SNU-8/Fac and SNU-8/Pac, which demonstrated multi-drug resistant by anti-cancer drug sensitivity test. We investigated the expression of resistance-related genes in SNU-8/Fac and SNU-8/Pac. We found that *MDR1* and *ATP7B* were over expressed in both protein and mRNA levels in SNU-8/Fac and SNU-8/Pac cells, but expression of *MRP1* and *BCRP* was somewhat decreased in

the SNU-8/Pac. SNU-8/Fac cells showed high sensitivity to anti-cancer drug paclitaxel after treatment of cyclosporine A, a MDR1 suppresser.

Taken together, we established a new drug-resistant cell line SNU-8/Fac and SNU-8/Pac from original SNU-8/WT, which demonstrated over expression of multi-drug resistant genes P-gp/*MDR1* and ATP7B. In addition, this drug resistance could be inhibited by MDR1 suppresser.

Key Words: Ovarian cancer, Multidrug resistance, P-gp/*MDR1*, ATP7B, Cisplatin



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가 (Parker
 SL, *et al.*, 1996). 가
 70-75%가 FIGO - (Hong WK, *et al.*,
 2005), 1
 75 %,

50 %가 18 24 (Gadducci
 A *et al.*, 1998; Mano MS, *et al.*, 2004). 20 30 % 1
 (McGuire WP and RF Ozols 1998). 80 %
 2 (Conte PF, *et al.*,
 1999).

90 %가
 (Pereira F, *et al.*, 1994).

multidrug resistance ABC(ATP-binding cassette)
 drug transporter *MDR1* (Brangi M, *et al.*, 1999; Chen CJ, *et al.*,
 1986; Gottesman MM and I Pastan 1993; Juliano RL and V Ling

1976; Riordan JR, *et al.*, 1985; Roninson IB, 1992), multidrug resistance-associated protein(*MRP*) (Cole SP, *et al.*, 1992), breast cancer resistance protein(*BCRP*) (Doyle LA, *et al.*, 1998; Izquierdo MA, *et al.*, 1996; Izquierdo MA, *et al.*, 1995)

170KD P-glycoprotein(P-gp)

ATP-dependent transporter

. *MDR1* multidrug resistance

(Brangi M, *et al.*, 1999; Chen CJ, *et al.*,

1986; Juliano RL and V Ling 1976; Riordan JR, *et al.*, 1985).

MR
(Flens  제주대학교 중앙도서관
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MRP MRP1 MRP2 MRP1

MDR1

. *BCRP* Mitoxantron, doxorubicin verapamil

(Allen

JD, *et al.*, 1999),

MDR1 MRP1

(Volk EL, *et al.*,

2000).

110KD major vault protein lung resistance protein(*LRP*) (Scheffer GL, *et al.*, 1995)

(Izquierdo MA, *et al.*, 1996),

MDR

가 (Chen CJ, *et al.*, 1986).

LRP

(Beck J, *et al.*, 1995).

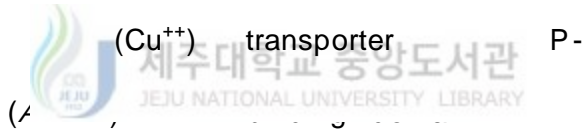
5-FU

thymidylate

synthetase(*TS*)(Lambert E, *et al.*, 1996; Newman EM, *et al.*, 1988;

Takeishi K, *et al.*, 1985), cisplatin

metallothionein(*MT*)(Lambert E, *et al.*, 1996)



(Petrukhin K, *et al.*, 1994),

cisplatin

(Beck J, *et al.*, 1995).

(*MDR1*) (p-gp)

가 (Tsuruo T, *et al.*, 1981). Verapamil PSC

833 1 P-gp

(Krishna R and LD Mayer, 2000; Oza AM, 2002).

Cyclosporine A P-gp

. Cyclosporine A P-gp

. Cyclosporine A (Coley HM, *et al.*, 1989; Naito M, *et al.*, 1993; Nooter K, *et al.*, 1990) (AML)
, (List AF, *et al.*, 2001; Smeets M, *et al.*, 2001).



2

serous cystadenocarcinoma 1

cisplatin SNU-8

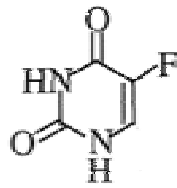
2

1

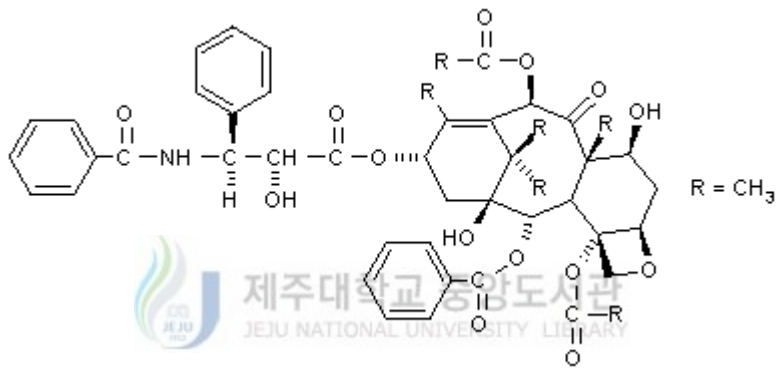
SNU-8/WT 2

SNU-8/Fac *MDR1, MRP1, BCRP, LRP, TS,*

MT ATP7B



5-FU



Paclitaxel

Fig. 1. Structure of 5-FU and Paclitaxel.

1.

SNU-8 (seoul,
korea) 37°C, 5 % CO₂ (Sanyo)
56 30 fetal
bovine serum(FBS, GibcoBRL) 10 % (antibiotic-
antimycotic, GibcoBRL)가 RPMI 1640(RPMI medium 1640,
GibcoBRL)



(-196)

가

가 (Grout , 1990).

dimethyl sulfoxide (DMSO, Sigma) 10 %, FBS 23.5 %, 66.5 %

37

70 %

DMSO

가

10

6

2.



5-fluorouracil(5-FU)(Sigma Chemical, St. Louis, MO, USA)

IC₅₀ (50%)

SNU-8

3

confluent

SNU-8/Fac

6

3.

MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay(Pieters R et al., , 1988)

. 96 well microplate(Falcon) SNU-8 3
SNU-8/Fac 2.5×10^4
90 $\mu\ell$ 24
10 $\mu\ell$ PBS
well , blank .
3 37 , 5 % CO₂ well
MTT (5mg/ $\mu\ell$ 37 , 5 %
CO₂ 4-5 MTT가 . well
80 $\mu\ell$ 150 $\mu\ell$ DMSO 10min
Shaking micro plate reader(Bio-Tek, USA)
540nm .
Sigma Chemical(St. Louis, MO, USA) .

4. Hematoxylin and Eosin staining

trypsin 5×10^4 8 Well permanox

slide(Nalge Nunc Internation) 3 95 %
 ethyl alcohol 1min Hematoxylin & Eosin
 staining

**5. Total RNA reverse transcription-polymerase chain
 reaction(RT-PCR) assay**

TRIZOL Reagent(Invitrogen) total RNA
 (Chomczynski P and N Sacchi 1987). Polypropylene tube
 5 × 10⁶ 5min 25
 incubation 200 μℓ chloroform 15sec
 가 2-3min 25 incubation . 4
 14,000rpm 15min tube
 . Tube 500 μℓ isopropyl alcohol
 10min 25 incubation . 4 14,000rpm
 15min 75 %
 RNA pellet tapping 4 14,000rpm 5min
 . pellet 0.1 % DEPC
 water . RNA (1 A260 unit of single-stranded

RNA = 40µg/mL) 260nm (DUR 650 spectrophotometer, Beckman), RNA A260(260nm)/A280(280nm) , RNA 5 µg RNA

37

121 20 0.1 % DEPC water

RT-PCR assay first strand cDNA 1U/µl RNasin (Promega), oligo(dT) 450ng, 40mM Tris-HCl(pH 8.4), 100mM KCl, 10mM MgCl₂, 1mM each dNTP, 10mM DTT(GibcoBRL) MMLV reverse transc , 200U가 20µl total RNA 1 µg

PCR 25ng RNA cDNA 1×PCR (10mM Tris-HCl pH 8.3, 50mM KCl, 1mM MgCl₂, 100 µg/ml gelatin, 0.05 % triton X-100), primers 10 pmole, 50 µM dNTP, taq DNA polymerase (Perkin Elmer) 2.5unit 가 25 µl

PCR primer PCR Table 1, 2

PCR Gene Amp PCR system 2400(Perkin Elmer)

PCR 25 $\mu\ell$ *MDR1, MRP, LRP, BCRP, ATP7B, MT,*
TS 10 $\mu\ell$ -*actin* 5 $\mu\ell$ 1.5 % agarose gels 15
 . 0.5 $\mu\text{g/ml}$ ethidium bromide 10min
 3 15 .

6. Western blot analysis

drug - sensitive SNU - 8/WT drug - resistant SNU -
 8/Fac 2×10^5 100mm
 가 60-70 % trypsin(GibcoBRL)
 가 BRL) 3

lysis buffer(50mM Tris-HCl, 150mM NaCl, 1mM EDTA,
 0.5 % NP-40, 10 mg/mL aprotinin, 1 mM phenylmethylsulfonyl
 fluoride) 250 $\mu\ell$ (ultrasonicator)

sample 20 vortexing .
 4 14,000rpm 10min .
 tube bicinchoninic assay(BCA)

loading buffer (0.2 mM Tris-HCl (pH 6.8), 3 % (w/v) SDS,
 30 % (v/v) glycerol, 15% (v/v) mercaptoethanol 0.01 % (w/v)
 bromophenol blue) 95 5min . 7 %

12 % SDS -PAGE gel(polyacrylamide gels) 50 μ g loading

1 30 . nitrocellulose
membranes 80 V 3 electrotransfer .
electrotransfer 5 % skim milk 1 blocking
membranes anti -P -gp antibody(Signet)(1:1,000) rabbit
polyclonal anti -ATP7B antibody(1:1,000)(orbigen) 1
. TBS -T membranes 10 4 2
rabbit -antimouse IgG(diluted 1:5000, Sigma), horse -antirabbit
IgG (diluted 1:5000, Sigma) 1 . TBS -T
membrane 10 4
(E



Table 1. Primer sequences used for PCR.

Gene ^a	S&AS ^b	Nucleotide sequences	Sequences region ^c	Length of PCR products	References ^d
<i>MDR1</i>	S	5'-CTGGTTTGATGTGCACGATGTTGG-3'	907-930	295bp	Chen, <i>et al.</i> , 1986
	AS	3'-TGCCAAGACCTCTTCAGCTACTG-3'	1179-1201		
<i>MRP</i>	S	5'-GACGGGAGCTGGGAAGTC	4180-4197	355bp	Cole, <i>et al.</i> , 1992
	AS	3'-ACAACCTACTCCGGTGCC-5'	4551-4568		
<i>LRP</i>	S	5'-ACAACCTACTGCGTGATTCTC-3'	941-960	390bp	Scheffer GL, <i>et al.</i> , 1995
	AS	5'-ACCTTCGTGGATGTACGACT	1330-1311		
<i>BCRP</i>	S	5'-CGACCGACGACACAGA	2136-2151	475bp	Doyle LA, <i>et al.</i> , 1998
	AS	5'-CTTAAAATGAATGCGATTGAT	2590-2610		
<i>MT</i>	S	5'-CCGGCTCCTGCAAATGCAA	47-66	220bp	Lambert, <i>et al.</i> , 1996
	AS	5'-TGTACAACCCTGACCGTGAC	247-266		
<i>TS</i>	S	5'-GGGCAGATCCAACACATCCTC	208-228	1111bp	Takeishi K, 1985
	AS	5'-AAGAGCACATACATTTCACTCTCCTCAC	1291-1318		
<i>ATP7B</i>	S	5'-TCCTGGTGGCTATTGACGGTGT	3539-3560	833bp	Petrukhin K, <i>et al.</i> , 1994
	AS	5'-CATTGAGGCGCAGAGACCACTT	4349-4372		
<i>-actin</i>	S	5'-GACTATGACTTAGTTGCGTTA	1912-1932	501bp	Nakajimalijima, <i>et al.</i> , 1985
	AS	5'-GTTGAACTCTCTACATACTCCG	2392-2412		

^a *MDR1*, multidrug resistance; *MRP*, multidrug resistance-associated protein; *BCRP*, breast cancer resistance protein; *LRP*, lung resistance protein; *MT*, Metallothionein; *TS*, thymidylate synthetase.

^b Sense and antisense.

^c The oligonucleotide primers constructed for PCR correspond to the sense and antisense based within these reported sequences.

^d References for primer sequences.

Table 2. PCR condition of *MDR1*, *MRP*, *BCRP*, *LRP*, *MT*, *TS*, *ATP7B* and *-actin*.

Gene	No. of cycles	Hot start	Denaturation	Annealing	Extension
MDR1	34			52 30sec	
MRP	33			56 30sec	
BCRP	35			53 30sec	
LRP	35			53 30sec	
MT	35	94 , 12 min	94 , 30 sec	57 30sec	72 , 1 min
TS	35			66 30sec	
ATP7B	33			63 30sec	
-actin	30			56 30sec	

Abbreviations for genes are the same as those in Table 1.

1.

CAP(cisplatin, cyclophosphamide, adriamycin)

SNU-8

SNU-8 가 cisplatin cyclophosphamide
cisplatin cyclophosphamide

(Figure. 2.).



2. 5-FU paclitaxel

SNU-8

5-FU paclitaxel

(Fig. 3, 4.). 5-FU paclitaxel IC₅₀

(table 3.) 1.79µg/ml 0.335µg/ml . 5-FU

paclitaxel 2µg/ml 1µg/ml

5-FU paclitaxel

SNU-8/Fac

SNU-8/Pac

3. 2

SNU-8/Fac SNU-8/Pac

SNU-8/WT

. Trypsin SNU-8/WT 2-3

SNU-8/Fac 3-4 , SNU-8/Pac

4-5 . SNU-8/Fac

가 SNU-8/Pac 가

. SNU-8/WT flask

SNU-8/Fac 가 가

SNU-8/Pac 가 가

(Fig. 5. A).

(Fig. 5. B).

4.

SNU-8/WT SNU-8/Fac

5-FU 4 (Fig. 6, table 4.)

doxorubicin paclitaxel 2 10000

	SNU-8/WT		SNU-8/Pac
paclitaxel	36 (Fig. 7, table 5.)		
	doxorubicin	5-FU	4 12

5.

MDR1, MRP1, BCRP, LRP, TS, MT

ATP7B RT-PCR mRNA

LRP SNU-8/WT, SNU-8/Fac

MRP1, B

SNU-8/Fac

SNU-8/WT

MDR1

ATP7B

SNU-8/Fac

SNU-8/WT

mRNA가 2.14 1.57

(Fig.8, table 6.).

LRP SNU-8/WT, SNU-8/Pac

MDR1, MRP1, BCRP, TS, MT SNU-8/WT

SNU-8/Fac

SNU-8/WT

ATP7B

SNU-8/Fac


SNU-8/WT

mRNA 1.57

(Fig.9, table

7.).

6. Western blot

	mRNA		protein	
	p-glycoprotein	ATP7B		
Western blot analysis				
	P-glycoprotein	SNU-8/Fac	SNU-8/WT	1.7
	(Fig. 10.) ATP7B	SNU-8/Fac	SNU-8/WT	1.3
ATP7B				
	(Fig. 12.)			

7. 2 SNU-8/Fac cytosporin A

2	SNU-8/Fac	cytosporin A	2
SNU-8/Fac	IC ₉₀	(90 % 가)	0.98 μ g/ml
(Fig13, table 8.).			

8. cycosphorin A

cycosphorin A IC₉₀ 1 μg/ml

5-FU, Doxorubicin paclitaxel

(Fig.14.). 5-FU Doxorubicin

paclitaxel .



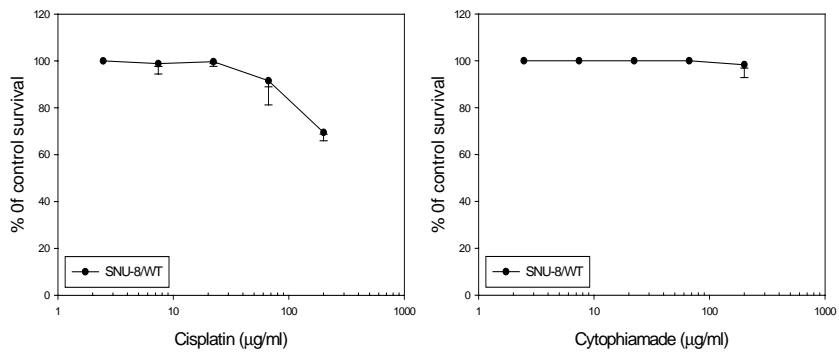


Figure. 2. Cytotoxicity of cisplatin and cytophiamide in the SNU-

8

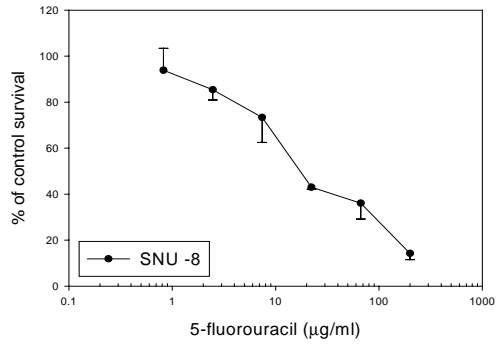


Fig. 3. Cytotoxicity of 5-FU in the SNU-8.

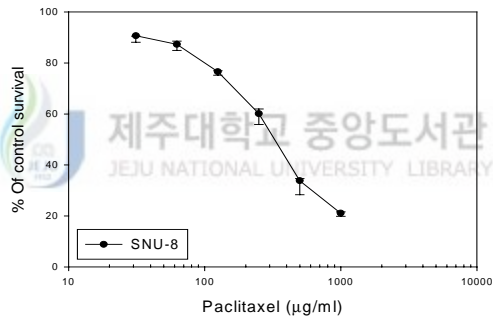
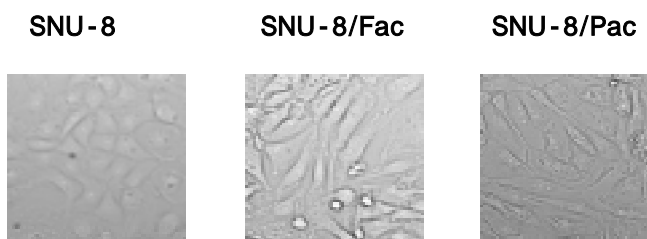


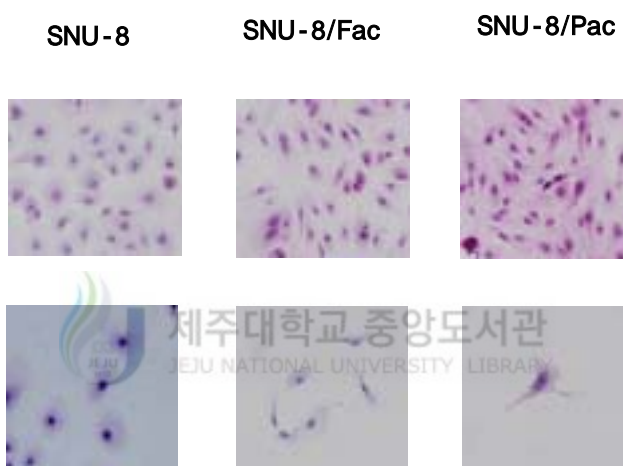
Fig. 4. Cytotoxicity of paclitaxel in the SNU-8.

Table 3. Sensitivity of SNU-8 to 5-FU and paclitaxel.

Cell	Drugs	IC50 (µg/ml)
SNU-8	5-FU	1.79
SNU-8	paclitaxel	0.335



(A)



(B)

Figure. 5. The morphological changes of drug- sensitive and drug- resistant cell lines(A: phase-contrast images, $\times 200$, B: Hematoxylin & eosin staining, $\times 100$, $\times 200$).

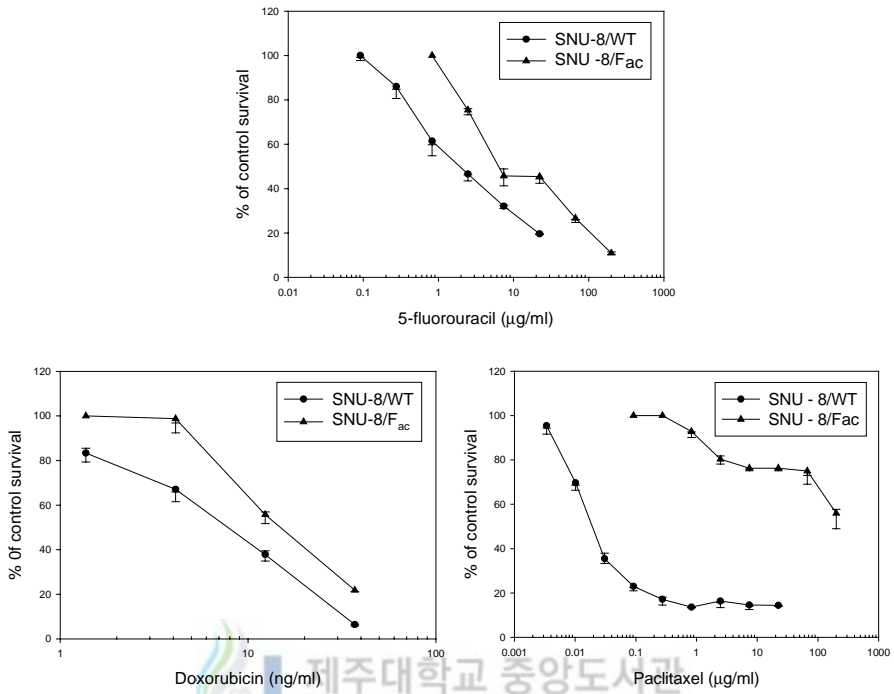


Fig. 6. Cytotoxicity of 5-fluorouracil, Doxorubicin, and Paclitaxel on SNU-8/WT and SNU-8/Fac. Cytotoxicity was determined using an MTT assay.

Table 4. Sensitivity of SNU-8/WT and its drug-resistant subline to various anticancer drugs

Drugs	IC ₅₀ ^b (ng/ml)	Relative resistance (fold) ^c
	SNU-8/WT	SNU-8/Fac a
5-FU	1760.25	7080.86(4.02)
Doxorubicin	8.0556	15.42 (1.80)
Paclitaxel	20	>200000 (>10000)

a, Numbers refer to selecting drug concentration in ng/ml.

b, Relative resistance was calculated as the ratio of drug concentrations (IC₅₀) which inhibited %-growth of each drug-resistant subline and the drug-sensitive parental line.

c, ratio of IC₅₀ of drug-resistant SNU-8 subline to IC₅₀ of SNU-8/WT.

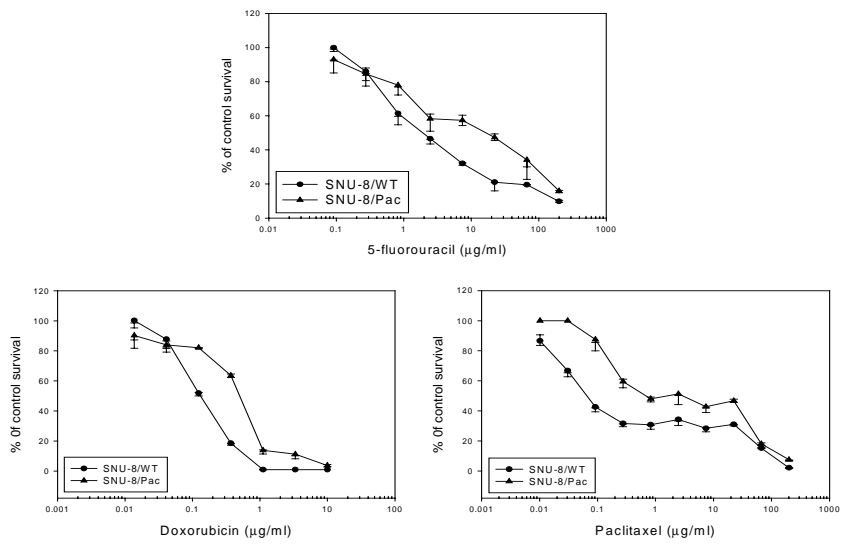


Fig. 7. Cytotoxicity of anticancer drugs in the SNU-8/WT and SNU-8/Pac. Cytotoxicity was determined using an MTT assay.

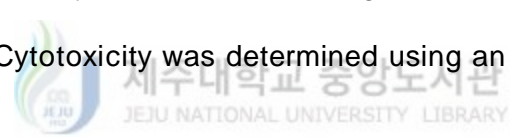


Table 5. Sensitivity of SNU-8/WT and its drug-resistant subline to various anticancer drugs

Drugs	IC ₅₀ ^b (μg/ml)	Relative resistance (fold) ^c
	SNU-8/WT	SNU-8/Pac ^a
5-FU	1.718	20.647 (12.02)
Doxorubicin	0.133	0.504 (3.78)
Paclitaxel	0.02	0.717 (35.85)

a, Numbers refer to selecting drug concentration in μg/ml.

b, Relative resistance was calculated as the ratio of drug concentrations (IC₅₀) which inhibited %-growth of each drug-resistant subline and the drug-sensitive parental line.

c, ratio of IC₅₀ of drug-resistant SNU-8 subline to IC₅₀ of SNU-8/WT.

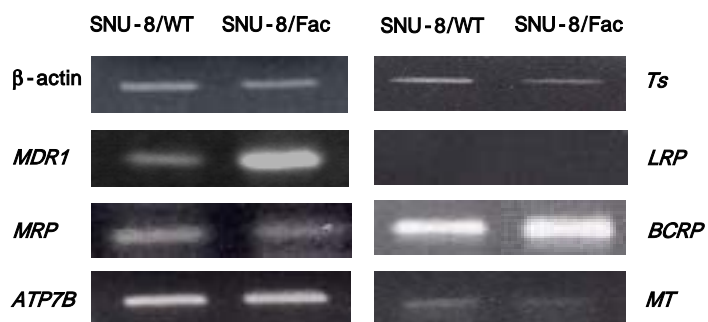


Fig. 8. Expression of drug resistance-related genes in the SNU-8/WT cells and their 5-FU-resistant cell subline. Gene expression was determined using a RT-PCR assay. β -actin was used as control for RAN. Abbreviations for gene names are described in a table.



Table 6. Sensitivity of SNU-8/WT and its drug-resistant subline to various resistant genes expressions.

	Transcripts/ β -actin (fold) ^a	
	SNU-8/WT	SNU-8/Fac
MDR1	0.07	0.15 (2.14)
ATP7B	1.07	1.67 (1.57)
MRP	++	+
LRP	-	-
BCRP	+	+
MT	+	+
Ts		+

a, Increased fold in the resistant cells as compared with that of drug-sensitive cells

Density of autoradiogram was determined using the densitometry.

-, not detected

+, trace that means visible density

Abbreviations for genes are the same as those in Table 1.

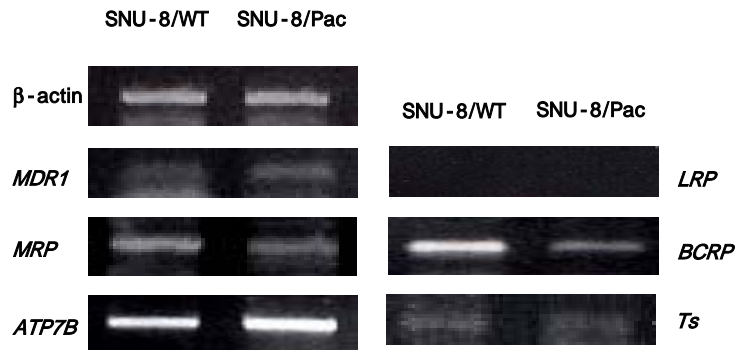


Fig. 9. Expression of drug resistance-related genes in the SNU-8/WT cells and their Paclitaxel-resistant cell subline. Gene expression was determined using a RT-PCR assay. β -actin was used as control for RAN. Abbreviations for gene names are described in a ... istant cells as compared with that of drug-sensitive cells.

Table 7. Sensitivity of SNU-8/WT and its drug-resistant subline to various resistant gene expressions.

	Transcripts/ β -actin	
	SNU-8/WT	SNU-8/Pac
MDR1	+	++
ATP7B	+	+++
MRP	++	+
LRP	-	-
BCRP	++	+
TS	+	+

Density of auto densitometry.

-, not detected

+. trace that means visible density.

Abbreviations for genes are the same as those in Table 1.

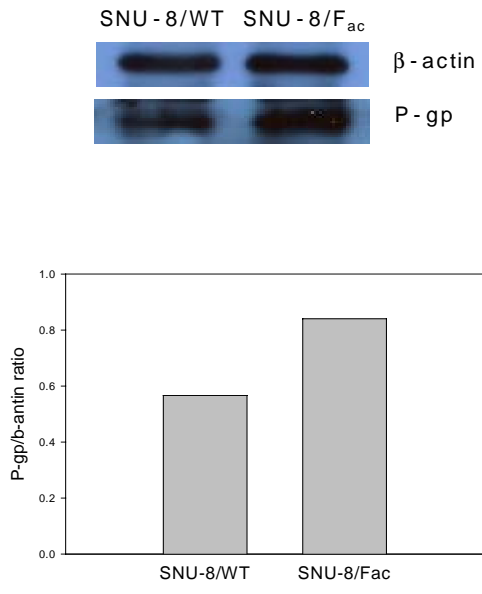


Fig. 10. Comparison of protein level of between ovarian cancer cell and that subline by western bolt analysis.

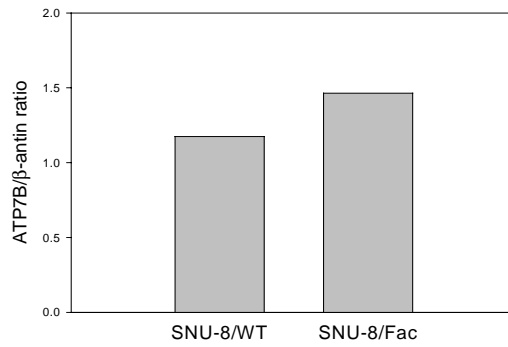
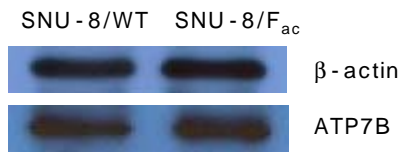


Fig. 11. Comparison of protein level of between ovarian cancer cell and that subline by western bolt analysis.

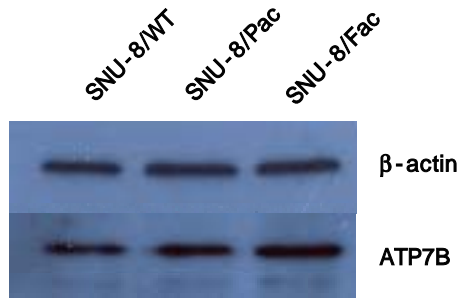


Fig. 12. Comparison of protein level of between ovarian cancer cell and that subline by western bolt analysis.

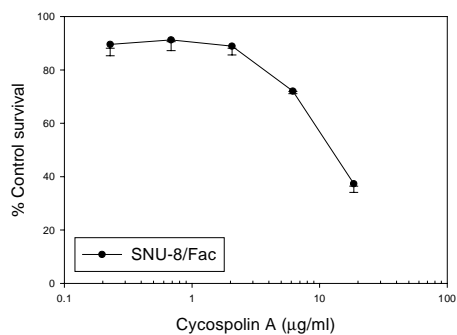


Fig. 13. Cytotoxicity of cyclosporine A in the SNU-8/Fac.

Table 8. Sensitivity of SNU-8/Fac to cyclosporine A.

Cell	Drugs	IC ₉₀ (µg/ml)
SNU-8/Fac	cyclosporine A	0.98

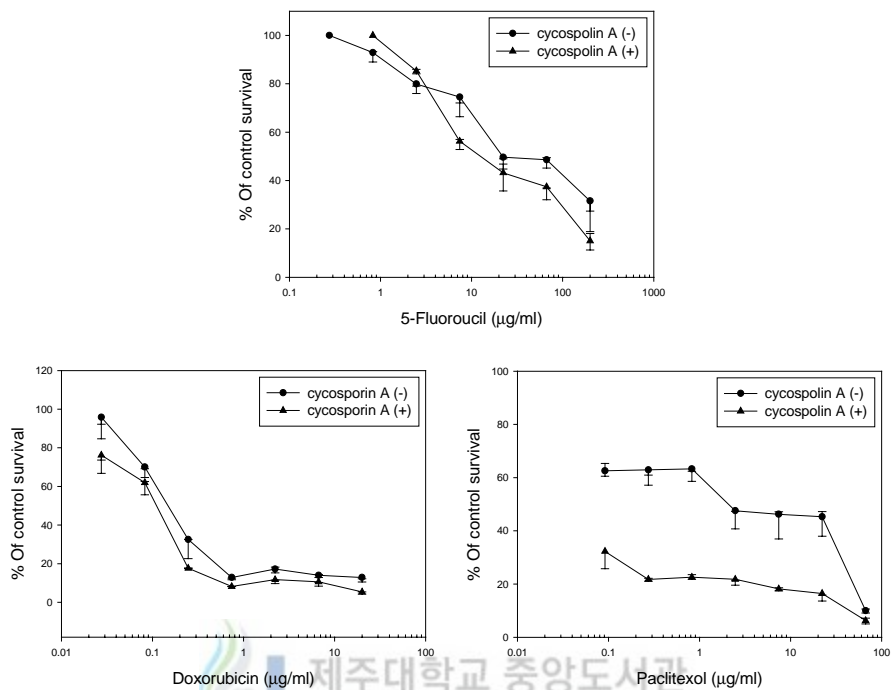


Fig. 14. Effect of cyclosporin A on the cytotoxicity of anticancer drugs in the SNU-8/ Fac. Cytotoxicity was determined using MTT assay.

가

가

5



가

1990

CP(cyclophosphamide, cisplatin),

CAP(cyclophosphamide, adriamycin, cisplatin), FAM(5-fluorouracil,

adriamycin mitomycin C)

1

80 %

2

2

1 (cisplatin)
 2 10 %
 6-7 (Poveda A
 2001).
 cisplatin
 2 5-FU Paclitaxel SNU-8/Fac
 SNU-8/Pac
 mRNA protein



. MTT assay
 test cisplatin cyclophosphamide
 IC₉₅ 200 µg/ml .
 . 5-FU paclitaxel IC₅₀ 1.79 µg/ml
 0.335 µg/ml . 5-FU

5-FU paclitaxel

SNU-8/Fac SNU-8/Pac .

가 (母) flask

cell membrane fibronectin adhesion

(Xio, Yang, *et al.*, 1994).

SNU-8/Fac 가

(Nielsen D and

T Skovsgaard 1992). SNU-8/Pac 가

Marianne Schmidt paclitaxel

가
(Schr  제주대학교 중앙도서관
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1 가 가 ,

multidrug resistance가 (Choi

CH, *et al.*, 2004; Conte PF, *et al.*, 1999; Mano MS, *et al.*, 2004;

McGuire WP and RF Ozols 1998; Parker SL, , *et al.*, 1996).

5-FU 2 SNU-8/Fac

paclitaxel 2 SNU-8/Pac 5-FU,

doxorubicin paclitaxel

multidrug resistance가

SNU-8/Fac paclitaxel

SNU-8/Pac 5-FU 12

SNU-8/Fac

paclitaxel

multidrug resistance

ABC(ATP-binding cassette) drug transporter *MDR1*

(Chen CJ, , *et al.*, 1986), *MRP1* (Cole SP, *et al.*, 1992), *BCRP*

(Chen CJ, *et al.*, 1986)가 *LRP* (Scheffer GL ,

et al., 1995), (Lambert E, *et*

al., 1996) *ATP7B* (Petrukhin K, *et al.*, 1994)

(Choi

CH, *et al.*, 2003).

2

5-FU

paclitaxel

SNU-8/Fac

SNU-8/Pac

mRNA

protein

multidrug resistance

multifactorial mechanism

MDR1

transmembrane proteins

(Riordan JR, *et al.*, 1985). P-gp efflux pumps protein MDR1

(Riordan JR and V Ling 1979). P-gp/MDR1 immunohistochemistry

(Baekelandt MM, *et al.*, 2000; Brinkhuis M, *et al.*, 2002; Goff BA, *et al.*, 2001),

가 (Barrand MA, *et al.*, 1994; Choi CH, *et al.*, 2004; Riordan JR and V Ling 1979). P-gp/MDR1 cisplatin

 제주대학교 중앙도서관 (Nakayama K, *et al.*, 2002), carcinoma cell

lines P-gp/MDR1 (Cole SP, *et al.*, 1992). Liang paclitaxel P-gp/MDR1

(Liang Y, *et al.*, 2001).

2 P-gp/MDR1

1 2 5-FU paclitaxel P-gp/MDR1 2

P-gp/MDR1

MRP1 *MDR1*, ATP-binding cassette transmembrane transporter superfamily member *P-gp/MDR1* 가 (Zaman GJ, *et al.*, 1993). *P-gp/MDR1* cell line *MRP1* (Barrand MA, *et al.*, 1994), 5-FU colon adenocarcinoma cell lines (Kuranaga N, *et al.*, 2001),

2



SNU-8/Fac

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SNU-8/Fac가 *MRP1*

. Liang paclitaxel *MRP1* (Liang Y, *et al.*, 2001). SNU-8/Pac

LRP

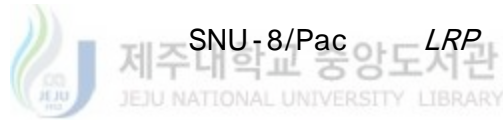
(Cole SP, *et al.*, 1992).

Mayr

(Riordan JR, *et al.*, 1985),

가 (Arts HJ, *et al.*, 1999).

cisplatin
 (van der Valk P, *et al.*, 1990). Tao nasopharyngeal carcinoma
 cisplatin 5-FU
 (Tao ZQ, *et al.*, 2005), SNU-
 8/Fac LRP, 2
 5-FU P-gp가
 . Liang paclitaxel LRP
 (Liang Y, *et al.*, 2001).
 2 SNU-8/Pac LRP
 Scheffer BCRP (Izquierdo
 MA, *et al.*, 1995) cisplatin
 (Goff BA, *et al.*, 2001)
 (Barrand MA, *et al.*,
 1994). MT cisplatin (Lambert E,
et al., 1996) TS 5-FU, methotrexate cisplatin
 (Pieters R, *et al.*, 1988), Tao nasopharyngeal
 carcinoma cisplatin 5-FU
 (Tao ZQ, *et al.*,



2005). SNU-8/Fac *BCRP, MT TS*

SNU-8/Fac

SNU-8/Fac

P-gp

. Baoqing Guo

paclitaxel

BCRP 가

(Guo B, *et al.*, 2004).

BCRP TS

SNU-8/Pac



ATP7B

(Stavrovskaya AA 2000)

cisplatin

가

(Beck J, *et al.*, 1995) ovarian

carcinoma

cisplatin

*ATP7B*가

(Zaman GJ, *et al.*, 1993).

ATP7B

2

5-FU paclitaxel

ATP7B

P-gp

ATP7B

Cycosporin A P-gp specific

(Ross

DD, *et al.*, 1993). Ito C T B leukemic lymphoblasts

(Ito C, *et al.*,

1998).

Cyclosporine A

5-FU
gp(MDR1)가



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SNU-8/Fac
-FU

P-

P-gp/MDR1

P-

gp/MDR1

Cycosporin A

Cycosporin A

IC₉₀ 0.98 µg/ml

Cycosporin A

IC₉₀ 1 µg/ml

5-FU

5-

FU

가

Cycosporin A가 P-gp/MDR1

가

2

SNU-8/Fac

P-gp/MDR1

(Choi CH, *et al.*, 2003), Cycosporin A

2가

가



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Cisplatin 5-FU

MDR1

Doxorubicin paclitaxel

5-FU, Doxorubicin

paclitaxel

5-Fu

Doxorubicin

Paclitaxel 2

SNU-8/Fac

P-gp/MDR1

5-FU

Paclitaxel

2

5-FU

SNU-8/Fac

가

5-FU

P-gp/*MDR1* *ATP7B*

가

2

P-gp/*MDR1* *ATP7B*



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▪

1. SNU-8
SNU-8 1 cisplatin

4. 2 5-FU Paclitaxel :
SNU-8/Fac SNU-8/Pac .



3. 2
SNU-8/Fac 5-Fu, Doxorubicin paclitaxel 4 , 2

SNU-8/Pac paclitaxel, Doxorubicin 5-Fu 36 , 4
12

4. SNU-8/Fac mRNA LRP
MRP1, BCRP, TS MT SNU- 8/Fac SNU-8/WT
MDR1 ATP7B .

SNU-8/Pac mRNA *LRP*
MDR1, MRP1, BCRP, TS *MT* SNU-8/Pac SNU-
 8/WT *ATP7B* .

5.

SNU-8/Fac protein P-gp ATP7B가
 . SNU-8/Pac protein ATP7B가
 .

6. MDR



cycosporin A

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SNU-8/Fac

5-Fu Doxorubicin
 paclitaxel .

2

1

2

▪

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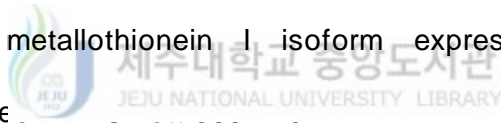
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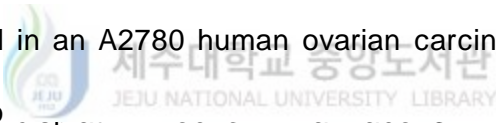
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Leukemia 15:80-88.

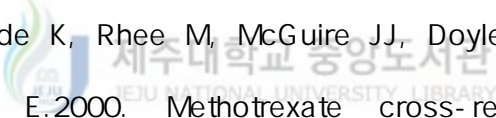
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1

2

2

5-fluorouracil(5-FU)

SNU-8/Fac

MDR1

Cyclosporin A

2

SNU-

8/Fac SNU-8/Pac MTT assay

RT-PCR western blot

2

SNU-8/Fac

SNU-8/Pac multidrug

resistance SNU-8/Fac

mRNA *MDR1* *ATP7B* protein

P-gp ATP7B가 SNU-8/Pac

mRNA *MRP1*, *BCRP*

, *MDR1* *ATP7B*

protein ATP7B가 SNU-8/Fac MDR

cyclosporin A

paclitaxel

cisplatin

5-FU

paclitaxel

SNU-8/Fac

SNU-8/Pac

P-gp/*MDR1*

ATP7B

가

: , , P-gp/*MDR1*, ATP7B, Cisplatin



가

가



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